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Renal biopsy pathology

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- c. Tubulointerstitial renal diseases
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1. Terminology for Renal Pathology

Routine processing of renal biopsies includes evaluation by light microscopy, direct immunofluorescence and electron microscopy. Immunofluorescence (IF) and electron microscopy (EM) are methods established decades ago in animal models as methods to establish the presence, type and precise location of immune complexes. Additional roles for these methods have emerged from experience with their application to clinical material since the 1960s and these are noted below.

The biopsy findings often have meaning only in the context of the clinical question. The minimum data that is required is the age of the patient, the presenting renal syndrome and the presence or absence of systemic disease. Often the final diagnosis requires incorporation of serologic data into consideration after the pattern of injury on biopsy is determined.

a. Light microscopy (LM) is used to evaluate each compartment (glomeruli, tubulointerstitial areas and blood vessels) for injury, inflammation, scarring or accumulation of abnormal material. The standard H&E stain is routinely supplemented with stains to highlight basement membranes (Periodic-acid Schiff = PAS and Jones' silver) and a stain to evaluate scarring (Masson trichrome which stains collagen blue).

i) Glomeruli

The most common indication for a renal biopsy is diagnosis of glomerular disease. Although glomerular pathology follows the same general principles of injury, inflammation and repair as disease in any organ system, a complex terminology has evolved to describe patterns of glomerular disease. This terminology has an added importance because, in several instances where knowledge about the underlying cause is lacking, diseases are named for the pathologic pattern.

Terms related to extent of glomerular involvement:

Focal = less than 50% of glomeruli involved
Diffuse = more than 50% of glomeruli involved
Global = all of an individual glomerulus involved
Segmental = portion of an individual glomerulus involved

Glomerulonephritis (GN) = inflammation in the glomerulus.

Evaluation of the activity and chronicity of inflammatory glomerular injury:

Acute or active injury (accumulation of circulating inflammatory cells; necrosis; exudates of neutrophils or fibrin)
Chronic persistent immune complex deposition
(cellular "proliferation" = mononuclear hypercellularity; abnormal excess GBM production)
Repair of injury with scarring (fibrosis, sclerosis)

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Terms related to location of inflammatory cells, immune complexes or scarring:

Mesangial = process confined to mesangial region with capillary loops easily defined and intact

Endocapillary = process confined within GBM in glomerular tuft

Extracapillary = abnormalities seen in Bowman's space outside of the GBM.

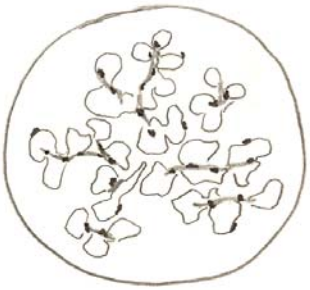
Extracapillary hypercellularity is more commonly referred to as a "**crescent**"

Terms related to type of inflammation:

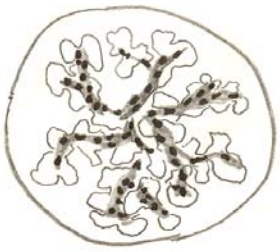
Exudative = prominent neutrophil component in the hypercellular glomerulus

Proliferative = mononuclear glomerular hypercellularity; generally the term is used without regard to whether the cells are endogenous proliferating glomerular cells or infiltrating monocyte/macrophages since the distinction cannot be reliably made on routine H&E sections; the term is often modified as to location as described above.

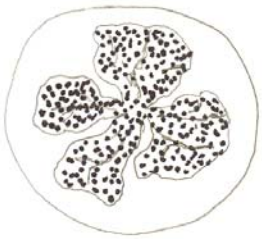
Necrotizing = evidence of karyorrhexis or fibrin exudate



Normal

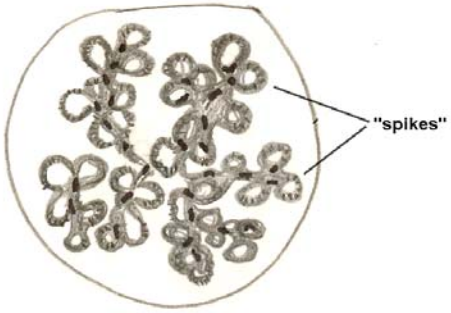


Mesangial Proliferative

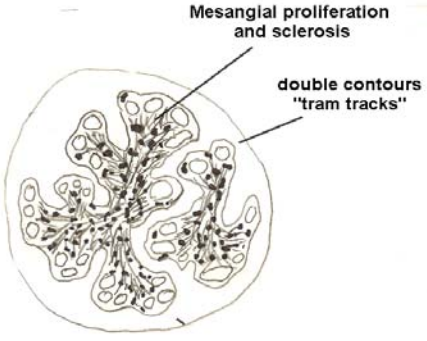


Endocapillary Proliferative

Extra capillary Proliferative (Crescent)



Membranous



MEMBRANO PROLIFERATIVE

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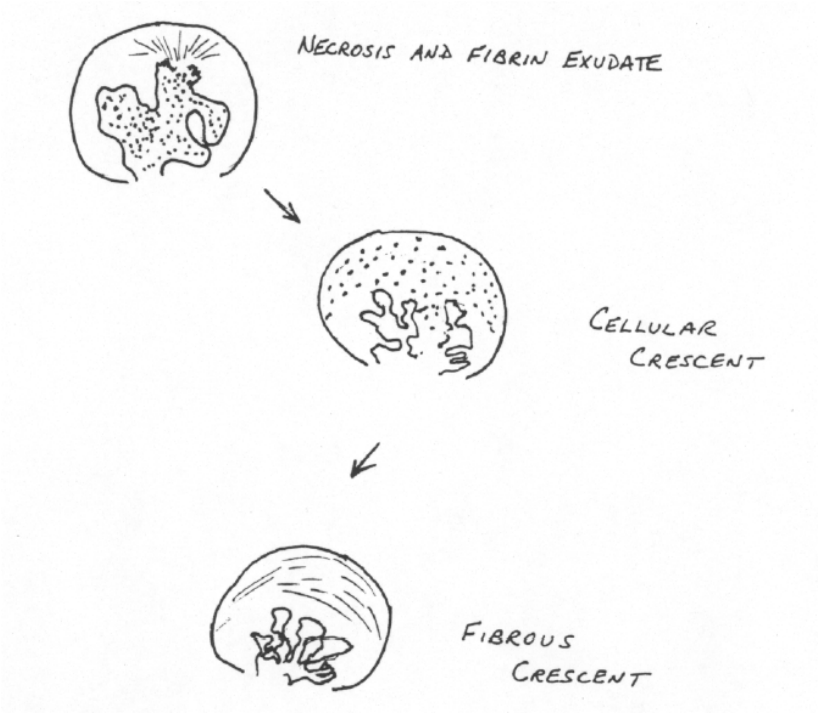
Glomerular necrosis and crescent formation

There is a characteristic sequence of lesions resulting from severe glomerular injury irrespective of cause:

1. "**necrosis**" = rupture of the GBM, presence of karyorrhectic debris, fibrin exudate and neutrophils
2. "**cellular crescent**" = early phase of reaction to necrotic debris and fibrin in Bowman's space due mostly to infiltration of macrophages
3. "**fibrocellular crescent**" = organization of the reaction in Bowman's space with persistent cellular response and ongoing collagen production
4. "**fibrous crescent**" = end stage scar in Bowman's space after necrotizing lesion (difficult to distinguish from scarring due to glomerular ischemia if no inflammation persists)



Terms related to excess collagen production in response to damage:

Excess glomerular basement membrane (GBM) production ("basement membrane reactions")

Membranous = thickening of the GBM in the peripheral capillary loop;
the term is currently used exclusively to refer to the thickening which results from "subepithelial" immune complex (deposition on the epithelial (podocyte) side of the GBM) associated with projection of excess basement membrane material between the immune complexes toward the podocytes (seen as "**spikes**" on silver stain).

Mesangiocapillary pattern = widening of the capillary loop & expansion of the mesangium;
the term is used almost interchangeably with **membranoproliferative GN** referring to the reaction to deposition of immune complexes in the mesangial and endothelial side of the peripheral loop GBM; in the mesangiocapillary pattern of response there is mesangial sclerosis and the subendothelial capillary loop thickening is associated with mesangial cell interposition and reactive duplication of the GBM giving a "**double contour**" or "**tram track**" appearance on silver or PAS stains

Sclerosis = Type of glomerular scarring due to collapse of the GBM or accumulation of mesangial matrix and collapsed GBM or basement membrane-like material (type IV collagen).

Fibrosis = scarring with accumulation of type I or III collagen fibers

Sclerosis and fibrosis are non-specific stereotypic responses to any type of injury, but it seems that fibrosis is more likely when basement membranes are disrupted and regeneration is not possible while sclerosis is sometimes reversible. The distinction between glomerulosclerosis and glomerular fibrosis is not strictly held even by pathologists.

ii) Tubules and interstitium

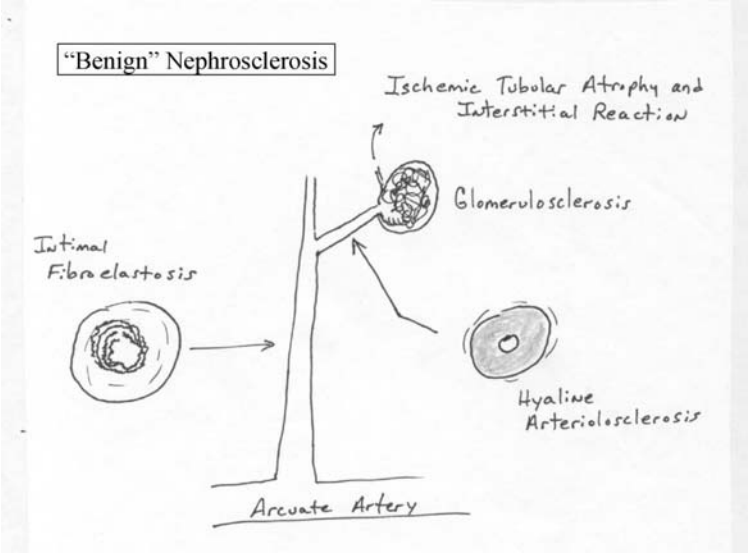
Acute tubular injury is manifest as raggedness or attenuation of the epithelial lining, sloughing of cells or cytoplasmic blebs into the tubule lumens or, less commonly, overt coagulative necrosis. Tubule cells do not ordinarily proliferate but can be stimulated to do so by injury. Tubules can regenerate completely if the tubular basement membrane (TBM) is left intact. **Regenerative nuclear changes** (mitoses or enlarged, irregular hyperchromatic nuclei) are an indication of recent tubular injury.

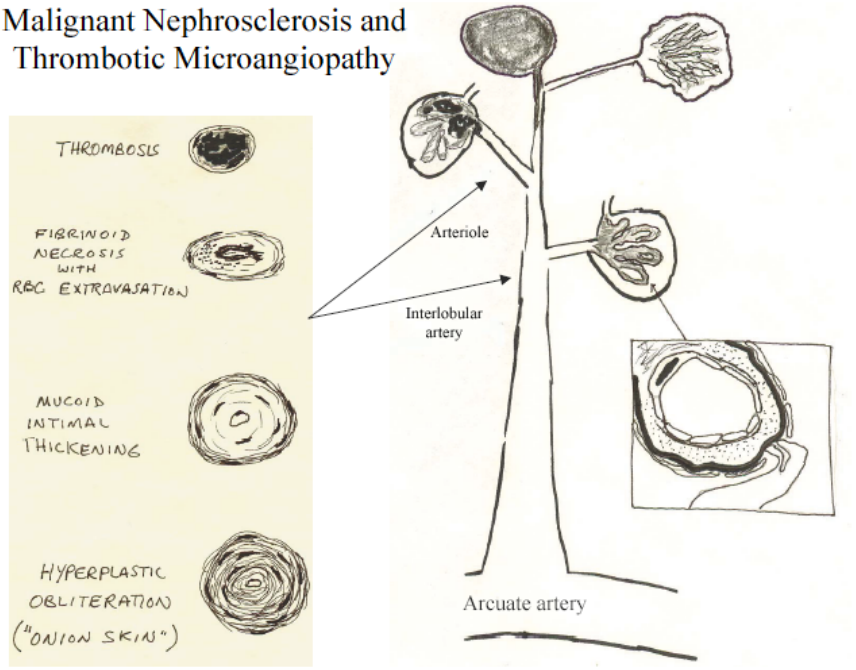
The normal interstitium consists of peritubular capillaries and spindle peritubular fibroblast-like cells. It is a virtual space barely perceptible in routine sections. The interstitium may be widened due to inflammation or fibrosis. In **acute interstitial nephritis**, there is edema and an accumulation of infiltrating inflammatory cells. The nature of the inflammatory cells will depend on the insult. There will be a prominent neutrophil component in bacterial infection and a predominant lymphocyte component in type IV hypersensitivity reactions. **Chronic interstitial nephritis** will consist of mostly mononuclear inflammatory cells along with varying degrees of fibrosis.

Chronic injury of any etiology causes **tubular atrophy** and **interstitial fibrosis**. Tubular atrophy is often accompanied by TBM sclerosis (thickening and duplication presumably from repetitive cycles of epithelial loss and re-epithelialization with new basement membrane production). With advanced nephron loss, there is usually some degree of **hypertrophy in the residual tubules**.

iii) Blood vessels

Renal biopsies generally represent only the microvasculature (interlobular arteries and arterioles, occasionally arcuate arteries at the cortico-medullary junction). Lesions in these vessels include inflammation (**vasculitis**), thrombosis (**thrombotic microangiopathy**) and sclerosis. **Arteriosclerosis** (intimal sclerosis, mural fibrosis and microvascular hyalinosis) is a non-specific pattern related to healing of any acute injury or chronic wear and tear (e.g., due to aging which may be accelerated by hypertension or diabetes mellitus).





b. Immunofluorescence (IF) is used primarily to detect the presence of immunoglobulins (Ig) and complement (C') components. Separate tissue sections are evaluated for each of the following: IgG, IgA, IgM, C3, C1q, kappa Ig light chains, lambda Ig light chains, fibrinogen and albumin. The presence of each of these is assessed individually by the direct IF technique on frozen sections. In the direct technique a fluorescent-labeled anti-human antibody with specificity against one of the reactants of interest (for example anti-human IgG) is layered on a slide of the patient's kidney and will highlight areas in the kidney where the patient's IgG has abnormally accumulated. The tissue is washed extensively beforehand to remove non-bound reactants that the patient would have had in the circulation.

The stains for immunoglobulins and complement are done primarily to detect the presence of immune complex deposits or autoantibodies against the GBM. **Immune complexes stain in a "granular" pattern** and can be further characterized as to dominant Ig class in the complexes (IgA, IgG etc). The presence of complement in the complexes is assessed primarily by staining for the common pathway reactant, C3. Evidence of classic complement pathway activation is obtained by staining for C1q. The general location of the deposits (mesangium, glomerular capillary loop, interstitium, blood vessels) is also noted. **Anti-GBM antibodies stain in a smooth "linear" pattern** outlining the glomerular basement membrane. The stains for kappa and lambda can detect the presence of a paraprotein (evidenced by staining for only one of the light chains = "monoclonal staining"). The stain for fibrinogen will highlight thrombi or exudation in areas of necrosis as seen in vasculitis or crescentic GN. The stain for albumin is used as a control for the background presence of serum proteins in the tissue.

c. **Electron microscopy (EM)** is used primarily to identify:

1. Abnormal deposition of extracellular material including:
    a) **Immune complexes**: EM is less sensitive than IF for detecting immune complexes but adds specificity to the IF findings and can precisely localize the deposits. Immune complexes are seen as densities in the mesangium and/or peripheral capillary loop. In the peripheral loop they are said to be "subepithelial" (between the GBM and the podocyte) or as "subendothelial" (between the GBM and the endothelial cells).
    b) Fibrils such as **amyloid** or crystalized immunoglobulin precipitates (**immunotactoid or fibrillary GN**, **cryoglobulins**)

2. The characteristic intramembranous deposit (within the lamina densa of the GBM) in **Dense Deposit Disease**.
3. **Viral inclusions** in cells or **intracellular accumulations storage material** as in Fabry disease
4. Characteristic **hereditary GBM lesions** (Alport's syndrome and thin GBM disease).
5. **Tubuloreticular structures in endothelial cells** which indicate interferon effect (characteristic of viral infections (especially HIV) and commonly seen in Lupus nephritis).
6. **Podocyte foot process fusion** as supporting (but not specific) evidence of a primary podocyte disease in nephrotic syndrome.

d. **Serologic workup** in a patient with glomerular disease might include the following (depending on the situation):

1) Anti-nuclear antibodies (ANA) and "subpanel"
   The ANA is a screen for connective tissue diseases, especially SLE. The "subpanel" is testing for a number of specific antibodies as detailed in Robbins Table 6-9 (pg 215).
2) Anti-neutrophil cytoplasmic antibodies (ANCA)
   ANCA is a marker of microscopic forms of vasculitis (Wegener's granulomatosis, microscopic polyangiitis and sometimes Churg-Strauss syndrome) which typically cause necrotizing and crescentic GN when the kidney is involved.
3) Anti-Glomerular Basement Membrane antibodies
   Circulating anti-GBM antibodies bind to the GBM, activate complement and cause a destructive GN (anti-GBM nephritis). In Goodpasture's disease, the antibodies cross-react with lung basement membranes and cause pulmonary hemorrhage.
4) ASO and anti-DNase B titers
   Rising or very high titers suggest recent Streptococcal infection
5) Hepatitis B and Hepatitis C virus antibodies and antigens
6) Complement profile (CH50, C3 and C4 levels)
   Hypocomplementemia is characteristic of active immune complex glomerulonephritis in post-infectious GN, Lupus nephritis and MPGN. Hypocomplementemia does not usually occur in IgA nephropathy or Membranous glomerulopathy.
7) C3 nephritic factor (C3NeF)
   Frequently found in Dense Deposit Disease and may be the cause.
8) Anti-phospholipid antibodies (Lupus anticoagulant; anti-cardiolipin antibodies)

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2. Patterns of Injury in the Kidney

a. Causes of Glomerular Hematuria

Pathogenesis	**Morphologic Pattern**	**"Primary" Renal Disease**	**"Secondary" renal disease (associated diseases)**
Immune complex diseases	Acute exudative GN		Post-infectious GN One-shot serum sickness Early, acute phase of a Potentially chronic GN
	Various patterns of glomerulonephritis as detailed in the section on terminology (Hypercellularity with variable degrees of activity and chronicity)	Proliferative immune complex GN --- MPGN type I	Chronic Infections (SBE, Hep B or C) Collagen vascular diseases (especially SLE) Drug-associated
		IgA nephropathy	Henoch-Schönlein Purpura Liver disease (cirrhosis) Celiac disease Dermatitis Herpetiformis
	Necrotizing and crescentic glomerulonephritis	Severe destructive instances of any of the immune complex diseases listed above	
Anti-GBM autoantibodies		Primary anti-GBM antibody-mediated glomerulonephritis	Goodpasture's disease
ANCA autoantibodies		Pauci-immune necrotizing and crescentic GN	Wegener's granulomatosis Churg-Strauss Microscopic polyangiitis
Genetic abnormalities of GBM		Thin GBM disease ("Benign familial hematuria") Alport Syndrome (Hereditary nephritis)	

b. Causes of Glomerular Proteinuria and Nephrotic Syndrome

Glomerular Pattern of Injury	"Primary" Renal Disease	"Secondary" Renal Disease (Associated Diseases)
Thick GBM with "spikes"	**Membranous immune complex glomerulopathy**	Chronic immune complex deposition • Chronic infection (viral hepatitis, SBE) • Connective tissue disease (SLE) • Carcinoma (lung, GI, breast, kidney, ovary) • Drugs
Mesangiocapillary proliferative with thickened capillary loops having double contour GBM ("tram tracks")	Membranoproliferative immune complex glomerulonephritis (**MPGN type I**)	
Nodular mesangial expansion		**Diabetic nephropathy** **Amyloid nephropathy** Monoclonal light chain nephropathy (Kappa)
Podocyte foot process fusion with normal glomeruli by light microscopy	**Minimal Change Disease** (in "idiopathic" nephrotic syndrome)	**NSAIDs** Interferon-alpha therapy Lymphoproliferative malignancies (esp. Hodgkins)
Podocyte foot process fusion with focal and segmental glomerulosclerosis (FSGS)	**"Primary" FSGS** (in "idiopathic" nephrotic syndrome)	**Hyperfiltration FSGS** Post-inflammatory scarring
Collapsing pattern FSGS	Primary collapsing glomerulopathy	**HIV (AIDS) nephropathy**
Homogeneous tapering intramembranous densities	**Dense Deposit Disease** (MPGN type 2)	Association with partial lipodystrophy

Most nephrotic children have minimal change disease. Adults are likely to have a systemic illness or, if the disease is a "primary" glomerulopathy, either membranous or FSGS.

Adults with nephrosis usually get a renal biopsy for diagnosis and appropriate management unless the obvious context is diabetes mellitus. In children, minimal change disease is assumed and corticosteroid therapy is initiated without biopsy. The response to steroids (steroid-responsive, steroid-dependent, steroid-resistant) is monitored and biopsy is done if nephrosis does not resolve.

c. Tubulointerstitial Renal Diseases

Morphologic Pattern of Injury	**Associated Causes or Conditions**
Acute Tubular Necrosis (ATN)	Ischemia (hypoperfusion; shock) Nephrotoxins (direct tubular toxins)
Interstitial Nephritis	Hypersensitivity reactions (drug allergy, allograft rejection) Viral infection (CMV) Sarcoidosis Sjögren's Anti-TBM disease Idiopathic interstitial nephritis-uveitis syndrome
Pyelonephritis	Bacterial infection
Intrarenal Tubular Obstruction	**Myeloma cast nephropathy** Pigment nephropathy (hemolysis, rhabdomyolysis) Urate nephropathy Oxalosis Nephrocalcinosis
Chronic Tubulointerstitial Nephropathy	Chronic ischemic nephropathy Drugs (analgesics, lithium) Heavy metals (lead) Obstructive uropathy Reflux nephropathy Nephronophthisis- Medullary cystic disease complex

d. Vascular Disease and Patterns of Renal Ischemia

Cause/ Associated Condition	Vascular Lesion	Parenchymal Lesion
Shock Renal hypoperfusion	None (vasoconstriction)	**Acute Tubular Necrosis (ATN)**
Fibromuscular Dysplasia ---------- Atherosclerosis	Renal Artery Stenosis (**Renovascular hypertension**)	Chronic Ischemic Nephropathy (bilateral) Goldblatt Kidney (unilateral)
	Cholesterol emboli (**Atheroembolic renal failure**)	Microinfarctions
Thrombus in left heart - Valve vegetation - Mural over MI	Thromboembolic occlusion of intrarenal arteries	**Renal infarction**
Diabetes Analgesic abuse Sickle cell anemia Urinary tract obstruction Pyelonephritis	Occlusion of vasa recta	**Papillary necrosis**
Hypertension Diabetes Aging	Arteriosclerosis	**Nephrosclerosis**
HUS (various causes) TTP "Malignant" hypertension Scleroderma	**Thrombotic Microangiopathy** Chronic microangiopathy with intimal fibroplasia	Multiple cortical hemorrhagic microinfarctions ("flea-bitten" appearance)
Disseminated Intravascular Coagulation (DIC) - Sepsis - Complication of pregnancy	Fibrin thrombi in arterioles and glomerular capillaries	**Diffuse cortical necrosis**