Disclaimer- This guide is intended as an overview with salient details only. In order to provide high quality patient care it is important to maintain close and appropriate supervision.

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Chapter 3: Approach to Kidney Pathology

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Common indications for a kidney biopsy

- Acute kidney injury of unknown etiology
- Nephrotic syndrome
- Proteinuria > 1 g/day
- Isolated hematuria (lower diagnostic yield than proteinuria, exclude urologic causes)
- Reduced kidney function in the setting of a monoclonal paraprotein [(to assess for monoclonal gammopathies of renal significance (MGRS)]
- Rapid rise in serum creatinine or rapid rise of proteinuria in a patient with diabetes with/ without retinopathy
- AKI in an allograft
- Transplant surveillance in a high risk patient (high PRA, positive DSA)

Please see previous chapter for further kidney biopsy details and references but here is a summary:

**Biopsy Needle Size:** 16 gauge needle is preferred for a kidney biopsy. Biopsies from 18 gauge needles have fewer glomeruli and increased rates of complications and 14 gauge needle biopsies have been associated with an increased risk of hematoma formation (Peters B et al, 2017).

Complications are divided into two categories: Major (bleeding requiring transfusions, obstructive nephropathy, septicemia) and minor (gross hematuria, perinephric hematoma)

**Presence of Pathologist or Technician:** When obtaining a kidney biopsy, consider having a pathologist (or technician) at the procedure to provide on-site assessment. Evaluating the biopsy cores under a dissecting microscope will determine approximate numbers of glomeruli and will reduce inadequacy rate, as they can assist if additional passes are required. (Sometimes when you look at the sample looks like “pepperoni” -from perfused tissue, with the glomeruli picking up blood from engorged capillaries, with medullary areas appearing pale- on the gross sample even while still on your needle or when put on gauze afterwards)
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**Biopsy Adequacy:** Minimum adequacy is 7 glomeruli and 1 artery. This criteria was established as part of the Banff classification for transplants, but generally applies to native biopsies as well. With small samples, the possibility of missing focal lesions (such as segmental sclerosis or a crescent) increases. If scant cortex available may preclude assessment of interstitial fibrosis/tubular atrophy

The biopsy should be placed in 2 separate containers immediately after on-site assessment:

1. **Formalin** (10% buffered formaldehyde) - to be processed for light and electron microscopy
2. **Michel’s solution** - to be used for immunofluorescence microscopy, the tissue in Michel’s solution is washed, embedded in optimal cutting medium (OCT) and when sectioned for immunofluorescence microscopy, immunofluorescence is critical in the evaluation of immune complex and paraprotein-mediated disease.

Placing a biopsy in saline solution or moistened gauze upon transfer to the pathology lab can create artifacts and should be avoided. The biopsy will be grossed in the pathology lab, where one or more glomeruli will be cut from formalin fixed samples to send for electron microscopy. The remaining light microscopy tissue will be processed and embedded in paraffin wax, and cut into 3 um sections. Many 3 um sections are required for evaluation by the nephropathologist.

These sections are stained with the following special stains to highlight different histopathologic features:

- **Hematoxylin and Eosin (H&E)** - H&E is useful for nuclear detail and evaluation of inflammatory infiltrates. While H & E is the most common stain used by pathologists overall, it’s main limitation in interpreting kidney biopsies is that it does not highlight the glomerular and tubular basement membranes well.

- **Periodic Acid Schiff (PAS)** - The most important histochemical stain for a kidney biopsy. PAS highlights glomerular basement membranes, tubular basement membranes, and peritubular capillaries. It can be used for the assessment of proliferative changes within glomeruli and glomerulitis, as well as highlight tubulitis or peritubular capillaryitis within transplants.

- **Jones Methenamine Silver (JMS)** - Highlights glomerular basement membranes and tubular basement membranes. The mesangium stains black, which is pale in mesangiolysis or when replaced by non-matrix material (such as in amyloidosis or fibrillary glomerulonephritis). A spicular nature to the mesangium can occur with rapid deposition of fibrillary deposits, which occurs in amyloidosis and occasionally fibrillary glomerulopathy. Additionally, this stain can be used to assess the amount of interstitial fibrosis and tubular atrophy. Interstitial fibrosis will appear dark on JMS. Classic-type tubular atrophy will be highlighted by thickened tubular
basement membranes, and is commonly seen in arterionephrosclerosis. Endocrine-type tubular atrophy (consists of small tubules with narrow lumina, clear cells, and relatively thin basement membranes commonly seen in ischemic cortex in the setting of thrombotic microangiopathy or accelerated hypertension) will show attenuation of tubular basement membrane staining, which commonly occurs in the context of thrombotic microangiopathies.

- **Masson Trichrome**- Using low power, is used to quantify the percentage of interstitial fibrosis and the degree of intimal fibrosis of arteries. The presence of fibrosis stains blue on Masson Trichrome. Interstitial edema appears as light blue and has a fluffy or myxoid quality. At high power, immune deposits can be identified, which stain hot pink on Masson’s Trichrome.

- **Silver Methenamine Masson Trichrome (SMMT)**- This is a combination of a Jones methenamine silver and a masson trichrome stain is used as the counterstain. Some laboratories offer this stain, others will do an additional JMS level instead. This is useful to identify segmentally sclerotic and crescentic lesions within glomeruli, as the glomerular basement membrane disruption is highlighted by the silver stain.

- **Congo Red**- Assesses for the presence of amyloid. Amyloid stains salmon pink to red on Congo Red and displays apple green birefringence on polarization. The red color can be accentuated by fluorescence under yellow light/red filter. Collagen fibrils in areas of fibrosis will also polarize, but will not show apple-green birefringence.

### Standard immunofluorescence for every case:

<table>
<thead>
<tr>
<th><strong>Albumin</strong> (control)</th>
<th>Outlines the glomeruli, tubules, vessels, and interstitium. Highlights protein resorption droplets within Bowman’s space of glomeruli or within proximal tubules, which can indicate high-grade proteinuria.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IgA</strong></td>
<td>Granular mesangial staining 2+ or greater, with predominance over other immune reactants is indicative of IgA nephropathy or IgA-dominant infection-associated glomerulonephritis. Secondary causes of IgA deposition include inflammatory bowel disease, liver disease/cirrhosis, and infections.</td>
</tr>
<tr>
<td><strong>IgG</strong></td>
<td>Granular staining along glomerular basement membranes is seen in membranous glomerulopathy and infection-associated glomerulonephritis. Linear staining along glomerular basement membranes is seen in anti-GBM nephritis and atypical anti-GBM nephritis. Granular mesangial staining is seen in immune complex glomerulonephritides, such as lupus nephritis. Smudgy mesangial and capillary loop staining is seen in fibrillary glomerulopathy.</td>
</tr>
<tr>
<td><strong>IgM</strong></td>
<td>Granular mesangial staining occurs in many immune complex-mediated glomerulonephritis. When IgM-dominant with kappa light chain staining &gt; lambda light chain staining, consider cryoglobulinemic glomerulonephritis.</td>
</tr>
</tbody>
</table>
C3- Granular mesangial staining occurs in infection-associated glomerulonephritis and C3 glomerulonephritis. Granular capillary loop C3 staining is common in membranous glomerulonephritis.

C1q- Granular mesangial and capillary loop staining occurs in lupus nephritis and C1q glomerulonephritis.

Fibrinogen- Segmental to global staining is seen within glomeruli with crescents. Staining within vessels can highlight thrombi or foci of necrotizing arteritis.

Kappa and Lambda

*In addition, an unstained control and a H & E on the frozen tissue should be performed. The frozen H&E is examined by both brightfield and darkfield microscopy.

Additional immunohistochemical or immunofluorescence stains that may be used on select cases include:

SV40 (by immunofluorescence or immunohistochemistry)- Evaluates for the presence of polyomavirus. Should be performed on ALL transplant biopsies, as some cases of polyomavirus nephritis can have little to no viral cytopathic effects. SV-40 positivity can indicate the presence of BK or JC nephritis.

C4d (by immunofluorescence or immunohistochemistry)- C4d positivity within peritubular capillaries are a criterion in antibody-mediated rejection (of which includes positivity for donor specific antibodies, microvascular inflammation within tissue, and a positive DSA). While helpful when positive, negative C4d staining occurs in up to ⅓ of biopsies showing antibody mediated rejection.

Hemoglobin- Used to evaluate eosinophilic to brown pigmented casts. Hemoglobin casts indicate intravascular hemolysis and require identification and treatment of the underlying condition.

Myoglobin- Used to evaluate pigmented casts, of which can be morphologically identical to hemoglobin casts. Their presence is indicative of rhabdomyolysis.

Uromodulin or Tamm-Horsfall protein- Can be used in the evaluation of atypical casts, as well as distal tubular epithelium in autosomal dominant tubulointerstitial disease.

Phospholipase A2 Receptor (PLA2R)- Used for evaluation of primary membranous nephropathy. Positive cases show granular capillary loop deposits.

Thrombospondin type 1 domain containing 7a (THSD7A)- Used for the evaluation of membranous nephropathy. Positive cases show granular capillary loop deposits.

DNAJB9- Used for the evaluation of fibrillary glomerulonephritis. Positive cases show mesangial staining.

Fibronectin- Used for the evaluation of fibronectin glomerulopathy.
Additional special stains that may be used on select cases include:

• **Acid fast bacilli (AFB)**- A special stain used to identify the presence of mycobacteria. This is used in the workup of cases with granulomatous interstitial nephritis to rule out Mycobacterium tuberculosis and atypical mycobacteria.

• **Von Kossa stain**- A special stain used to identify calcium deposition. This can be used to highlight Michaelis Gutman bodies in Malakoplakia.

• **Hale’s stain**- Used for evaluation of bile casts.

• **Perl’s stain or prussian blue**- Highlights iron and hemosiderosis.

• **Phosphotungstic acid-haematoxylin (PTAH)**- Highlights fibrin deposition. May be useful in identifying fibrin thrombi in donor kidneys or in cases of thrombotic microangiopathy.

### Evaluating the kidney biopsy:

To read a **kidney biopsy**, assess the 4 main compartments of the kidney parenchyma:

1. Glomeruli
2. Interstitium
3. Tubules
4. Vasculature

**Summary of pathological findings in glomerular diseases (From 2009)**

Developing a systematic approach when studying a kidney biopsy will help you not miss anything:

1. Look at the H & E of the frozen tissue for a general overview of the biopsy. This can be reviewed under either **lightfield** or darkfield microscopy, while it’s optimal to do both. The eosin stain on the frozen H & E fluoresces and highlights glomerular and tubular basement membranes, similar to a silver stain (such as JMS or SMMT).
2. Review the **immunofluorescent** stained slides. These have a FITC-label and positivity appears in green (excited by blue light). Immunofluorescence is reviewed in both glomeruli, tubulointerstitium, and vessels and is graded from 0 (not present) to 3+ (strong positivity). Immune deposits are described as granular, linear, or smudgy. Within glomeruli, specify if the deposits are along capillary loops, mesangium, or both.

3. It is difficult to identify the location along capillary loops (subepithelial, intramembranous, or subendothelial), and this distinction can be made either by review at high power (40x or 50x objective for 400x or 500x magnification overall) or by **electron microscopy**.

**Common patterns:**

1. Granular
2. Linear
3. Pauci-immune

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**Granular mesangial**- IgA nephropathy (IgA, kappa < lambda, +/- C3), lupus nephritis (full house staining with IgA, IgG, IgM, C3, C1q, kappa, and lambda), infection-associated glomerulonephritis (IgG, C3, kappa, lambda, +/- IgA)

**Granular capillary loop**- membranous glomerulopathy (usually only IgG, C3, kappa, and lambda; but can provide “full house staining in membranous lupus nephritis)

Granular mesangial and granular capillary loop staining in a case of membranous lupus nephritis (C1q shown). Lupus, post-Infectious, Membranous, IgA nephropathy (LIMA) (LIMA Granules)
Look for extraglomerular staining on immunofluorescence, including tubular basement membrane or vascular immune deposits. Tubular basement membrane deposits can occur in: lupus nephritis, anti-brush border antibody disease (also known as LRP2 nephropathy), idiopathic hypocomplementemtic tubulointerstitial nephritis, and IgG4-associated disease. Vascular immune deposits can occur in lupus vasculopathy and cryoglobulinemia. Vascular staining for IgM and fibrinogen is common in thrombotic microangiopathies.

Carefully compare kappa and lambda immunofluorescent stains, by keeping both slides on the stage at once. Compare staining in glomeruli, tubular basement membranes, proximal tubule protein reabsorption droplets, and casts. A 2+ difference in immunofluorescent intensity is significant, small differences typically are not.

After review of immunofluorescence microscopy, you will have ruled in or ruled out immune complex-mediated diseases or paraprotein mediated diseases.

**Linear-** anti-GBM nephritis (IgG, kappa, lambda), light chain deposition disease (kappa or lambda only), monoclonal immunoglobulin deposition disease (IgA or IgG or IgM plus kappa or lambda).

**Smudgy-** fibrillary glomerulonephritis (IgG, kappa, lambda +/- C3 or IgA), amyloid (kappa or lambda only, but can “trap” all immune reactants).
Next, review the light microscopy. Start at low power under the 4 or 5x objective (40-50x magnification overall). At low power, you can determine the amount of cortex versus medulla, tubulointerstitial scarring, and interstitial inflammation.

Low power of a Masson Trichrome stain on a kidney biopsy showing normal glomeruli, no significant interstitial inflammation, no significant interstitial edema, and no significant interstitial fibrosis.

At higher power (20x objective), review glomeruli. Determine if there is increased cellularity within the glomerulus. Increased cellularity can be due to extracapillary proliferation (seen in crescent formation), mesangial proliferation, endocapillary proliferation, or all of the above. Mesangial hypercellularity is defined as > 4 cells in a single mesangial area (by the Oxford classification of IgA nephropathy, but can serve as a guideline for other diseases as well). Endocapillary hypercellularity shows inflammatory cells within the glomerular capillary loops. This is usually accompanied by endothelial cell swelling, which causes closure of the glomerular capillary loops. If you cannot see a lumen inside a glomerular capillary in a non-ischemic, non-sclerotic glomerulus, there is endocapillary hypercellularity present.
Next, look at the glomerular basement membranes on a silver stain (JMS or SMMT). Prominent glomerular basement membranes can occur in membranous glomerulopathy, light chain deposition disease, diabetes, gout, and smoking-related glomerulopathy. If the glomerular basement membranes are prominent (and likely thickened on electron microscopy), review them at high power (40x - 50x objective, or 400x to 500x magnification overall). This can reveal spikes or holes (seen in membranous glomerulopathy or other causes of capillary loop deposits), or double contours (seen with glomerular basement membrane duplication). Review the mesangium on silver stain. Compare to the appearance on PAS, and it should be similar. If there is less silver staining in the mesangium, consider mesangiolysis (occurs in thrombotic microangiopathies and in diabetes) or mesangial replacement with non-matrix material (such as fibrillary glomerulonephritis or amyloidosis). The silver stain can also help define breaks in the glomerular basement membrane caused by fibrinoid necrosis and crescent formation. If fibrinoid necrosis and/or crescents are present, consider a differential diagnosis of immune complex glomerulonephritis, anti-GBM nephritis, or pauci-immune crescentic glomerulonephritis (seen most commonly with positive serologies for antineutrophil cytoplasmic antibodies, ANCA). A crescentic lesion can be present with or without fibrinoid necrosis. Early, active crescents are cellular. Older crescents are fibrocellular to fibrous, with mimic segmentally sclerotic scars. A cellular crescent is shown below.
Then review the interstitium. Within the interstitium, assess the amount of interstitial fibrosis, tubular atrophy, and whether there is presence of edema or inflammation.

**Fibrosis**

Use the Masson-Trichrome stain to assess the percentage of interstitial fibrosis (appears blue) or edema (lighter blue). Edema is typically present when there is increased spacing between tubular profiles without fibrosis. Evaluation of interstitial fibrosis using silver stains (JMS or SMMT) typically underestimates the amount of fibrosis, but can be useful to determine the degree of tubular atrophy. Types of tubular atrophy include classic type (with thick and duplicated tubular basement membranes), endocrine type (small tubules with simplification adjacent to areas of vascular scarring), and thyroidization-type (multiple simplified tubules containing hyaline casts).

**Inflammation**

Use the H&E stain to assess the amount of interstitial inflammation. If interstitial inflammation is present, evaluate for any associated tubulitis, as well as what types of cells make up the tubulitis (lymphocytes or plasma cells in cellular rejection, neutrophils in acute pyelonephritis, eosinophils in a drug reaction, etc). Tubulitis is best assessed on a PAS stain, where you examine for inflammatory cells underneath the tubular basement membranes. Lymphocytes appear hyperchromatic and are generally smaller in size than tubular epithelial cell nuclei. If there are interstitial inflammatory infiltrates, examine them for their composition (neutrophil-rich, lymphocyte rich, plasma cell-rich, eosinophil-rich, histiocyte-rich, mixed, or granulomatous), as well as whether they are present in the cortex, medulla, or both.

The composition of inflammatory infiltrates can give clues to their etiology. For example, eosinophil-rich infiltrates occur in an acute interstitial nephritis due to an allergen or a drug reaction. Plasma-cell rich inflammatory infiltrates can occur in IgG4-associated disease or in rejection of an allograft.
Neutrophil-rich infiltrates present in a greater extent in the medulla than cortex occurs in ascending infections and in tubulointerstitial nephritis due to checkpoint inhibitors.

**Tubular injury and atrophy**

The tubules should be examined for injury (vacuolation, apical cytoplasmic blebbing, nuclear pseudostratification, loss of brush borders, thinning, and dilatation), as well as regeneration (prominent nucleoli, nuclear enlargement, mitotic figures within tubular epithelial cells). Casts should be examined within tubular lumens. While a few Tamm-Horsfall protein casts and hyaline casts are acceptable in almost any biopsy, atypical casts should prompt careful evaluation and possibly further workup. Eosinophilic granular to globular casts may represent myoglobin or hemoglobin and could indicate rhabdomyolysis or systemic hemolysis, respectively. Fractured casts, casts with a cellular reaction, or casts that are PAS-pale could indicate light chain casts in light chain cast nephropathy. Paraffin immunofluorescence for kappa and lambda light chains could be performed to prove these are light chain restricted if not already identified on the frozen tissue.

**Vessels**

Finally, examine the blood vessels. Estimate the amount of fibrosis of arteries as mild, moderate, or severe. This can be done by assessing the patency of the vascular lumen (<25% occluded = mild, 25-50% occluded = moderate, >50% luminal stenosis = severe). The amount of arteriolar hyalinosis is also assessed. Whether arteriolar hyalinosis is nodular or concentric may also be important, as concentric arteriolar hyalinosis occurs in diabetic nephropathy, while nodular arteriolar hyalinosis occurs in calcineurin inhibitor toxicity. Examine the intima within vessels for intimal arteritis. If intimal arteritis is present, determine if this extends transmurally or if there is fibrinoid necrosis.

**Electron microscopy**

For electron microscopy images, first look at the lowest power image taken to assess to look at the integrity of the glomerulus. Interpret higher power images in the context of how the glomerulus looked on lower power. Ischemic glomeruli can have basement membranes appear thickened due to corrugation, and may not appear thickened in an intact glomerulus that is without wrinkled basement membranes. Segmentally sclerotic glomeruli often have global podocyte foot process effacement. If the finding is critical, such as evaluation of podocyte foot process effacement in focal segmental glomerulosclerosis, submitting a non-sclerotic glomerulus for electron microscopy is key.

**Orient yourself in each high power image by identifying key structures, such as the urinary space, the subepithelial (containing podocytes) and subendothelial (containing fenestrated endothelium) sides of the glomerular basement membranes, the intracapillary space, and the mesangium.** A labeled electron photomicrograph as an example is below:
Next, evaluate for electron-dense deposits. Electron dense immune-type deposits can be seen along glomerular basement membranes in subepithelial, intramembranous, and subendothelial locations, or within the mesangium. Large subendothelial electron dense deposits can create the appearance of “wire loops” by light microscopy. The overlying glomerular basement membrane can be easily delineated, and this distinguishes this finding from electron dense transformation that occurs in dense deposit disease. An image of a wire-loop due to massive subendothelial deposits is shown below:

An electron photomicrograph with labeled subepithelial, subendothelial, and mesangial deposits is shown below:
*in situ (in situ means “in kidney” because the antigen is in the kidney already) Formation of Immune Complex:

- Usually subepithelial (area containing podocytes)
- Membranous, Anti-GBM disease

*Deposition of Immune Complexes formed in circulation:

- Can be subendothelial (area containing the fenestrated endothelium) sides of the glomerular basement membranes
- Larger Immune complexes will not pass through the GBM and are stuck on the endothelial side and so deposit there like in MPGN
- Can however be both subendothelial and subepithelial if antigen and Ab pass through GBM separately as in SLE.

Reading a kidney pathology report:

**Diagnosis:** There may be multiple kidney biopsy diagnoses provided. The top line should represent the disease process that is driving the patient’s clinical presentation. Secondary or tertiary diagnoses are listed thereafter. In addition, the amount of global glomerulosclerosis, interstitial fibrosis, tubular atrophy, arteriosclerosis, and arteriolar hyalinosis is often included in the diagnostic lines or in a table of chronicity indices. If these prognostic indicators are not included in the main diagnosis, you should be able to find them in the microscopic description.

In this case, a patient’s presentation (let’s say AKI in a patient with a kidney transplant), is likely due to a combination of chronic active antibody mediated rejection and acute T-cell mediated rejection. In this case, the most severe would be listed first.
After the diagnosis, sometimes a diagnostic comment is provided to provide more information on the diagnosis, give references, or to describe a differential diagnosis for a non-specific pattern of injury. Non-specific patterns of injury that may have a more general differential diagnosis include membranoproliferative glomerulonephritis, which may have a large differential diagnosis, acute or chronic active tubulointerstitial nephritis, thrombotic microangiopathy, and others. In these cases, integration of clinical and laboratory information may be critical to identify the etiology for the pattern of injury on the biopsy and to achieve a clearer diagnosis.

The microscopic description should have information regarding light microscopy, immunofluorescence microscopy, and electron microscopy. This should delineate all the major details of the histopathology of the 4 compartments, as described earlier in this chapter. Any special stains, immunohistochemistry, or additional immunofluorescence studies should be indicated with their results.

Communication with the nephrologist and pathologist is important before, during, and after the biopsy to make clinicopathologic correlations and to optimize patient care.

Other useful resources:
Path 101 at NephSim - Basics on looking at a slide under the microscope
Atlas of Kidney Pathology
GlomCon - series of web based interactive seminars for trainees and physicians in nephrology and nephropathology, click under “For Healthcare Professionals”
Washington University School of Medicine in St. Louis’s Dr. Tim Yau’s video series on nephropathology.

Example:
Chronic Active Antibody Mediated Rejection.
- Chronic Transplant Glomerulopathy, Severe.
- Peritubular Capillaritis, Moderate.
- C4d Diffusely Positive in Peritubular Capillaries.
Acute T-Cell Mediated Rejection, Class 1A.
Global (13/53) and Segmental Glomerulosclerosis.
Interstitial Fibrosis and Tubular Atrophy, Moderate (Banff Grade 2).
Arteriosclerosis, Moderate.
Arteriolar Hyalinosis, Moderate.