Renal Pathology

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Contents

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Learning Objectives

- To understand the histopathological approach to a renal biopsy.
- To know the differential diagnosis of light microscopic, immunohistological/immunofluorescent and electron microscopic pathological features and patterns of injury.
- To understand the use of some common histological classification systems.

7.1 Introduction

This chapter aims to provide an introduction to medical renal pathology, including native and transplant pathology. Definitions and differentials for the most common pathological features, a description of the various patterns of injury, the immunohistochemical/immunofluorescence and electron microscopic findings of the most commonly seen entities are provided. The most frequently encountered classification systems are described. Some cases are provided at the end of the chapter to provide fully worked examples and to allow the reader to practise their pathological interpretation of renal biopsies.

7.2 The Pathologist's Approach to a Medical Renal Biopsy

Each pathologist will have their own preferences, but the following provides a general outline of how the biopsy will be handled.

7.2.1 Clinical Details

This crucial information will provide the basis for the interpretation of all subsequent findings and often guides the pathologist as to how the tissue should best be utilised. Useful information includes:

- Demographics.
  - Age.
  - Sex.
  - Native/transplant.
- Indication for biopsy.
  - Nephrotic syndrome.
  - Acute kidney injury.
  - Chronic kidney disease.
  - Haematuria.
  - Proteinuria.
  - Transplant dysfunction.
- The result of any previous biopsies.
- Available test results, e.g. autoantibodies.
- If a transplant biopsy:
  - Duration of transplant.
  - Cause of ESKD, if known.
  - Donor information.
    - Living related/altruistic, DCD/DBD.
    - Age.
- The clinical question, e.g. rejection? vasculitis? cause of CKD?

At this stage, the pathologist will decide how the tissue will be treated in the laboratory:

- Urgency.
  - Routine or urgent.
- Whether electron microscopy is required.
  - Samples are ideally taken from the core before the tissue is processed into a wax block to preserve the ultrastructural features (reprocessing tissue from the wax block causes artefacts which can make interpretation difficult).

7.2.2 Initial Sections

Serial 3–5 um sections will be cut from the tissue core. A particular use of serial sections is that many views through a single glomerulus are visible, which allows for better orientation and localisation of lesions. The pathologist will first receive one or two haematoxylin and eosin (H&E) stained slides with between three to six sections on each. At this point further decisions will be made:

- Is there renal tissue?
  - Capsule, cortex, medulla, pelvi-calyceal system, vessels.
  - If not, what is there, and is it pathological? Possibilities include fat, connective tissue, skeletal muscle, liver, adrenal or bowel.
- Is it adequate for diagnosis? (see below).
- If an urgent result is required or the biopsy shows unexpected features (e.g. unexpected vasculitis), a provisional written or verbal report can be issued at this point.

The adequacy of a biopsy will vary depending on the findings; for example, it may be possible to diagnose membranous glomerulopathy with one patent glomerulus, whereas if a lesion is focal, the probability of detection will depend on the number of glomeruli sampled [1].

7.2.3 Further Stains

Further tinctorial stains and immunofluorescence/immunohistochemistry will follow, usually taking 1 to 3 days. A range of complementary stains are used, each of which highlights different aspects of the biopsy. The

stains used vary slightly depending on personal preference, but as a guide, these may include the following:

Stain	Staining pattern	Applications
H&E (haematoxylin and eosin)	Pink cytoplasm Blue nuclei	Overall assessment The most commonly used stain in histopathology
PAS (periodic acid-Schiff)	Pink staining of basement membranes, mesangial matrix, hyaline material	Overall assessment Glomerular cellularity and matrix Hyaline casts, arteriolosclerosis, glomerular deposits
PAMS (periodic acid methenamine silver)	Black staining of collagen (mesangial matrix, basement membranes, fibrosis)	Areas of chronic damage (interstitial fibrosis) Assessment of glomerular capillary walls Mesangial matrix Glomerular sclerosis Tubular basement membranes
HVG/EVG (haematoxylin/elastic Van Gieson)	Connective tissue and elastin	Assessment of vessels Areas of chronic damage
Congo red	Positive areas indicating amyloid deposits appear 'salmon pink' with 'apple green' birefringence under polarised light Eosinophil cytoplasm Elastic fibres Calcium phosphate	Identification of amyloid Also useful for: Eosinophils (pink cytoplasm) Interstitial calcium phosphate (pale purple) Vascular elastic lamina (pink)

Other stains that may be used include MSB (Martius scarlet blue) for fibrin, Von Kossa for calcium phosphate and Perl's stain for iron.

7.2.4 Immunohistochemistry and Immunofluorescence

Either immunohistochemistry (IHC) or immunofluorescence (IMF) is used to identify immunoglobulin and complement deposition. Each method has advantages and disadvantages; thus, local preferences and availability will determine which is used. The specific antibodies used will vary slightly, but immunoglobulins (M, A, G), two complement components (C3, C1q or C6–C9) and kappa and lambda light chains are fairly standard in native biopsies. Some centres also routinely use fibrinogen.

The native IHC/MIF panel:

- Immunoglobulin.
  - IgM.
  - IgA.
  - IgG.
- Complement component.
  - C3.
  - C1q.
- Kappa/lambda light chains.

In transplant biopsies, a different panel is used. C4d positivity of the peritubular capillaries is a feature of antibody-mediated rejection. BKV stain highlights tubular epithelial cell nuclei containing viral replication. In some cases, both the native and transplant panels will be used, particularly if there is concern of a recurrent glomerulopathy.

The transplant IHC/IMF panel:

- C4d.
- BKV.

Assessment of positive staining by IHC/IMF includes:

- Glomerular distribution.
  - Focal or diffuse.
  - Segmental or global.
- Glomerular location.
  - Mesangial.
  - Subepithelial capillary wall.
  - Subendothelial capillary wall.
- Extra-glomerular staining.
  - Tubular.
  - Vascular.
- Pattern of staining.
  - Granular (coarse/fine).
  - Linear.
- Intensity of staining.
  - Weak or strong.
  - Dominant or codominant staining (comparing the relative intensity).

7.2.5 Electron Microscopy

Electron microscopic (EM) examination requires separate processing and may take longer than LM and IHC/IMF, and so may be reported at a later date. EM allows for assessment of various features:

- Presence and location of electron dense deposits (usually visible as immunoglobulin/complement positivity on IHC/IMF).

- Presence, morphology and location of organised deposits (e.g. amyloid fibrils).
- Glomerular basement membrane (GBM) thickness and alterations, e.g. duplications.
- Podocyte alterations, e.g. foot process effacement (FPE).
- Identification of other structures, e.g. tubuloreticular inclusions.
- Peritubular capillary alterations, e.g. lamination in transplant biopsies.

The ultrastructural appearances often corroborate the light microscopic and IHC/IMF features and help confirm or provide a more precise diagnosis, but in some cases, EM is essential for a diagnosis to be made:

- Minimal change disease.
- Thin basement membrane disease.
- Fibrillary glomerulopathy (if DNAJB9 IHC is not available).
- Immunotactoid glomerulopathy.
- Alport syndrome.
- Early diabetic glomerulopathy.
- Early membranous glomerulopathy.
- Some cases of Lupus Nephropathy (e.g. lupus podocytopathy).

7.2.6 Reaching a Diagnosis

The combination of the clinical, light microscopic, IHC/IMF and electron microscopic information will allow the pathologist to reach a diagnosis in most cases. Often a summary of findings and discussion explaining the basis for the diagnosis will be useful, and a comparison to any previous biopsies should be included if possible. Many centres will have regular meetings of the nephrologists, transplant surgeons and pathologists, to allow discussion of the findings and to provide in-depth clinicopathological correlation. This is particularly useful in difficult cases, where it may not be possible to reach a definite diagnosis. This situation may arise because the findings are non-specific or complex, the various methodologies have not worked optimally or there is insufficient tissue available to perform all the required tests. In these situations, in discussion with the clinicians, the pathologist will give as definitive a diagnosis as possible.

7.3 What Is Normal?

Within cortical tissue:

Glomeruli (Figs. 7.1 and 7.2)

- Normal size (not noticeably enlarged or shrunken).
- Thin capsule, separate from the glomerular tuft (no adhesions).

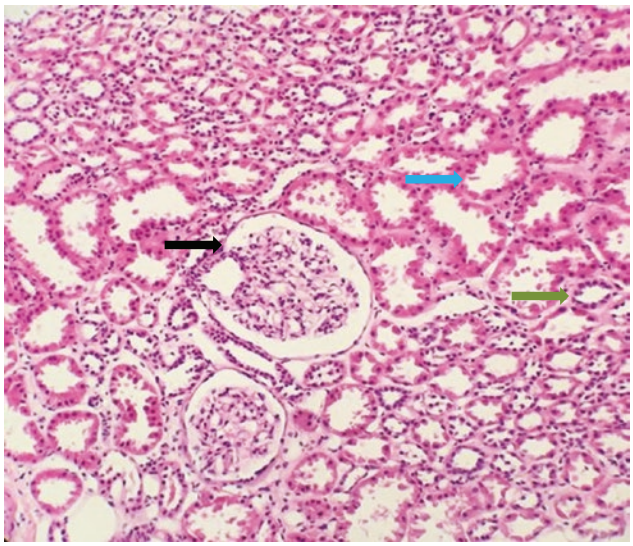


Fig. 7.1 Normal cortex showing glomeruli (black arrow), proximal tubules (blue arrow), distal tubules (green arrow). Interstitium is inconspicuous. H&E x100

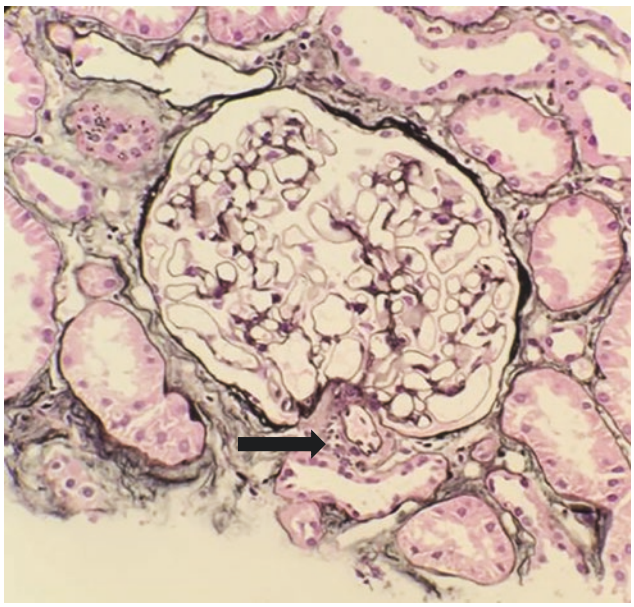


Fig. 7.2 Normal glomerulus with vascular pole (black arrow). PAMS x400

- A single layer of parietal epithelial cells.
- Glomerular tuft filling the urinary space.
- No cells filling the urinary space.
- Thin, uniform glomerular capillary walls.
- Patent capillary lumens containing occasional red blood cells and endothelial cell nuclei.
- A small amount of mesangial matrix with <4 mesangial cells per peripheral mesangial area (excluding hilar regions).

Tubules

- Back-to-back arrangement of predominantly proximal tubules, with some distal tubules and collecting ducts.
- Proximal tubules; columnar cells with abundant eosinophilic (pink) cytoplasm and an apical brush border.

Interstitium

- Very little or none is visible.

Extra-glomerular Vessels

- Patent arteries, arterioles, veins and capillaries, containing blood.
- No thromboemboli, necrosis, inflammation or degenerative changes (see below).

Within medullary tissue (Fig. 7.3).

Glomeruli

- None are present.

Tubules

- A mixed population of proximal and distal tubules, loops of Henle and collecting ducts.
- Distal tubules; low cuboidal cells with eosinophilic cytoplasm and apical nucleus, lacking a brush border.
- Loops of Henle; very thin epithelial cells, difficult to distinguish from capillaries.
- Collecting ducts; low cuboidal cells with pale cytoplasm, central nucleus and distinct cell borders, lacking a brush border, with a larger lumen.
- Not back-to-back; separated by interstitium.

Interstitium

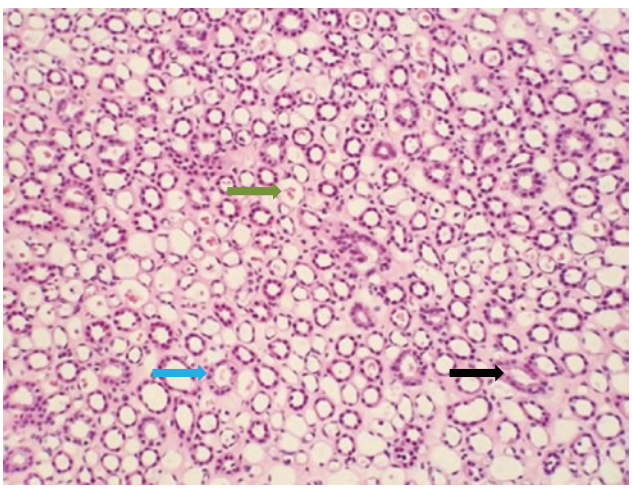


Fig. 7.3 Normal medulla showing distal tubules (black arrow), collecting ducts (blue arrow) and capillaries (green arrow). H&E x100

- Increased compared to the cortex (particularly within the inner medulla).
- Paucicellular collagenous matrix.
- Contains a few lymphocytes, fibroblasts and vessels.

Normal renal parenchyma will vary in appearance depending on the patient's age. Some age-related chronic damage, known as IFTA (interstitial fibrosis and tubular atrophy), is to be expected in older patients. An estimate of the percentage of chronic damage within the renal cortex can be given using the following equation

Age / 2–10 = % of acceptable sclerosis.

Thus, a biopsy from a 20-year-old patient should have only very minimal chronic damage, whereas one from an 80-year-old patient may have up to 30% IFTA acceptable as a normal feature [2].

7.4 Pathologies of each Compartment

During the assessment of a renal biopsy, each 'compartment' (i.e. glomeruli, tubules, interstitium and extra-glomerular vessels) should be reviewed. As the compartments are interdependent, injury to one will lead to secondary injury in the others, particularly if this injury is long-standing. When there is advanced multicompartmental damage, determining the primary site of injury can be difficult.

7.4.1 Active Versus Chronic

Whether the abnormalities are active/acute or chronic is useful for guiding prognosis and management. Active lesions imply that the injury is current, may benefit from intervention and could recover. Chronic lesions imply that the injury is remote and has healed. These lesions are generally considered irreversible and will not respond to treatment. Chronic lesions are non-specific as the scarring response is similar in any cause of injury, so it can be impossible to determine the original cause if only chronic lesions are present.

Active features	Cellular proliferation within the glomerular tuft (endocapillary and/or mesangial hypercellularity) Cellular proliferation out with the glomerular tuft (parietal epithelial cell proliferation/extracapillary hypercellularity/cellular crescent formation) Necrosis (karyorrhexis, fibrin) Interstitial oedema Inflammation (glomerulitis, tubulitis, interstitial nephritis, vasculitis)
Chronic features	Glomerular sclerosis (segmental or global) Fibrous tissue within the urinary space (fibrous crescents) Interstitial fibrosis and tubular atrophy ('IFTA')

7.4.2 Glomerulus

Each glomerulus should be assessed systematically, examining each component (cells and matrix): capsule, the urinary space, capillary walls, capillary lumens, mesangial regions and tubular and vascular poles (where visible) (Figs. 7.4 7.5, 7.6, 7.7, 7.8, 7.9, 7.10, 7.11, 7.12, 7.13, and 7.14).

	Terminology	Definition
Describes all glomeruli	Focal	Involves <50% of all glomeruli
	Diffuse	Involves ≥50% of all glomeruli
Describes one glomerulus	Segmental	Involves <50% of a glomerulus
	Global	Involves ≥50% of a glomerulus

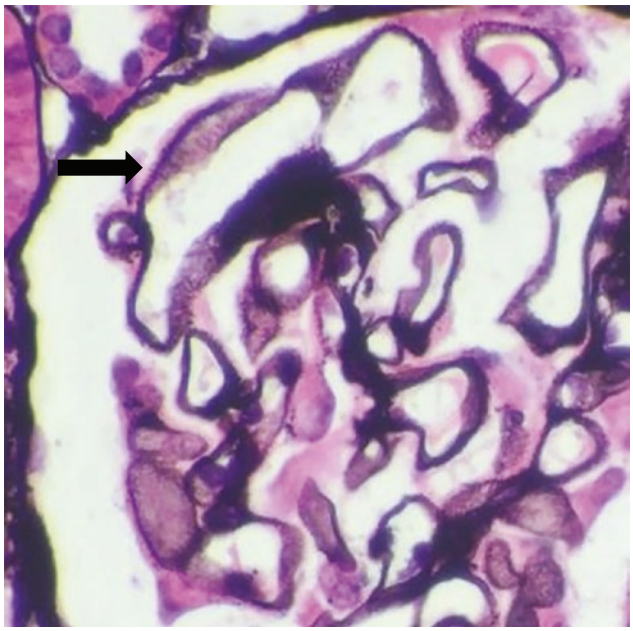


Fig. 7.4 Subepithelial spikes along capillary walls in a case of membranous glomerulopathy (black arrow). PAMS. x400 original magnification

Glomerulus		
Lesion	***Description***	***Differentials***
Capsule		
Fibrosis	Chronic lesion Thickened, multi-layered membrane best seen on PAMS or PAS	Sclerosed/sclerosing glomeruli Ischaemia
Adhesion	An area of attachment of the glomerular tuft to the capsule	Non-specific, represents a scar implying damage of the tuft has occurred Can be an early feature of FSGS
Rupture	Acute lesion A break in the capsule, usually with associated inflammation and possibly fibrin Best seen on PAMS	Necrotising glomerulonephritis, e.g. ANCA-associated GN, anti-GBM disease, IgA nephropathy
Urinary space, visceral and parietal epithelial cells		
Crescents (a form of extracapillary hypercellularity) (Fig. 7.11)	*Cellularity decreases as fibrosis increases over time, so the proportion of each indicates the age/maturity of the lesion* *Cellular (<25% fibrosis)* Acute lesion Proliferation of parietal epithelial cells extending into the urinary space from the capsule, at least two cells thick. Often includes karyorrhectic debris, inflammatory cells or fibrin from the damaged tuft *Fibrocellular (>25% of cells and fibrosis)* A subacute lesion A mixture of parietal epithelial cells and fibrosis (collagen) *Fibrous (<25% cells)* Chronic lesion Fibrosis in urinary space, containing few/no nuclei. Fibrosis is highlighted on PAMS	*Cellular* Many differentials, particularly vasculitis (ANCA, anti-GBM), immune complex-mediated glomerulopathy, e.g. IgA disease Implies a response of parietal epithelial cells to rupture of a capillary wall *Fibrocellular/fibrous* Non-specific. Healed/healing vasculitis, immune complex-mediated glomerulopathy, ischaemia, sclerosis

Glomerulus		
Lesion	***Description***	***Differentials***
Podocyte/visceral epithelial cell hyperplasia (a form of extracapillary hypercellularity)	Increased number of visceral epithelial cells, which may contain PAS-positive protein resorption droplets	Collapsing FSGS Seen to a lesser extent in many conditions as a non-specific feature (e.g. ischaemia)
Foamy visceral epithelial cells	Podocytes are enlarged with abundant foamy/bubbly cytoplasm	Lysosomal storage disorders, such as Fabry's disease
Capsular drops	Hyaline material attached to the capsule	Diabetic nephropathy
Capillary walls		
Spikes, chains (Fig. 7.4)	Spikes arranged perpendicular to the GBM, extending into the urinary space, or holes (chains) in the capillary wall when viewed obliquely, caused by basement membrane extending between or surrounding subepithelial immune deposits Best seen on PAMS	Membranous glomerulopathy (primary or secondary) Lupus nephropathy (class V) Amyloidosis involving capillary walls causes feathery spike formations/spicules
Double contour ('tram track', splitting, duplication) (Fig. 7.5)	A double-layered appearance of the capillary wall, caused by mesangial interposition and new basement membrane formation inside the original Best seen on PAMS	Subendothelial electron dense deposits: Immune complex-mediated (type I) MPGN, C3 glomerulopathies, SLE, cryoglobulinemia, PIGN Organised deposits, e.g. amyloid, fibrillary, immunotactoid or fibronectin glomerulopathy Chronic endothelial injury: Chronic TMA, pre-eclampsia, transplant glomerulopathy (CAMR)
Wire loop (Fig. 7.7)	Very thick, glassy capillary walls, caused by large subendothelial deposits	An active feature of SLE
Thickening	Thickened capillary walls without definite spikes or double contours	Diabetic nephropathy, an early form of any of the above capillary wall lesions If vacuolated, LCAT deficiency
Hyaline cap	Hyaline material deposited between the glomerular basement membrane and the endothelium, often in sclerotic areas, may occlude the capillary lumen	Diabetic nephropathy Non-specific in sclerosed foci
Endotheliosis	Endothelial cell swelling causing thickened capillary walls and shrinkage of the capillary lumen, which appears bloodless	Pre-eclampsia, eclampsia, other causes of thrombotic microangiopathy (TMA)
Capillary lumen		
Endocapillary hypercellularity (Fig. 7.10)	Lumen narrowed/occluded by cells (can be endothelial and/or inflammatory cells)	Acute PIGN (especially if diffuse and neutrophilic), C3GN, MPGN, SLE, IgA/HSP, vasculitis, infection-associated GN, glomerulitis (ABMR)
Microaneurysm (Fig. 7.13)	Ectasia of capillary loops due to destruction of the mesangial matrix	Diabetic nephropathy Heals to form mesangial nodules
Thrombus (Fig. 7.12)	Occlusion of the lumen by fibrin thrombus	Acute TMA, pre-eclampsia, renal vein thrombosis, sickle cell nephropathy, hyperacute rejection
Hyaline thrombus	A pseudothrombus, composed of cryoglobulins, with the glassy, eosinophilic appearance of hyaline (with IHC/IMF positivity)	Cryoglobulinaemia, an active feature of lupus nephritis
Sickle cells	Dysmorphic, sickle-shaped erythrocytes	Sickle cell disease

Glomerulus		
Lesion	***Description***	***Differentials***
Mesangium		
Proliferation (Fig. 7.9)	More than three cells in a group within a peripheral mesangial area (away from the hilum)	IgA nephropathy, lupus nephritis (class II), secondary membranous glomerulopathy
Increased matrix (Fig. 7.6.)	Diffuse; maintains the normal distribution of the matrix, but more material is present Nodular; rounded areas of matrix with a rim of mesangial cells	Diabetes, amyloid, monoclonal immunoglobulin deposition disease (MIDD), idiopathic nodular glomerulopathy
Mesangiolysis	Injury and destruction of mesangial matrix and cells releasing the anchoring points of adjacent capillary loops, which merge, forming one large, microaneurysmal capillary loop	Diabetes, TMA, malignant hypertension, radiation nephropathy, rarely a non-specific feature of various glomerulonephritides with mesangial deposits
Multicompartmental lesions		
Segmental sclerosis and hyalinosis (Fig. 7.8)	A segment of the glomerular tuft shows increased mesangial matrix with obliteration of capillary loops. Represents a segmental scar May be attached to the capsule forming an adhesion May contain foam cells May be adaptive enlargement of uninvolved glomeruli Begins at the cortico-medullary junction The morphology and location within the tuft determine the variant of FSGS (see 'classification systems') Hyaline is glassy acellular material which often forms part of a sclerosed area. Formed from insudated plasma proteins in response to endothelial injury	FSGS primary or secondary Most commonly a non-specific feature in many types of glomerulonephritis, which should be excluded before giving a diagnosis of FSGS
Global sclerosis	A chronic feature The end point of any glomerular injury Complete replacement of the tuft by fibrosis. Highlighted on PAMS	A non-specific feature Can be accepted as a normal feature depending on patient age and the number of glomeruli involved (see above)
Chronic ischaemic change	Small tuft, urinary space fibrosis (collagen deposition inside the capsule), wrinkled capillary walls (highlighted on PAMS), contracted mesangium, the urinary space may appear enlarged	Chronic hypoperfusion of any cause, e.g. renal artery stenosis, atherosclerosis, thromboemboli Ischaemic glomeruli are often seen as a non-specific feature of many renal diseases
Necrosis (Fig. 7.11)	Cell death (mesangial, epithelial, endocapillary or inflammatory) with associated karyorrhectic debris. Once capillary walls are involved, fibrin deposition, haemorrhage and crescents may be seen	Any highly active glomerulopathy, particularly vasculitic diseases
Hypertrophy	Enlargement of the glomerulus	A compensatory response to nephron loss of any cause Can be a helpful clue to suggest covert FSGS if sclerosing lesions are not evident

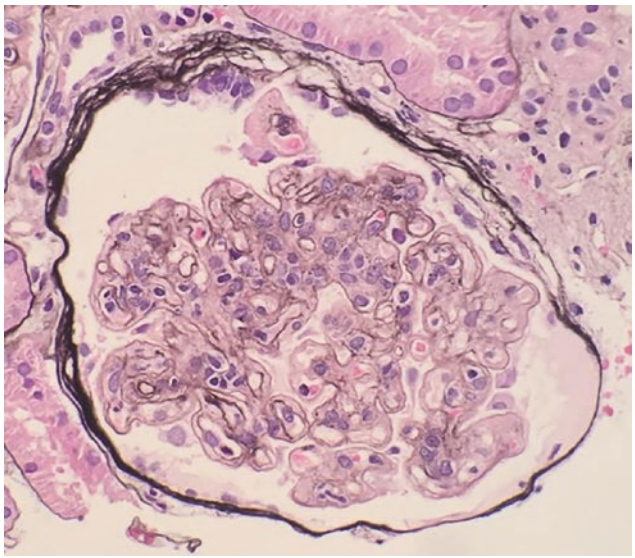


Fig. 7.5 Glomerulus showing diffuse capillary wall double contours in an MPGN. PAMS x400

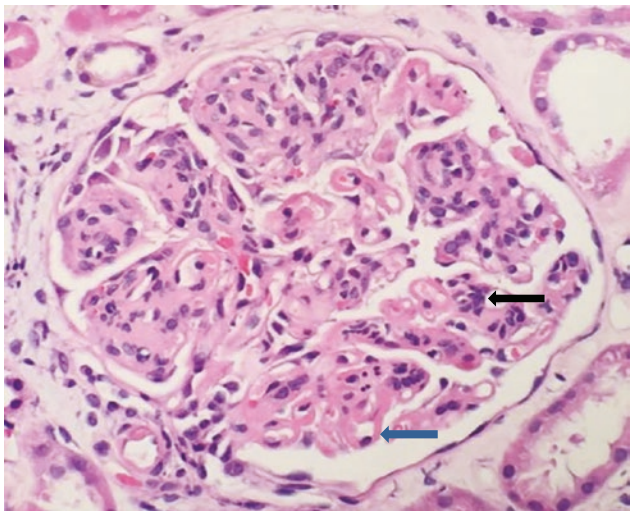


Fig. 7.7 Glomerulus showing an MPGN in a case of lupus nephritis, including mesangial hypercellularity (black arrow) and thickened glomerular capillary loops, including wire loops (blue arrow). H&E. x400

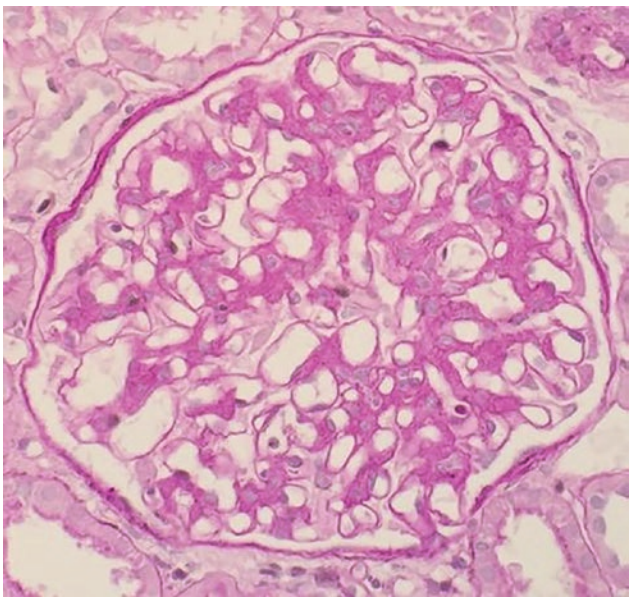


Fig. 7.6 Mesangial matrix expansion in a case of fibrillary glomerulopathy. PAS. X400

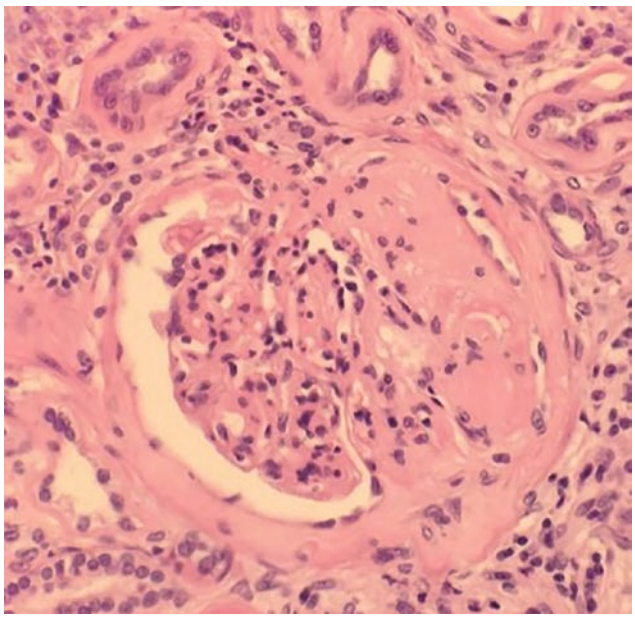


Fig. 7.8 Glomerulus with a segment of sclerosis. Surrounding tubules are atrophic. H&E. x400

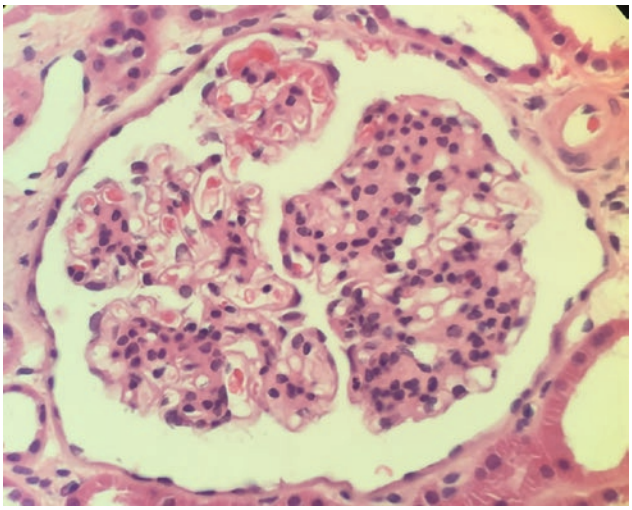


Fig. 7.9 Glomerulus showing mesangial proliferation in a case of IgA nephropathy. H&E. X400

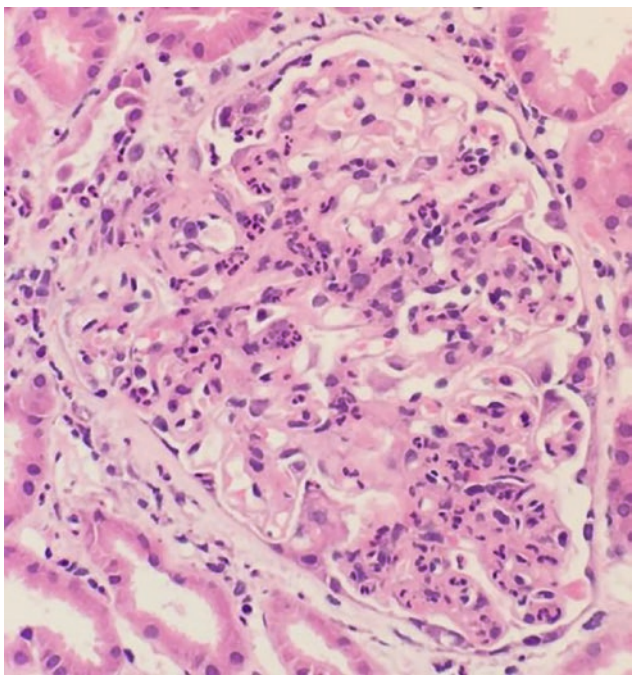


Fig. 7.10 Endocapillary, neutrophilic hypercellularity in a case of post-infectious glomerulonephritis. H&E. x400

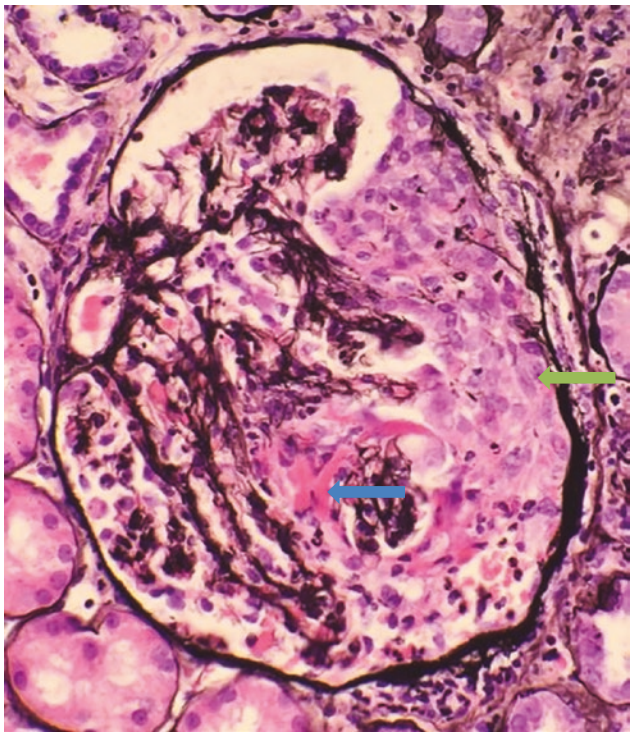


Fig. 7.11 Glomerulus with fibrinoid necrosis (blue arrow) and cellular crescent formation (green arrow). PAMS x400

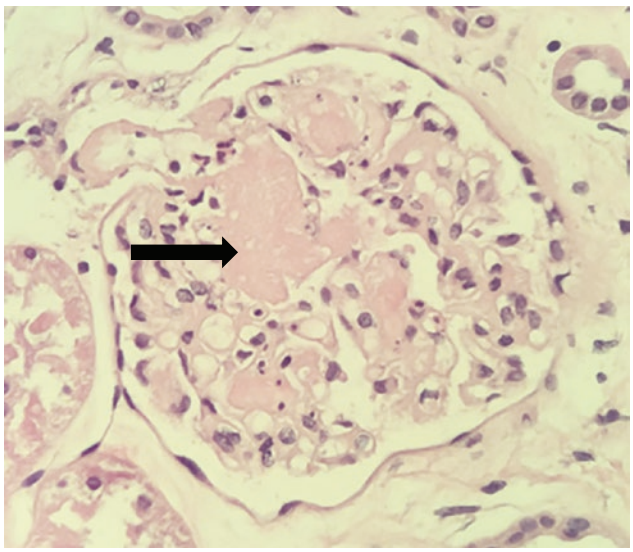


Fig. 7.12 Glomerulus showing fibrin thrombi (black arrow) within capillary loops in a case of acute thrombotic micorangiopathy. H&E. x400

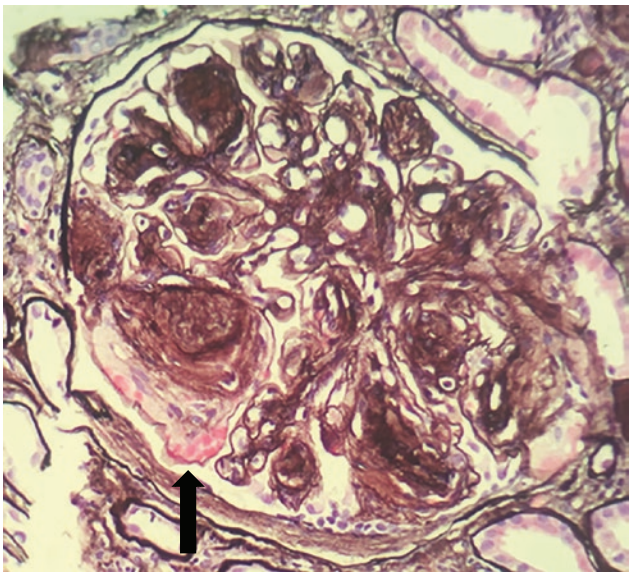


Fig. 7.13 Nodular glomerulopathy with a microaneurysm (arrow) in a case of diabetic nephropathy. PAMS X400

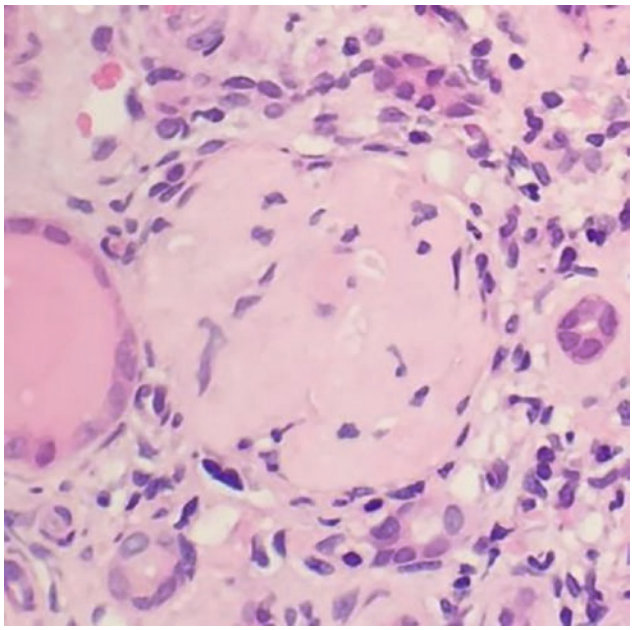


Fig. 7.14 Globally sclerosed glomerulus. H&E x200

7.4.3 Tubules (Figs. 7.15, 7.16, 7.17, 7.18, 7.19, 7.20, and 7.21)

Tubules		
Lesion	***Description***	***Differentials***
Tubular cells		
Acute tubular injury (ATI) (Fig. 7.15) Acute tubular necrosis (ATN) (Fig. 7.18)	ATI – Simplification (loss of the proximal tubular brush border and thinning/flattening of the cytoplasm), luminal ectasia, nuclear enlargement, prominent nucleoli, blebbing/sloughing of cytoplasm into the lumen (forming casts), vacuolisation and variation in cell size and shape ATN – Severe form of ATI involving loss of tubular nuclei and detachment, necrosis and fragmentation of tubular epithelial cells with denudation of the basement membrane Often associated with interstitial oedema	Primary; ATI/ATN due to toxic/ischaemic injury Secondary; acute glomerular injury or vascular injury of any cause Vacuolation may be particularly prominent in ATI due to particular toxins or hyperosmolar injury (hyperkalaemia, mannitol) Histological changes can be mild even in clinically severe AKI ATI can be seen in glomerular and vascular diseases, which should be excluded before giving a diagnosis of ATI
Tubulitis (Fig. 7.16)	Leukocytes (usually lymphocytes) infiltrating tubular epithelial cells (within the tubular basement membrane) with acute tubular injury	TIN (any cause), pyelonephritis, acute T cell-mediated rejection
Crystalline inclusions	Proximal tubular injury (as seen in ATI/ATN), with crystalline or needle-shaped inclusions and cytoplasmic monoclonal light chain deposition. May only be visible on electron microscopy	Light chain proximal tubulopathy
BK viral cytopathic changes	Appearances range from normal to marked cytopathic change (enlarged and hyperchromatic). Causes basophilic intranuclear inclusions Often also see tubulitis and interstitial inflammation	Acute T cell-mediated rejection TIN Other viral infections, e.g. CMV, adenovirus (with different viral cytopathic appearances) ATI/ATN

Tubules		
Lesion	***Description***	***Differentials***
Infarction (Fig. 7.18)	Areas of infarction are likely to involve all the compartments within the affected area, which will show coagulative necrosis, meaning that the architecture remains visible, but the cytological details (cytoplasm and nucleus) are lost, leaving a pale, ghostly outline. Often there is also extensive haemorrhage, particularly in infarction due to venous thrombosis	The cause is not usually present in the biopsy, but possibilities include hypoperfusion, vascular thrombosis, thromboembolism or vasculitis, and the included vessels may show evidence of these
Atrophy (Fig. 7.17)	A chronic feature Atrophic tubules appear small with thick basement membranes; over time, these tubules disappear and are replaced by fibrous tissue Another form of tubular atrophy is called 'thyroidisation' (because the histological appearance is similar to that of normal thyroid follicles). These tubules are dilated, with flattened epithelial cells and filled with hyaline cast material Another form of atrophy is endocrine type (because the appearance resembles parathyroid glands); small cuboidal tubular cells with very little/no visible lumen and no thickened basement membrane All types are usually associated with interstitial fibrosis	A non-specific finding, but the pattern of atrophy can help suggest aetiology. Patchy atrophy is classically seen in reflux nephropathy, whereas stripy atrophy suggests chronic CNI toxicity. The endocrine type can suggest renal artery stenosis
Hypertrophy	Large tubules with large tubular epithelial cells and an increased volume of cytoplasm Often also see glomerular hypertrophy	An adaptive change to a reduced number of tubules This may be because the kidney is small relative to body mass (e.g. low birth weight, obesity, some transplants), or due to loss of tubules due to chronic damage
Vacuolation	Fine (small) or coarse (large) vacuoles within the tubular cytoplasm	Can be a non-specific sign of acute tubular injury, specific causes include CNI toxicity, osmotic tubular injury, mannitol, contrast, IVIg, hypo/hyperkalaemia
Resorption droplets	Eosinophilic, PAS-positive, small, round cytoplasmic inclusion, formed from protein	Any cause of glomerular proteinuria
Luminal material		
Casts		
Hyaline casts (Fig. 7.19)	The most common type of cast, composed of Tamm-Horsfall protein. Appear glassy, eosinophilic, PAS-positive and solid	Non-specific, increased in chronically damaged tubules
Myeloma/light chain casts (Fig. 7.20)	Composed of monoclonal immunoglobulins mixed with Tamm-Horsfall protein, appears cracked/fractured/crystalline with a surrounding inflammatory cell reaction (giant cells, macrophages, neutrophils or lymphocytes), usually shows restriction on IHC/IMF for kappa or lambda light chains ATI also seen	Myeloma/plasma cell dyscrasia
Myoglobin casts (Figs. 7.21 and 7.22)	Red/brown granular cast material Myoglobin immunostain positive ATI also seen May see rhabdomyolysis in any skeletal muscle present	Myoglobinuria
Red blood cells or red cell casts (Fig. 7.23)	Red blood cells filling the tubular lumen	A few red blood cells are acceptable as part of biopsy-related trauma Larger numbers may be due to vasculitis or any necrotising glomerulopathy If there is no evidence of this, more levels should be examined

Tubules		
Lesion	***Description***	***Differentials***
Inflammatory cell casts	Inflammatory cells and cellular debris May be an associated interstitial infiltrate and ATI	Pyelonephritis, reflux nephropathy Can be seen occasionally in any cause of interstitial inflammation, e.g. TIN, ACR, GN
Bile casts	Brown cast material, stains with Fouchet, usually with ATI (a rare diagnosis)	Hyperbilirubinaemia of any aetiology
Crystals		
Calcium oxalate crystals (Fig. 7.24)	Fan-shaped colourless crystals within tubules, refractile under polarised light Do not dissolve during processing	A large number are seen in primary hyperoxaluria and ethylene glycol toxicity, whereas less are usually present in secondary hyperoxaluria. A small number can be seen as a non-specific finding in severely damaged/end-stage kidneys
2,8-dihydroxyadenine crystals (2,8-DHA)	Single or clusters of birefringent, brown needle/rod-shaped crystals in tubules, tubular cytoplasm and interstitium, predominantly within the cortex May see an inflammatory cell reaction including giant cells Do not dissolve during processing Appear black on PAMS and blue on trichrome	2,8-Dihydroxyadeninuria
Cystine crystals	Birefringent hexagonal or rhomboid colourless crystals within glomerular and tubular cells and in interstitial macrophages Multinucleated tubular epithelial cells and podocytes and atrophic proximal tubules ('swan neck' deformity) are also seen Crystals dissolve during processing so are best seen in frozen tissue. In processed tissue, empty clefts remain as evidence of crystal deposition	Cystinosis
Monosodium urate crystals (Fig. 7.25)	Clusters of birefringent, needle-shaped crystals in tubules or interstitium, predominantly in the medulla (within collecting ducts) May be surrounded by a granulomatous inflammatory response, forming a tophus Monosodium urate crystals are birefringent and needle-shaped, but dissolve during processing, so are best seen in frozen tissue. In processed tissue, empty clefts remain as evidence of crystal deposition	Uric acid nephropathy/gout
Calcium phosphate deposits	Granular, purple deposits within tubules and interstitium Not birefringent Do not dissolve during processing Stain black on von Kossa	Nephrocalcinosis (hypercalcaemia, hypercalciuria, hyperphosphataemia, hyperphosphaturia of any cause) Occasional incidental calcium phosphate deposits are often seen in areas of chronic damage

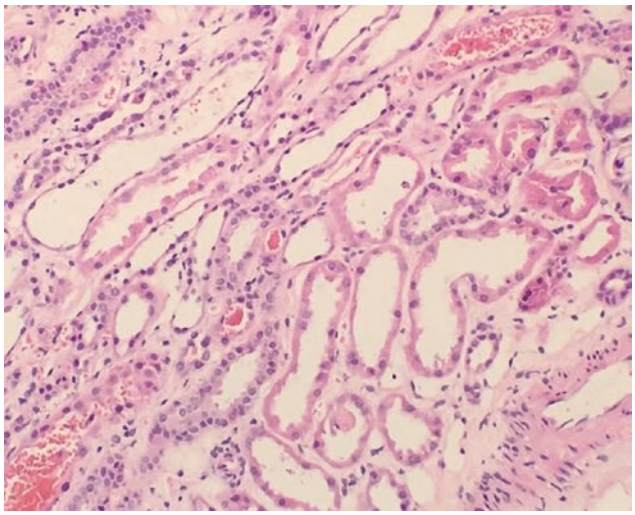


Fig. 7.15 Acute tubular injury and red cell casts in a case of pauci-immune glomerulonephritis. H&E x200

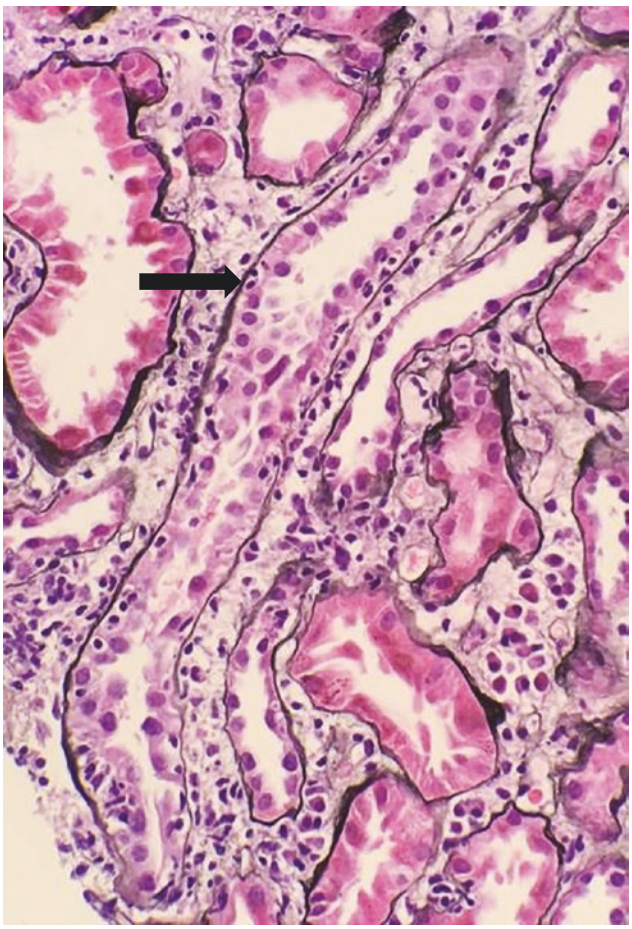


Fig. 7.16 A distal tubule showing infiltration by lymphocytes (tubulitis), in a case of acute cellular rejection. PAMS x200

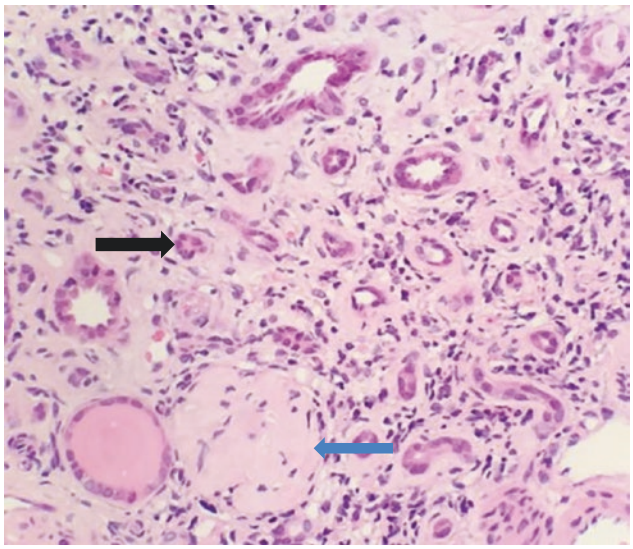


Fig. 7.17 Chronically damaged parenchyma showing atrophic tubules (black arrow), fibrotic interstitium and a globally sclerosed glomerulus (blue arrow). H&E. x100

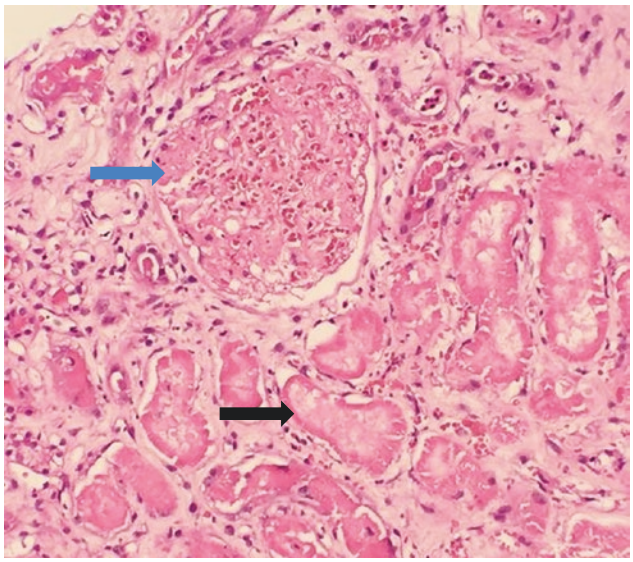


Fig. 7.18 Tubular necrosis (black arrow) and glomerular necrosis (blue arrow) in an area of cortical infarction in a case of active antibody-mediated transplant rejection. H&E. x100

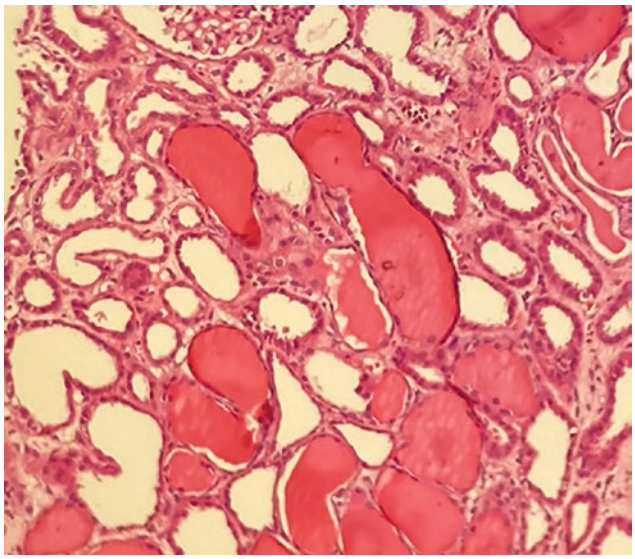


Fig. 7.19 Tubular hyaline casts. H&E X100

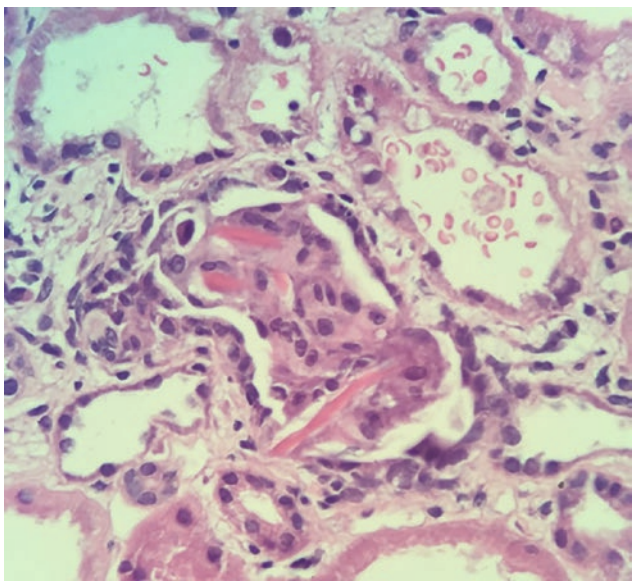


Fig. 7.20 A distal tubule containing angulated cast material with a surrounding multinucleate cell reaction. H&E X400

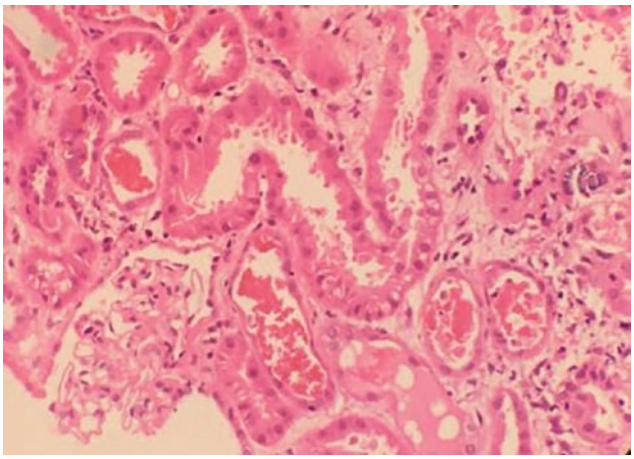


Fig. 7.21 Tubular myoglobin casts in a case of myoglobinuria due to rhabdomyolysis. H&E x200

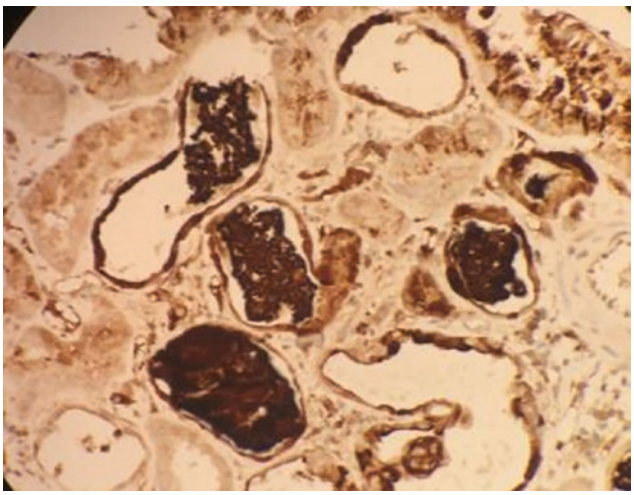


Fig. 7.22 Myoglobin immunohistochemistry showing positive staining of tubular myoglobin casts. x200

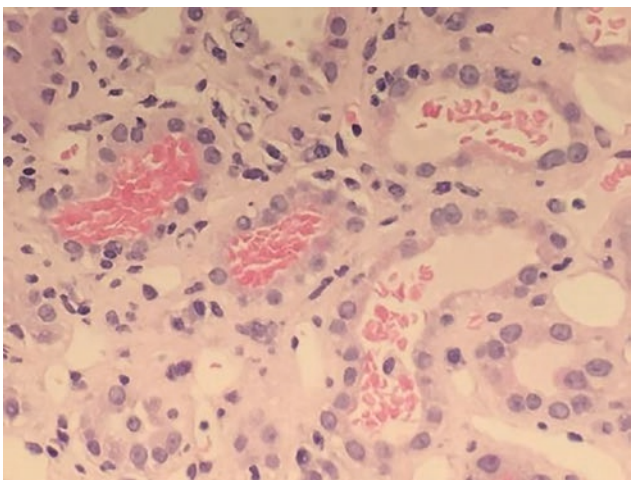


Fig. 7.23 Tubules containing red cell casts in case of crescentic glomerulonephritis. H&E x200

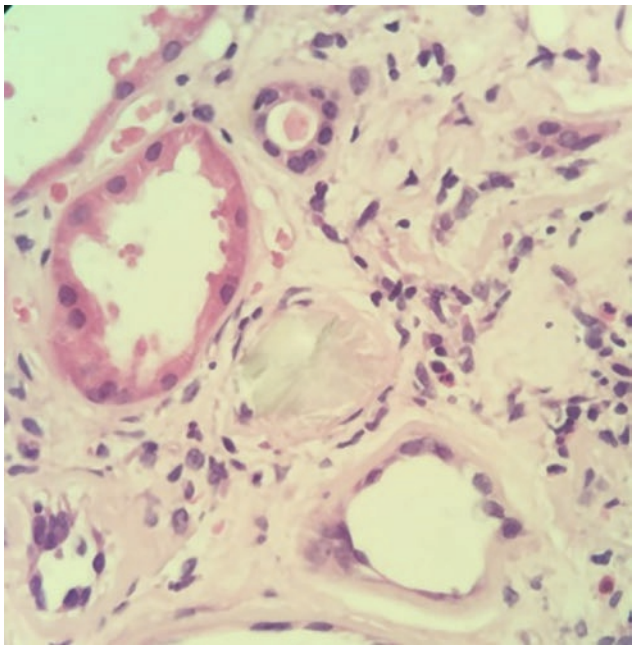


Fig. 7.24 Tubular oxalate crystal. H&E X400

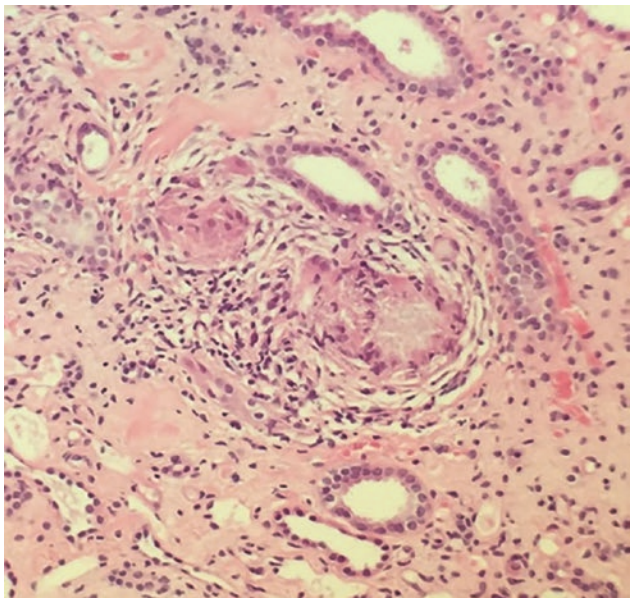


Fig. 7.25 Urate deposition in chronically damaged parenchyma. H&E X100

7.4.4 Interstitium

Interstitium		
Lesion	*Description*	*Differentials*
Inflammation (Fig. 7.26)	Any type of inflammatory cell may be seen. Lymphocytes usually predominate, often with a few plasma cells, but neutrophils, eosinophils, plasma cells or granulomas (aggregates of macrophages) may be conspicuous If inflammatory cells are seen infiltrating tubular epithelial cells, this is tubulointerstitial nephritis Inflammatory cells, particularly lymphocytes, can aggregate in areas of chronic damage (interstitial fibrosis); if it is limited to these areas, it is likely to be non-specific	**Lymphocytes**: Chronic parenchymal damage (e.g. chronic glomerulonephritis) TIN Acute cellular rejection Obstruction Lymphoproliferative disease (neoplastic lymphocytes) **Neutrophils**: Pyelonephritis Light chain cast nephropathy **Eosinophils**: Allergy/drug-related TIN ANCA GN Diabetic nephropathy **Granulomas**: ANCA GN Tuberculosis Fungal infection Sarcoidosis Xanthogranulomatous pyelonephritis Malakoplakia
Oedema	Acute and reversible Pale expansion of the interstitium by fluid, separating adjacent tubules	Any cause of acute parenchymal damage, e.g. ATI, acute TIN, RPGN, acute cellular rejection
Fibrosis (Fig. 7.17)	Chronic and irreversible Eosinophilic expansion of the interstitium, composed of collagen, often contains a few lymphocytes. Entrapped tubules and glomeruli may be atrophic/sclerosed	Any cause of chronic parenchymal damage
Amyloid	Eosinophilic amorphous material may also be seen in the glomerular mesangium and capillary walls, arteriolar walls and surrounding tubules	Amyloidosis
Foam cells	Large cells with central nuclei and abundant multi vacuolated cytoplasm (containing lipid, which is removed during processing) The cells are thought to be of macrophage/monocyte origin	Often seen in any cause of proteinuria, particularly in the nephrotic syndrome (hyperlipidaemia), also in Fabry's disease, Alport syndrome, lipoprotein glomerulopathy
Cellular infiltrate	Rarely, a neoplastic population can be seen infiltrating or replacing the renal parenchyma	Renal neoplasia, myeloma, lymphoma, metastatic carcinoma, adrenal inclusions

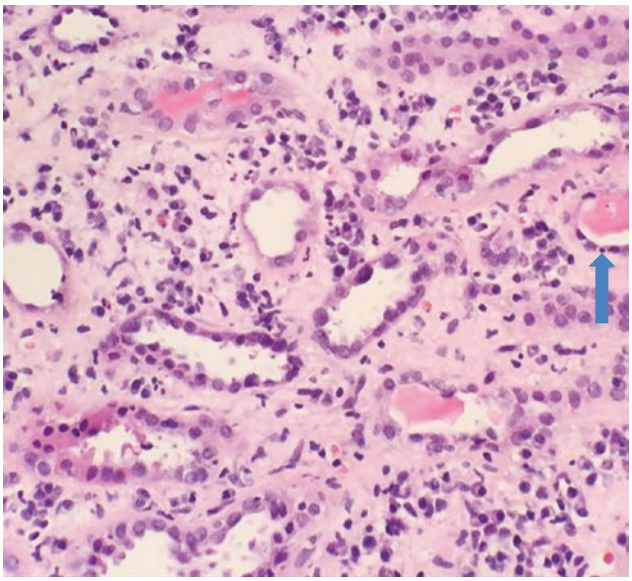


Fig. 7.26 Interstitial inflammation, including lymphocytes, plasma cells and neutrophils, with tubulitis (blue arrow). H&E x400

7.4.5 Vascular

Extra-glomerular vessels		
Lesion	***Description***	***Differentials***
Vasculitis (Fig. 7.27)	Inflammation within an arterial/arteriolar wall, with fibrinoid necrosis and schistocytes (fragmented erythrocytes) if severe Inflammation may be lymphocytic, neutrophilic, eosinophilic or granulomatous Can be seen with thrombi, interstitial inflammation, haemorrhage or infarction	Any cause of vasculitis, most commonly seen are ANCA vasculitis, anti-GBM disease, IgA vasculitis
Endothelialitis Endarteritis	Arterial/arteriolar subendothelial lymphocytes, extending through to the media if severe May see reactive (enlarged) endothelial nuclei and subendothelial swelling	Acute vascular rejection
Capillaritis	Increased numbers of leukocytes within peritubular capillaries	Acute antibody-mediated rejection
Thromboemboli	Lumenal material in vessels. Can be fibrin thrombus, atheroma (cholesterol crystals) (rare with others such as fat or tumour cells)	Most commonly embolisation of an atheromatous plaque in atherosclerosis
Hypertensive vasculopathy (Fig. 7.28)	Fibrointimal proliferation with multiplication of the elastic lamina (fibroelastosis), medial thickening of arterioles, hyaline arteriolosclerosis Highlighted on elastin stain	Hypertension (essential or secondary to any cause, including chronic renal disease) Renal artery stenosis Scleroderma
Hyaline arteriolosclerosis (Fig. 7.29)	Eosinophilic, glassy amorphous material within the arteriolar wall	Hypertension Diabetic nephropathy CNI-related, classically 'nodular' in appearance

Extra-glomerular vessels		
Lesion	***Description***	***Differentials***
Accelerated/ malignant hypertensive changes (Fig. 7.30)	Fibrinoid necrosis and thrombi, 'onion-skinning' (multilayered intima of arterioles), mucoid intimal thickening (pale bluish acellular matrix material)	Accelerated/malignant hypertension Thrombotic microangiopathy, Scleroderma
Amyloid	Eosinophilic material within the vessel wall, appears red/pink on Congo red stain. When the Congo red stain is viewed under polarised light, amyloid classically shows 'apple green' birefringence Often also present in glomeruli and interstitium	Amyloidosis
Atherosclerosis	Intimal thickening composed of foam cells (lipid-laden macrophages), cholesterol clefts, amorphous material, all present underneath the endothelium	Atherosclerosis
Acute thrombotic microangiopathy (Fig. 7.31)	Endothelial swelling obstructing the lumen, intramural schistocytes (fragmented erythrocytes), fibrin thrombi and fibrinoid necrosis	Many, including HUS, aHUS, TTP, malignant hypertension, pre-eclampsia, scleroderma, antiphospholipid syndrome, acute antibody-mediated rejection

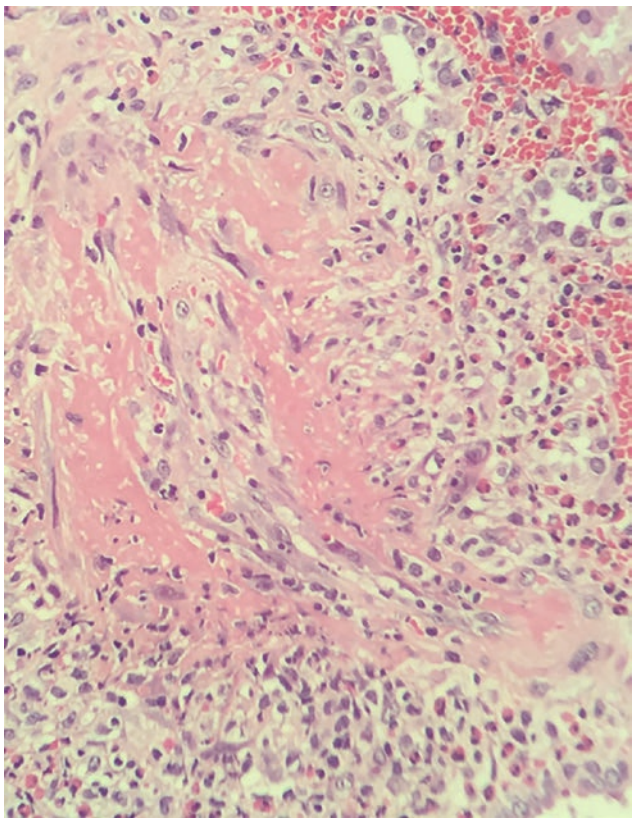


Fig. 7.27 Arterial vasculitis with fibrinoid necrosis in a case of ANCA vasculitis. H&E X100

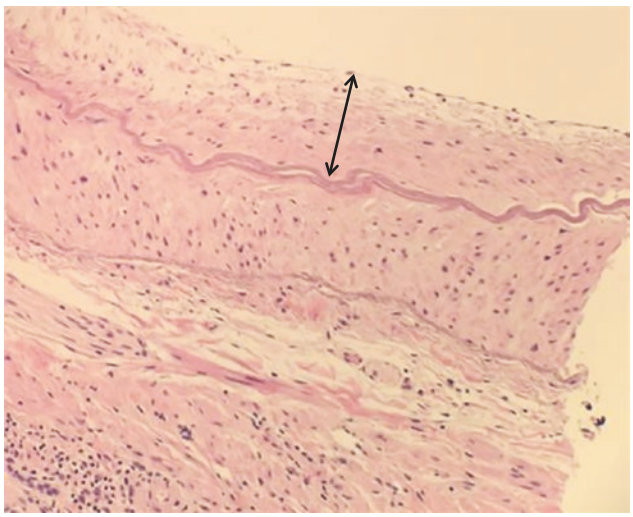


Fig. 7.28 Section of an artery showing fibrointimal proliferation (arrow spans the area of proliferation from the internal elastic lamina to the endothelium) H&E x400

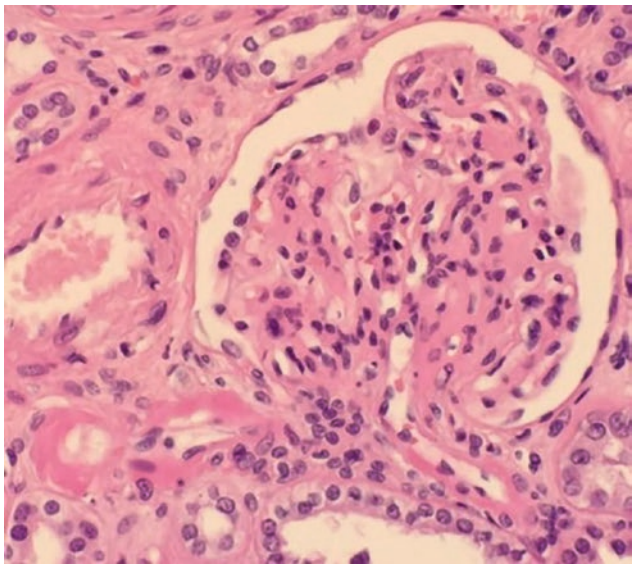


Fig. 7.29 Hyaline arteriolosclerosis of the afferent and efferent arterioles in a case of diabetic glomerulopathy. H&E x400

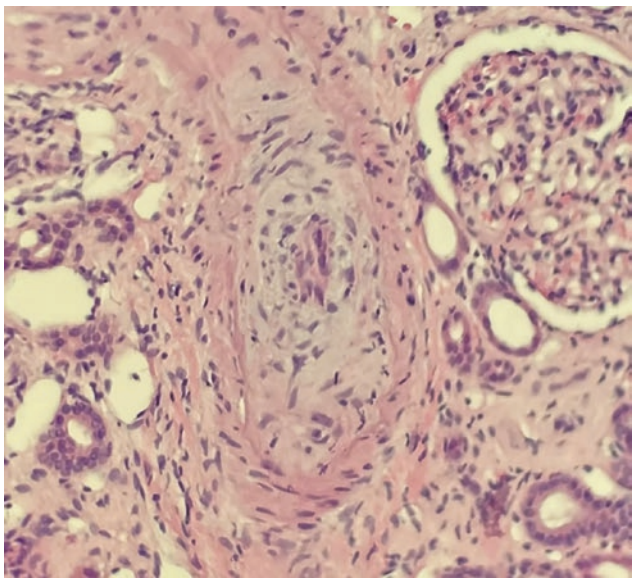


Fig. 7.30 An artery showing mucoid intimal thickening in a case of accelerated hypertension. H&E X100

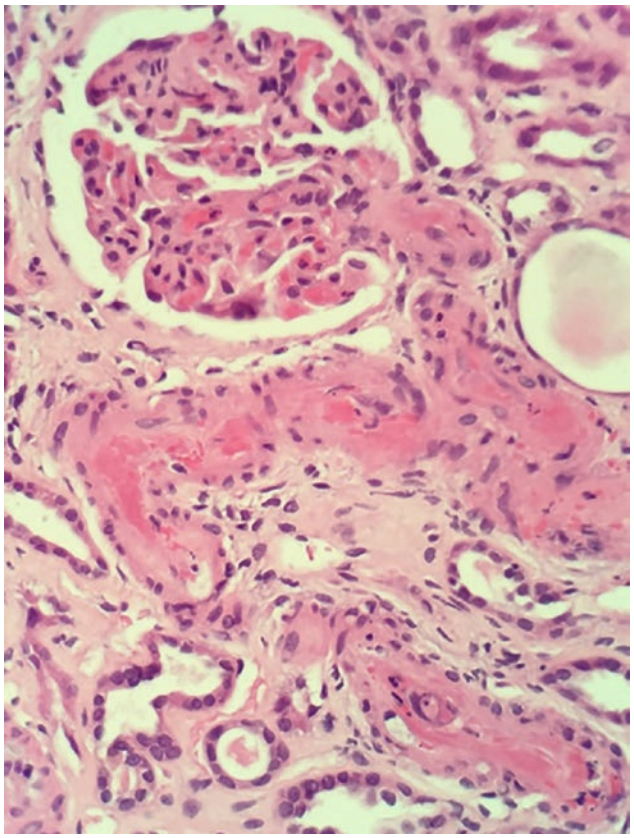


Fig. 7.31 Subendothelial fibrin and fragmented red cells in an arteriole in a case of acute TMA. H&E x200

7.4.6 Patterns of Injury

Once each compartment has been examined for the above features, the glomerular findings can be used to identify a pattern of injury, which provides a differential diagnosis. While this is often helpful, it is important to note that diseases do not always conform to their typical morphology and one disease can have more than one potential pattern (e.g. particularly lupus nephritis). Occasionally, more than one disease process will be present, complicating the interpretation.

Pattern of injury	Differential diagnosis
Normal on light microscopy (Fig. 7.2)	Normal glomerulus No light microscopic change: • Minimal change disease. • Thin basement membrane disease. • Unsampled focal segmental process. • Lupus podocytopathy. • Alport syndrome. Any early/mild glomerulopathy, particularly: • Membranous glomerulopathy. • IgA nephropathy. • Lupus nephritis. • Amyloidosis.
Capillary wall subepithelial 'spikes' (Fig. 7.4)	Membranous glomerulopathy (primary or secondary) Class V lupus nephritis Amyloidosis (spicules)
Endocapillary hypercellularity (Fig. 7.10)	IgA nephropathy/Henoch-Schonlein nephritis Lupus nephritis Acute postinfectious glomerulonephritis (neutrophilic) Cryoglobulinaemic glomerulonephritis C3 glomerulopathy HIV-associated immune complex kidney disease (HIVICK)
Diffuse mesangial matrix expansion (Fig. 7.6)	Monoclonal immunoglobulin deposition disease Amyloidosis Diabetic nephropathy Cryoglobulinaemic GN Immunotactoid GP Fibrillary GN
Nodular mesangial matrix expansion (Fig. 7.13)	Diabetic glomerulosclerosis Monoclonal immunoglobulin deposition disease Amyloidosis Idiopathic nodular sclerosis Advanced MPGN (also shows double contours) Fibronectin glomerulopathy
Mesangial proliferation (Fig. 7.9)	IgA disease/Henoch Schonlein purpura Lupus nephritis class II Late post-infectious glomerulonephritis PGNMID
Membranoproliferative glomerulonephritis (MPGN) (Mesangiocapillary glomerulonephritis) (Fig. 7.7)	Immune complex-related MPGN (of any cause) C3 glomerulopathy (C3 glomerulonephritis or dense deposit disease) Chronic endothelial injury, e.g.TMA Chronic antibody-mediated rejection (transplant glomerulopathy)
Segmental sclerosis (Fig. 7.8)	FSGS (primary or secondary) Sclerosis as part of any glomerular disease
Crescentic glomerulonephritis (Fig. 7.11)	Vasculitic glomerulonephritis: • Pauci-immune/ANCA-related. • Anti-glomerular basement membrane disease. Immune complex-mediated glomerulonephritis: • IgA vasculitis. • Lupus nephritis. • Any MPGN. • PIGN. Can rarely be seen in others, e.g. cryoglobulinaemia, TMA

7.5 Immunohistology and Immunofluorescence

Once a pattern has been identified, immunohistology (IHC) or immunofluorescence (IMF) allows for a more specific diagnosis. The typical glomerular findings are listed in the table below. IHC often show some background staining, which is non-specific, particularly within the mesangial regions and areas of sclerosis.

Disease	IHC/IF	Pattern and distribution
Immunoglobulin dominant		
IgA nephropathy (Fig. 7.32)	IgA dominant +/– C3	Granular Mesangial and paramesangial
Membranous glomerulopathy (Fig. 7.33)	IgG +/– C3 (IgA, IgM, C1q + in secondary forms)	Granular Subepithelial capillary wall +/– mesangial (secondary)
Anti-GBM disease (Fig. 7.34)	IgG +/– C3	Linear Capillary wall
Immune complex-related MPGN (Fig. 7.35)	IgG dominant + C3 (IgA, IgM and C1q may also be +)	Coarsely granular Subendothelial capillary wall +/– mesangial
Cryoglobulinaemic glomerulonephritis	Type I – Monoclonal (k > l) with IgG and C3 Type II – Monoclonal IgM (k > l) with polyclonal IgG and C3 Type III – Polyclonal IgG, IgM and C3	Granular Hyaline thrombi, subendothelial capillary walls, mesangial
Fibrillary glomerulonephritis	IgG dominant + C3 DNAJB9+	Coarse granular Mesangial, segmental subendothelial/subepithelial capillary wall
Immunotactoid glomerulonephritis	IgG +/– C3 May be monoclonal	Coarse granular Subendothelial/subepithelial capillary walls, mesangial
Complement dominant		
Post-infectious glomerulonephritis (Fig. 7.36)	C3 +/– IgG (IgA dominant in staphylococcal infections)	Coarse granular Irregular subepithelial 'humps' along capillary walls, mesangial 'Starry sky' pattern
Dense deposit disease	C3 Ig typically negative, can be some focal + but must be C3 dominant	Coarse granular Subendothelial capillary wall and mesangial
C3 glomerulonephritis	C3 Ig typically negative, can be some focal + but must be C3 dominant	Coarse granular Subendothelial/subepithelial capillary wall and mesangial
Immunoglobulin and complement		
Lupus (Fig. 7.35)	IgG IgA IgM C3 C1q (referred to as 'full house')	Granular Mesangial +/–subendothelial/subepithelial capillary wall
Other		
Amyloidosis	AL is monoclonal, usually lambda light chain restriction Subtypes may show SAA+ LECT2+ TTR+	Smudgy Mesangial, capillary wall, interstitial, vascular (same distribution as Congo red positivity)

Disease	IHC/IF	Pattern and distribution
MIDD	Monoclonal, usually kappa	Linear Capillary, mesangial, tubular basement membranes
Collagenofibrotic glomerulopathy	Collagen III +	Mesangium
Negative		
Pauci-immune/ANCA glomerulonephritis (also GPA, EGPA, MPO)	All negative	N/A
Diabetes		
TTP		
FSGS Including HIVAN		
Fabry's disease		
Minimal change disease		
Idiopathic nodular glomerulosclerosis		
Alport syndrome		
Thin basement membrane disease		
Sickle cell glomerulopathy		

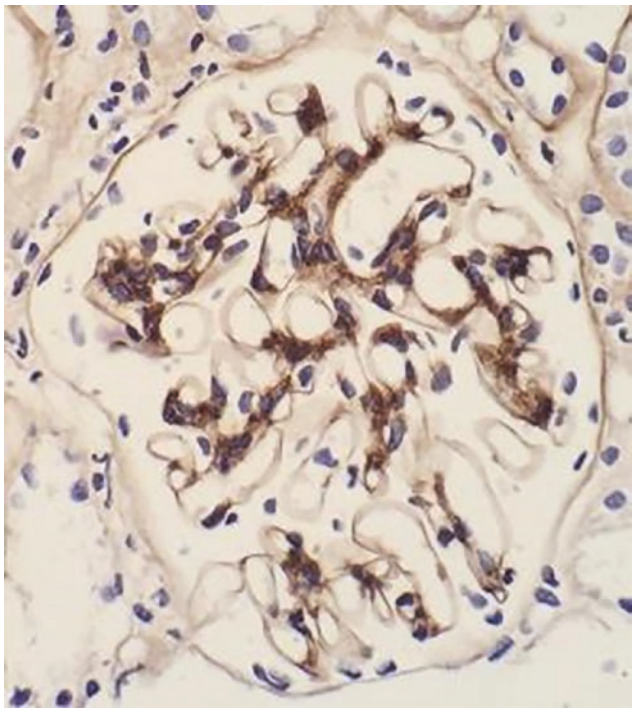


Fig. 7.32 IgA stain showing mesangial staining in a case of IgA nephropathy. X200

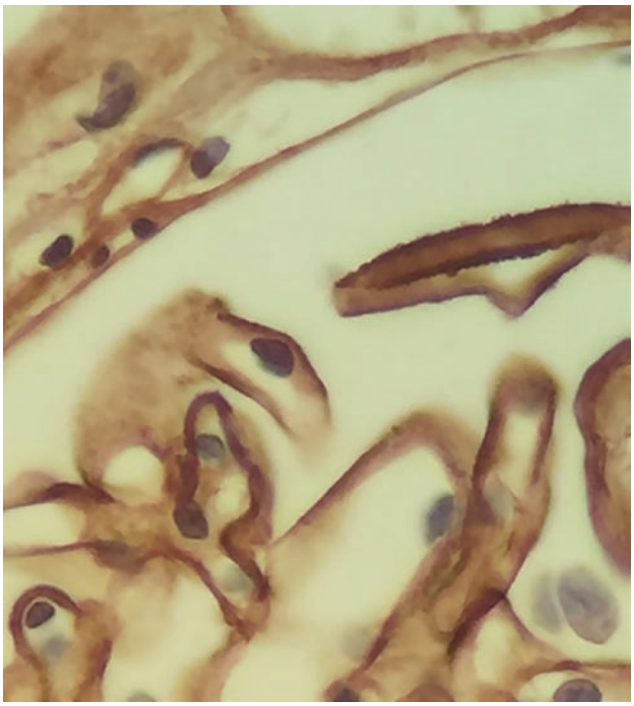


Fig. 7.33 An immunohistochemical stain for IgG shows granular subepithelial capillary wall positivity in a case of membranous glomerulopathy. x400

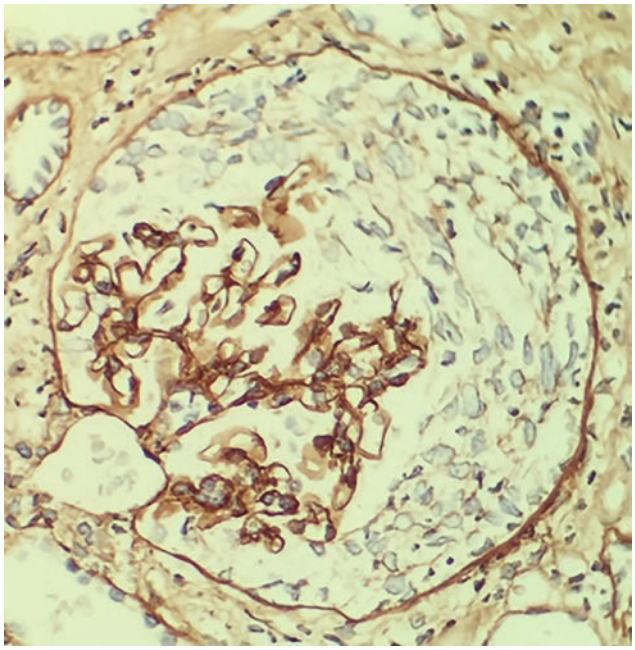


Fig. 7.34 Linear capillary wall positivity for IgG in a glomerulus with a cellular crescent, in a case of anti-GBM disease. X400

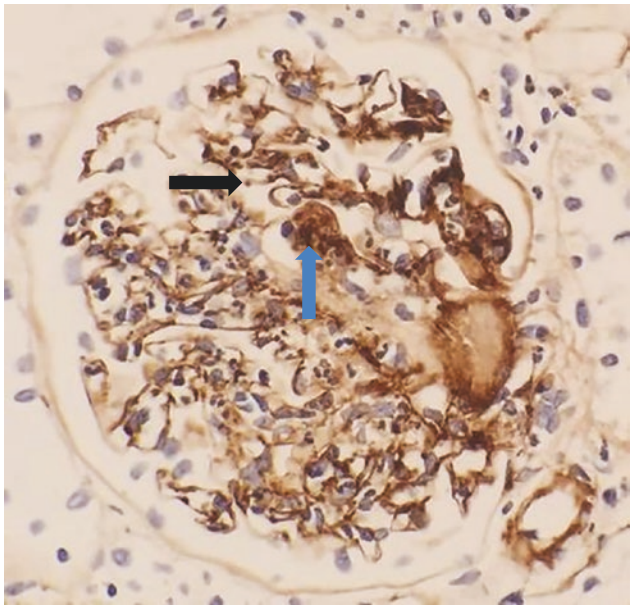


Fig. 7.36 Immunohistochemical stain for C3 shows positive subepithelial humps (black arrow) and granular mesangial positivity (blue arrow) in a case of post-infectious glomerulonephritis. X400

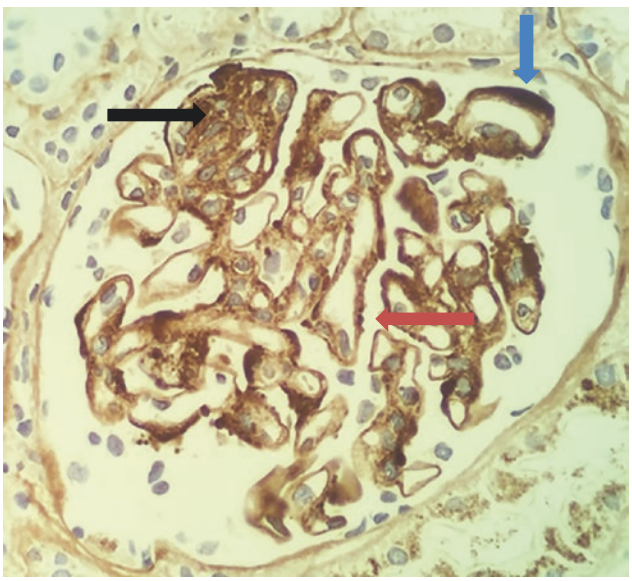


Fig. 7.35 C1q immunohistochemistry showing mesangial (black arrow), subendothelial (blue arrow) and subepithelial (red arrow) positivity in case of lupus nephritis. X400

While the glomerulus is usually the focus for assessment of immunohistology, some pathologies may show significant positivity in other compartments.

Disease	IHC	Pattern and distribution
Tubules		
Light chain cast nephropathy	Kappa or lambda restriction	Pathogenic casts
Monoclonal light chain mediated tubulointerstitial nephritis	Kappa or lambda restriction	Tubular basement membranes
Myoglobin cast nephropathy (Fig. 7.22)	Myoglobin positive	Pathogenic casts
Interstitium		
IgG4 disease	IgG4 positive	Plasma cells Ratio of IgG:IgG4 > 40% or >10 IgG4+ plasma cells per high-power field (x40 objective)

7.6 Electron Microscopic Findings

As stated above, EM is not always crucial for the diagnosis; however, it is helpful to confirm, refine and clarify the light microscopy findings, and in some cases, it is essential. The table below shows a description of the usual findings in the conditions listed. Usually only one or two glomeruli are assessed. As with the other modalities previously discussed, EM findings are not always specific and can be difficult to interpret. Also, focal and segmental lesions may not be represented (Figs. 7.37 and 7.38).

Organised deposits					
	Light microscopy			**Electron microscopy**	
	PAMS	**CR**	**IHC**	**Morphology**	**Distribution of deposits**
Glomerulosclerosis (of any cause, e.g. diabetic glomerulopathy, idiopathic nodular sclerosis)	+	–	Negative (may show non-specific entrapment within sclerosis)	Banded or randomly arranged collagen and precollagen fibrils	Areas of sclerosis
TMA (fibrin deposition)	+	–	Fibrinogen +	Fibrin; 6–8 nm fibrils	Mesangium
Collagenofibrotic/collagen 3 glomerulopathy	+	–	Collagen 3 +	Curved disorganised fibres with periodicity (regular transverse bands at 43–65 nm)	Subendothelium Mesangium
Fibronectin glomerulopathy	–	–	Fibronectin +	12–16 nm fibrils, but often amorphous or granular	Subendothelium Mesangium
Immunotactoid glomerulopathy	–	–	Monoclonal IgG+	10–50 nm microtubules arranged in parallel arrays	Subendothelium Subepithelium Mesangium
Cryoglobulin GN	–	–	IgG, IgM, C3, may be monoclonal (see above)	25–35 nm curved microtubules (not present in every case, can be amorphous)	Subendothelium Mesangium Intraluminal (hyaline thrombi)
Fibrillary GN (Figs. 7.39 and 7.40)	–	–	IgG+ C3+	15–30 nm randomly oriented non-branching fibrils	Subendothelium Subepithelium Mesangium
DM fibrillosis	- (often within + mesangial nodule)	–	–	10–25 nm random fibrils	Mesangium (less argyrophilic areas)
Amyloidosis (Figs. 7.37 and 7.38)	–	+	Subtypes AL, AA, beta-2-microglobulin etc.	8–12 nm randomly oriented non-branching fibrils	Subendothelium Subepithelium Mesangium Tubular basement membranes Arterioles Interstitium

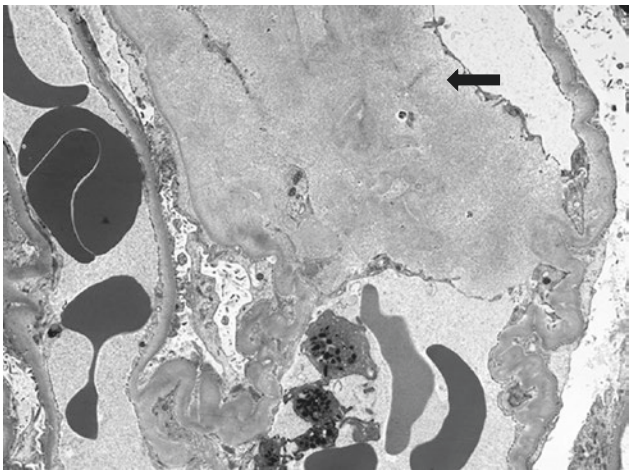


Fig. 7.37 Electron micrograph showing mesangial amyloid deposits (black arrow), in a case of amyloidosis. X1200. (Image courtesy of Leicester Royal Infirmary electron microscopy department)

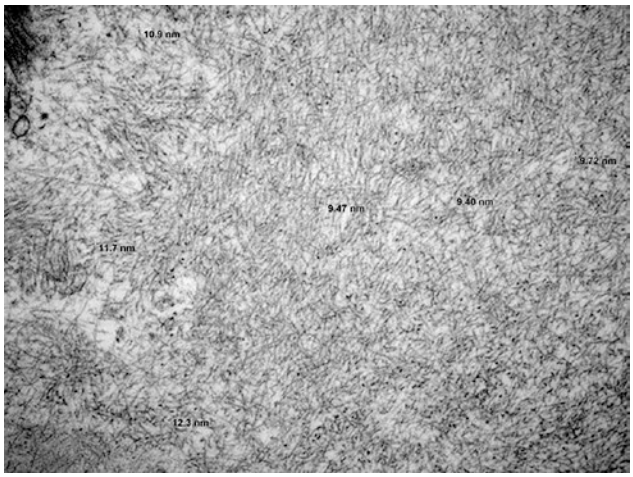


Fig. 7.38 Electron micrograph of amyloid fibrils with measurements, in a case of amyloidosis. X12000. (Image courtesy of Leicester Royal Infirmary electron microscopy department)

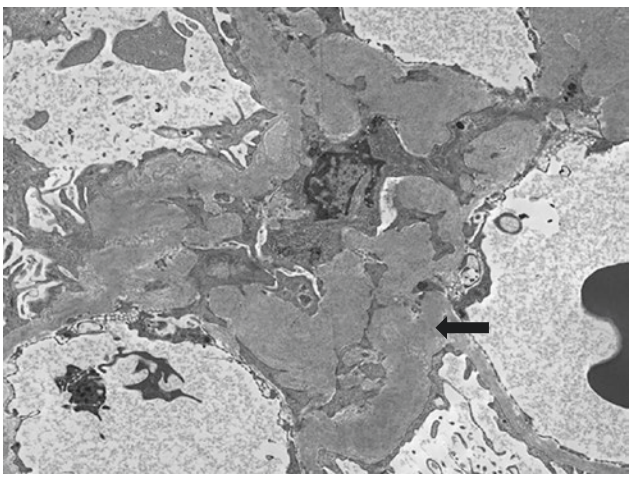


Fig. 7.39 Expanded mesangial region containing organised mesangial deposits (black arrow) in a case of fibrillary glomerulopathy. X1500. (Image courtesy of Leicester Royal Infirmary electron microscopy department)

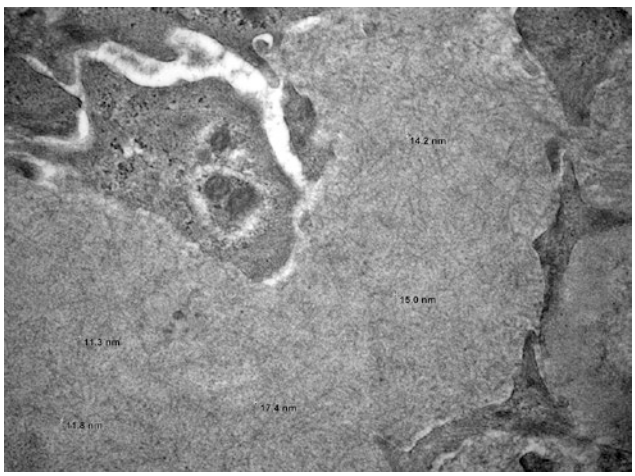


Fig. 7.40 A closer view showing the randomly organised fibrils, with an average diameter of 18 nm, in a case of fibrillary glomerulopathy. X8000. (Image courtesy of Leicester Royal Infirmary electron microscopy department)

Disease	EM Findings
Electron dense deposits (EDD)	
Post-infectious GN (Fig. 7.41)	Subepithelial hump-shaped electron dense deposits (EDD)
Dense deposit disease (Fig. 7.42)	Elongated "ribbon-like" very dense intramembranous and mesangial deposits
C3 glomerulonephritis	Subendothelial, subepithelial (can be hump-like) and mesangial EDD
Lupus nephritis (Fig. 7.43)	Mesangial, subendothelial and subepithelial EDD can all be present. Endothelial cell cytoplasm may contain tubuloreticular inclusions
IgA disease (Fig. 7.44)	Mesangial and paramesangial EDD
Membranous glomerulopathy	Subepithelial EDD Mesangial EDD may be present in secondary forms
PGNMID	Mesangial and subendothelial EDD
Other deposits/materials	
MIDD	Amorphous granular deposits along glomerular basement membrane (GBM), and within mesangium
LCAT deficiency	Lipid inclusions, basement membrane lacunae, striated membranous structures within the mesangium
Lipoprotein glomerulopathy	Capillary loop lipoprotein thrombi; lamellated with lipid vacuoles and granules, FPE
Fabry's disease (Fig. 7.45)	Lamellated lysosomal inclusions (myelin/zebra bodies) particularly within podocytes, but can be seen in all renal cells
BK nephropathy	Intranuclear viral particles 30 to 50 nm diameter (typically seen in tubular epithelial cells)
Tubuloreticular inclusion	Approximately 20 nm organised structures seen in lupus nephritis, viral infection (particularly HIV) and interferon therapy (typically found in the endoplasmic reticulum of endothelial cells)
Structural changes	
Minimal change nephropathy (Fig. 7.46)	Extensive foot process effacement (FPE) of podocytes, typically no other abnormalities
FSGS	Focal FPE overlying areas of sclerosis and in non-sclerotic glomeruli
Thin basement membrane disease (Fig. 7.47)	Diffusely thin GBM (compared with age-matched controls, generally <250 nm in adults [3]).
Alport syndrome	Variably thinned and thickened GBM with a multilaminated, 'basket weave' appearance of the lamina densa
Lupus podocytopathy	Extensive FPE (as in minimal change nephropathy), may see mesangial EDD but no capillary wall EDD
Diabetic nephropathy (Fig. 7.48)	Thickened glomerular basement membrane (often >600 nm), FPE, increased mesangial matrix, hyaline material (can resemble EDD)
Thrombotic microangiopathy	Acute; expansion of the lamina rara interna, endothelial cell swelling; may see fibrin tactoids and thrombi Chronic; duplication of theGBM, mesangial cell interposition
Chronic allograft glomerulopathy (Fig. 7.49)	Duplication of the GBM and lamination of the peritubular capillary basement membranes

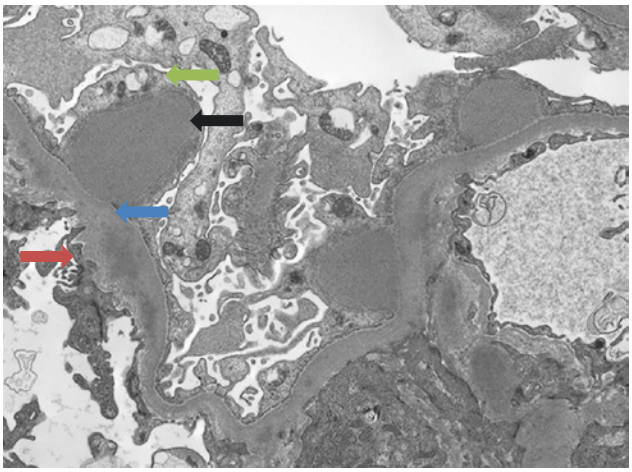


Fig. 7.41 Electron micrograph showing subepithelial hump deposits in a case of post-infectious glomerulonephritis. Black arrow, subepithelial deposit; blue arrow, basement membrane; green arrow, effaced podocyte foot process, X2500. (Image courtesy of Leicester Royal Infirmary electron microscopy department)

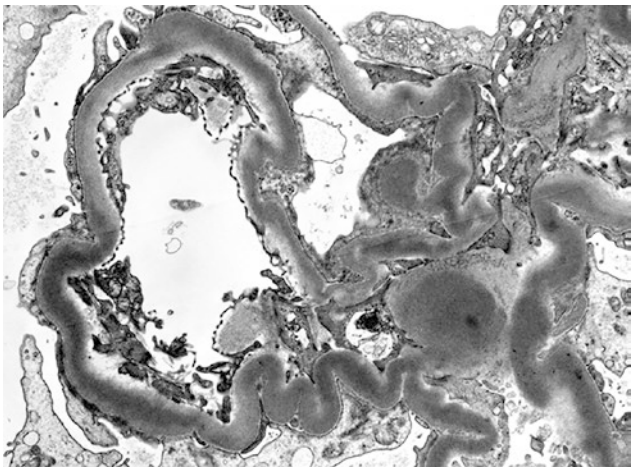
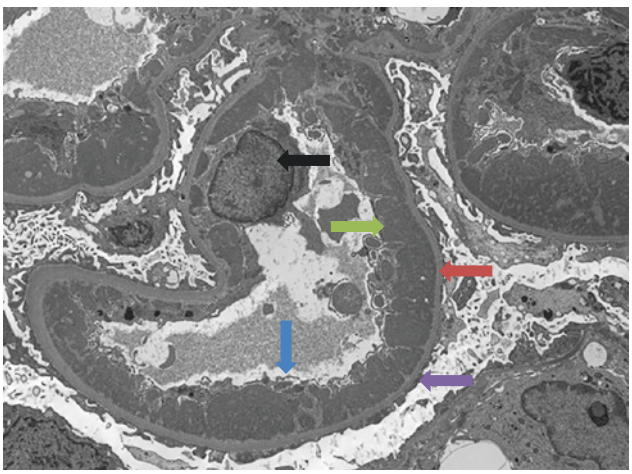


Fig. 7.42 Electron micrograph showing highly dense intramembranous and mesangial deposits. X2500. (Image courtesy of Leicester Royal Infirmary electron microscopy department)

Fig. 7.43 Electron micrograph showing numerous large subendothelial deposits in a case of lupus nephritis, visible as wire loops on light microscopy. Black arrow, endothelial cell nucleus; blue arrow, endothelium; green arrow, deposits; red arrow, basement membrane; purple arrow, effaced podocyte foot processes. X1000. (Image courtesy of Leicester Royal Infirmary electron microscopy department)



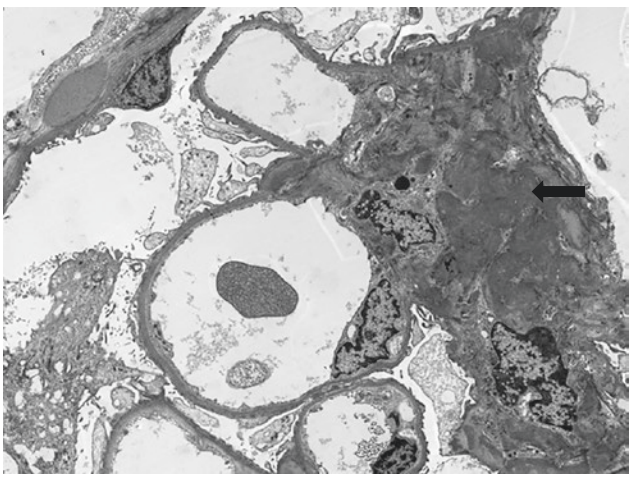


Fig. 7.44 Electron micrograph showing mesangial electron dense deposits (black arrow) in a case of IgA nephropathy. X1000. (Image courtesy of Leicester Royal Infirmary electron microscopy department)

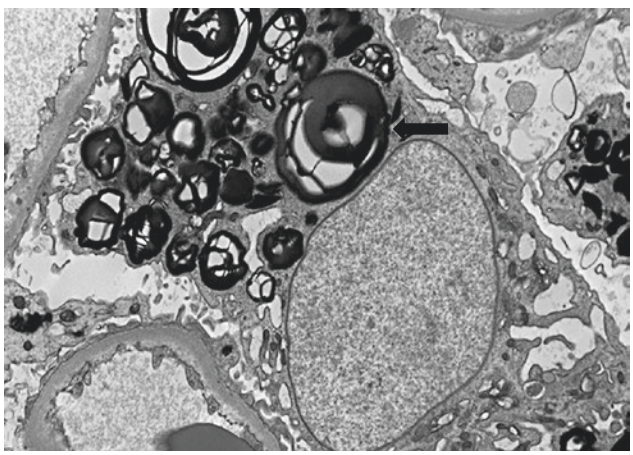


Fig. 7.45 Zebra bodies (black arrow) within podocyte cytoplasm in a case of Fabry's disease. X4400. (Image courtesy of Leicester Royal Infirmary electron microscopy department)

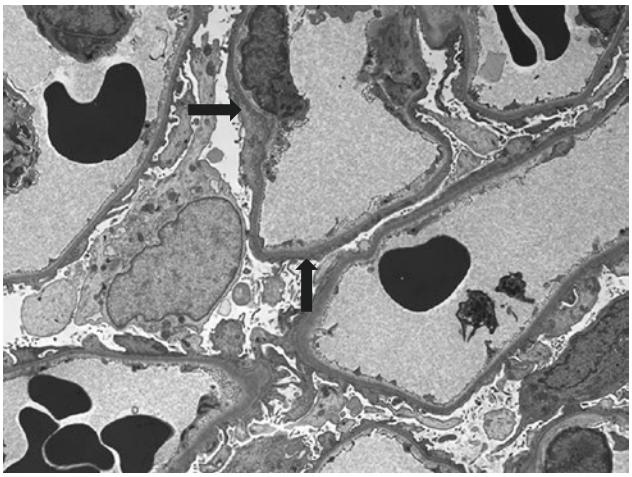


Fig. 7.46 Electron micrograph showing widespread podocyte foot process effacement (black arrows) in a case of minimal change nephropathy. X1000. (Image courtesy of Leicester Royal Infirmary electron microscopy department)

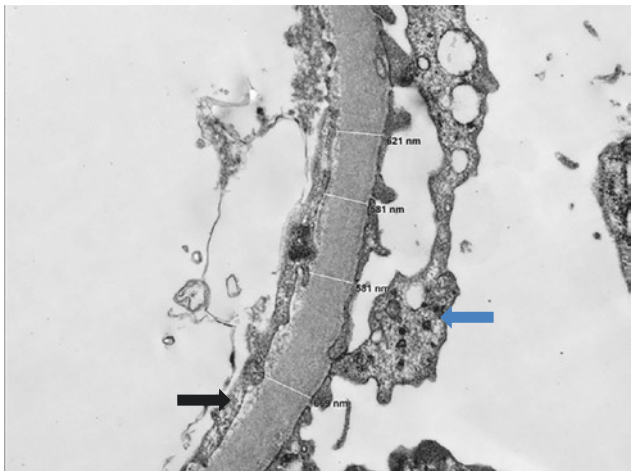


Fig. 7.48 Electron micrograph showing thickened glomerular basement membrane with measurements, in a case of diabetic nephropathy. Blue arrow, effaced podocyte foot process; black arrow, endothelium. X4000. (Image courtesy of Leicester Royal Infirmary electron microscopy department)

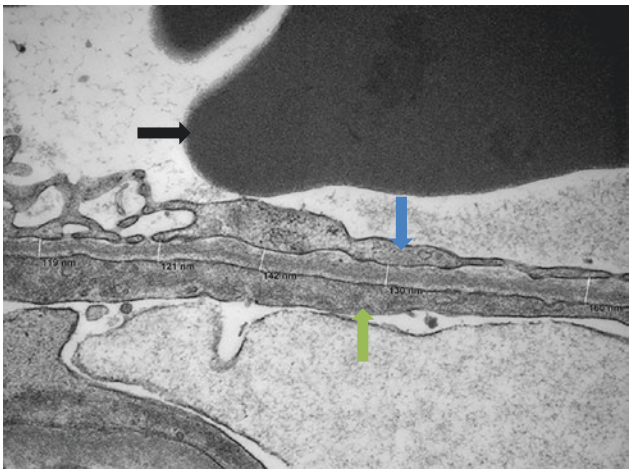


Fig. 7.47 Electron micrograph showing thinning of the glomerular basement membrane. Black arrow, erythrocyte; blue arrow, endothelium; green arrow, effaced podocyte foot process. X8000. (Image courtesy of Leicester Royal Infirmary electron microscopy department)

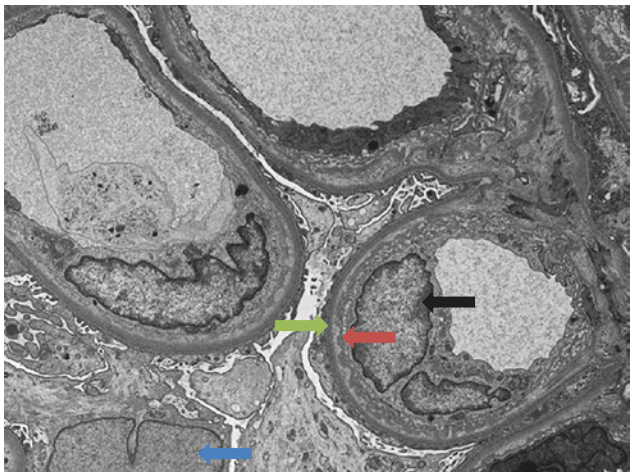


Fig. 7.49 Electron micrograph showing duplication of the glomerular basement membranes, visible as double contours on light microscopy, in a case of transplant glomerulopathy. Black arrow, endothelial cell nucleus; blue arrow, podocyte nucleus; green arrow, original basement membrane; red arrow, reduplicated basement membrane. X1200. (Image courtesy of Leicester Royal Infirmary electron microscopy department)

7.7 Reaching a Diagnosis

Given the variety of features on clinical, light microscopic, IHC/IMF and electron microscopy assessment of renal biopsies, and the fact that very few are specific for a single diagnosis, it is easy to see how each in isolation may not be diagnostic. However, the combination of each of these modalities with the clinical context will usually be sufficient for diagnosis. In some cases, there will be uncertainty, either due to unusual features, a lack of convincing or definitive features, suboptimal functionality of one or more tests or insufficient tissue. While all cases benefit from discussion in a multidisciplinary setting, this is particularly helpful for these 'difficult' or less conclusive cases, where the differential and clinical management options can be discussed.

7.8 Classification Systems

Once a diagnosis has been made, a relevant classification system can be applied if appropriate. The main benefit of classification systems is to provide a standardised system for a particular diagnosis, which should be given the same 'score' or category when viewed by different pathologists across hospitals, regions and countries. The standardised approach can be helpful in determining the treatment strategy, e.g. in lupus nephritis, and is useful in research. Potential drawbacks are that not all biopsies will fit neatly into one category; some may show poor concordance and systems change as they are updated. The following are examples of some of the most commonly used. Some are straightforward; others are more complex.

7.9 The Banff Classification of Renal Allograft Pathology [4]

Category 1: Normal biopsy or non-specific changes	
Requires exclusion of any diagnosis from the Banff diagnostic categories 2–4, 6	
Category 2: Antibody-mediated changes (use the diagnostic criteria groups to reach one diagnosis)	
Diagnoses	**Diagnostic criteria groups**
C4d staining without evidence of rejection Banff lesion score C4d > 1 (IF on fresh frozen tissue) OR C4d > 0 (IHC on paraffin-embedded tissue) AND Banff lesion scores t0, v0, no arterial intimal fibrosis with mononuclear cell inflammation in fibrosis and formation of neointima, no criterion from group 1 (AMR activity), no criterion from groups 4 (histologic features of AMR chronicity), no increased expression of thoroughly validated gene transcripts/classifiers in the biopsy tissue strongly associated with AMR	Criteria group 1 AMR activity: Banff lesion score g > 0 in the absence of glomerulonephritis and/or Banff lesion score ptc > 0 in the absence of TCMR or borderline Banff lesion score v > 0 Acute thrombotic microangiopathy in the absence of any other cause Acute tubular injury in the absence of any other apparent cause
Active AMR No criterion of AMR chronicity (criteria group 4) AND At least one criterion from criteria group 1 (AMR activity) AND At least one criterion from criteria group 2 (antibody interaction with tissue) AND At least one criterion from criteria group 3 (DSA or equivalents)	Criteria group 2 antibody interaction with tissue: Banff lesion score C4d > 1 (IF on fresh frozen tissue) OR C4d > 0 (IHC on paraffin-embedded tissue) At least moderate MVI (g + ptc >1) in the absence of recurrent or de novo glomerulonephritis; borderline (diagnostic category 3) or acute T cell-mediated rejection (TCMR; diagnostic category 4). If borderline, acute TCMR or infection is present (Banff lesion scores g + ptc) >1 is not sufficient and Banff lesion score g > 1 is required Increased expression of thoroughly validated gene transcripts/classifiers in the biopsy tissue strongly associated with AMR
Chronic active AMR At least one feature of AMR chronicity (criteria group 4) AND At least one criterion of antibody interaction with tissue (criteria group 2) AND At least one criterion of DSA or equivalents (criteria group 3)	Criteria group 3 DSA or equivalents: DSA (anti-HLA or other specificity) Banff lesion score C4d > 1 (IF on fresh frozen tissue) OR C4d > 0 (IHC on paraffin-embedded tissue) Increased expression of thoroughly validated gene transcripts/classifiers in the biopsy tissue strongly associated with AMR
Chronic AMR Banff 2017 permits the use of this term for biopsy specimens showing TG and/or peritubular capillary basement membrane multilayering in the absence of criterion of current/recent antibody interaction with the endothelium (Criteria Group 2) but with a prior documented diagnosis of Active or Chronic Active or documented prior evidence of DSA	Criteria group 4 histologic features of AMR chronicity Banff lesion score cg > 0 (by LM or EM), excluding biopsies with evidence of chronic thrombotic microangiopathy Seven or more layers in one cortical peritubular capillary and five or more in two additional capillaries, avoiding portions cut tangentially by EM in available Arterial intimal fibrosis of new onset, excluding other causes leukocytes within the sclerotic intima favour chronic AMR if there is not prior history of biopsy-proven TCMR but are not required

Category 3: Suspicious (borderline) for acute TCMR
Foci of Banff lesion score t > 0 AND Banff lesions score i ≤ 1 OR Foci of Banff lesion score t1 AND Banff lesion score i ≥ 2
Category 4: TCMR
Acute TCMR IA Banff lesion score i ≥ 2 AND Banff lesion score t2 **Acute TCMR IB** Banff lesion score i ≥ 2 AND Banff lesion score t3 **Acute TCMR IIA** Banff lesion score v1 regardless of Banff lesion scores i or t **Acute TCMR IIB** Banff lesion score v2 regardless of Banff lesion scores i or t **Acute TCMR III** Banff lesion score v3 regardless of Banff lesion scores i or t **Chronic active TCMR grade IA** Banff lesion score ti ≥ 2 AND Banff lesion score i-IFTA≥2; other known causes of i-IFTA (e.g. pyelonephritis, BK-virus nephritis, etc.) ruled out AND Banff lesion score t2 **Chronic active TCMR grade IB** Banff lesion score ti ≥ 2 AND Banff lesion score i-IFTA≥2; other known causes of IFTA ruled out AND Banff lesion score t3 **Chronic active TCMR grade II** Arterial intimal fibrosis with mononuclear cell inflammation on fibrosis and formation of neointima
Category 4: IFTA
Grade I (mild) Banff lesion score ci1 OR Banff lesion score ct1 **Grade II (moderate)** Banff lesion score ci2 OR Banff lesion score ct2 **Grade III (severe)** Banff lesion score ci3 OR Banff lesion score ct3
Category 6: Other changes not considered to be caused by acute or chronic rejection
BK virus nephropathy Post-transplant lymphoproliferative disorder Calcineurin inhibitor toxicity Acute tubular injury Recurrent disease De novo glomerulopathy (other than TG) Pyelonephritis Drug-induced interstitial nephritis

7.9.1 The Oxford Classification of IgA nephropathy [6]

Variable	Score
Mesangial hypercellularity (≥4 cells in a single mesangial area)	≤50% glomeruli M0 >50% glomeruli M1
Endocapillary hypercellularity	Absent E0 Present E1
Segmental glomerulosclerosis	Absent S0 Present S1 (with a comment indicating the presence/absence of podocytopathic features)
Tubular atrophy and interstitial fibrosis	<25% T0 26–50% T1 >50% T2
Cellular/fibrocellular crescents	Absent C0 In at least one glomerulus C1 In >25% of glomeruli C2

7.9.2 The Columbia Classification of Focal Segmental Glomerulosclerosis

The diagnosis of FSGS can be problematic, as segmental lesions are non-specific and there are many possible aetiologies (genetic, viral, drug, adaptive, underlying glomerulopathy). This classification can be used in primary or secondary forms and is based on light microscopic features. Given the focal nature of the diagnostic features, it has been suggested that 25 glomeruli and multiple sections are required to reliably detect lesions. The juxtamedullary region is thought to be affected initially; therefore, biopsies ideally will include this area. The NOS variant is the commonest and is thought to develop from the other variants [5].

Variant	Inclusion criteria	Exclusion criteria	Prognosis
FSGS NOS (not otherwise specified)	At least one glomerulus with segmental increase in matrix obliterating the capillary lumina There may be segmental glomerular capillary wall collapse without overlying podocyte hyperplasia	Exclude perihilar, cellular, tip and collapsing variants	Standard
Perihilar variant	At least one glomerulus with perihilar hyalinosis, with or without sclerosis >50% of glomeruli with segmental lesions must have perihilar sclerosis and/or hyalinosis	Exclude cellular, tip and collapsing variants	Good
Cellular variant	At least one glomerulus with segmental endocapillary hypercellularity occulding lumina, with or without foam cells and karyorrhexis	Exclude tip and collapsing variants	Intermediate between collapsing and NOS variants
Tip variant	At least one segmental lesion involving the tip domain (outer 25% of tuft next to the origin of the proximal tubule) The tubular pole must be identified in the defining lesion The lesion must have either an adhesion or confluence of podocytes with parietal or tubular cells at the tubular lumen or neck The tip lesion may be cellular or sclerosing	Exclude collapsing variant Exclude perihilar sclerosis	Excellent Highest rate of complete remission
Collapsing variant	At least one glomerulus with segmental or global collapse and overlying podocyte hypertrophy and hyperplasia	None	Poor Highest rate of ESRD

7.10 ISN/RPS Classification of Lupus Nephritis

The International Society of Nephrology/Renal Pathology Society lupus nephritis classification was originally proposed in 2004 [7], but has recently been updated [8]. Some of the most prominent changes are the elimination of the segmental and global (S/G) subdivisions of class IV, due to poor concordance and uncertain clinical significance, and the introduction of an activity/chronicity index, modified from the NIH activity and chronicity index, to replace the previously used A, C and A/C parameters. Class V can co-exist with class III or IV (i.e. lupus nephritis class III + V).

Classification	Description	Features
Class I	Minimal mesangial lupus nephritis	Normal glomeruli on light microscopy, with immune deposits detectable on IHC/IF
Class II	Mesangial proliferative lupus nephritis	Mesangial hypercellularity (four or more mesangial cells per mesangial region, surrounded by matrix, not including the central or hilar regions)
Class III	Focal lupus nephritis	Active or inactive, focal or global endo-extracapillary glomerulonephritis, involving <50% of glomeruli
Class IV	Diffuse lupus nephritis	Active or inactive, focal or global endo-extracapillary glomerulonephritis, involving ≥50% of glomeruli
Class V	Membranous lupus nephritis	Global or segmental subepithelial immune deposits, with or without mesangial changes
Class VI	Advanced sclerotic lupus nephritis	>90% of glomeruli globally sclerosed, with no residual activity

Modified NIH Activity Index	Definition	Score
Endocapillary hypercellularity	Endocapillary hypercellularity in % of glomeruli: <25% (1+) 25–50% (2+) >50% (3+)	0–3
Neutrophils/ karyorrhexis	Neutrophils and/or karyorrhexis in % of glomeruli: <25% (1+) 25–50% (2+) >50% (3+)	0–3
Hyaline deposits	Wire loops or hyaline thrombi in % of glomeruli: <25% (1+) 25–50% (2+) >50% (3+)	0–3
Fibrinoid necrosis	Fibrinoid necrosis in % of glomeruli: <25% (1+) 25–50% (2+) >50% (3+)	(0–3) x2
Cellular/ fibrocellular crescents	Cellular/fibrocellular crescents in % of glomeruli: <25% (1+) 25–50% (2+) >50% (3+)	(0–3) x2
Interstitial inflammation	Interstitial leukocytes in % of the cortex: <25% (1+) 25–50% (2+) >50% (3+)	0–3
Total		**0–24**
Modified NIH chronicity index	**Definition**	**Score**
Total glomerulosclerosis	Global and/or segmental glomerulosclerosis in % of glomeruli: <25% (1+) 25–50% (2+) >50% (3+)	0–3
Fibrous crescents	Fibrous crescents in % of glomeruli: <25% (1+) 25–50% (2+) >50% (3+)	0–3
Tubular atrophy	Tubular atrophy in % of the cortical tubules: <25% (1+) 25–50% (2+) >50% (3+)	0–3
Interstitial fibrosis	Interstitial fibrosis in % of the cortex: <25% (1+) 25–50% (2+) >50% (3+)	0–3
Total		**0–12**

7.10.1 The Modified Karpinski Score for Time Zero Renal Transplant Biopsies [9]

This scoring system is one of a number of similar systems used for biopsies taken at implantation of a transplant. The function of these biopsies is to provide a baseline of the condition of the kidney at the time of transplant (donor-related damage), which can be used for comparison on subsequent biopsies. This scoring system should be used in biopsies with more than 20 glomeruli present.

Glomerular score
0 – No globally sclerosed glomeruli 1 – <20% 2 – 20–50% 3 – >50%
Tubular score
0 – No atrophic tubules 1 – <20% 2 – 20–50% 3 – >50%
Interstitial score
0 – No interstitial fibrosis 1 – <20% 2 – 20–50% 3 – >50%

Vascular score (use arterial or arteriolar score – Whichever is greater)
0 – No arteriolar narrowing/hyaline arteriolosclerosis 1 – Increased wall thickness less than the diameter of the lumen 2 – Increased wall thickness the same as or slightly more than the diameter of the lumen 3 – Increased wall thickness much greater than the diameter of the lumen or occlusion 0 – No arterial sclerosis/intimal fibroplasia 1 – Increased wall thickness less than the diameter of the lumen 2 – Increased wall thickness the same as or slightly more than the diameter of the lumen 3 – Increased wall thickness much greater than the diameter of the lumen or occlusion

Tips and Tricks

- Look for a second diagnosis, particularly in common conditions such as diabetic or IgA nephropathy.
- Atrophic tubules are generally accepted as the main determining factor of irreversible damage in the kidney and so are a key prognostic indicator [10].
- The percentage of chronic parenchymal damage within a core biopsy is assumed to be representative of the whole kidney, but this is not always the case, particularly in subcapsular samples, which may overestimate the amount of damage [11].

Case Studies

Case 1

Clinical Scenario.

A 73-year-old male with renal impairment, haematuria and proteinuria.

The H&E image (Fig. 7.50) shows a nodular glomerulopathy. Differentials are diabetic glomerulopathy, amyloidosis, monoclonal light chain deposition disease or idiopathic nodular glomerulopathy (a diagnosis of exclusion).

The silver stain (PAMS, Fig. 7.51) shows that the mesangial material is silver negative (excluding diabetic glomerulopathy).

The Congo red stain shows that the mesangial material is Congo red positive and shows 'apple green' birefringence under polarised light (Fig. 7.52) diagnostic of amyloidosis.

Light chain immunohistochemistry shows stronger mesangial positivity for lambda than kappa (Fig. 7.53), giving a diagnosis of AL (lambda) amyloidosis.

Diagnosis: AL amyloidosis.

Clinical Correlation: The patient was found to have a plasma cell neoplasm on bone marrow biopsy.

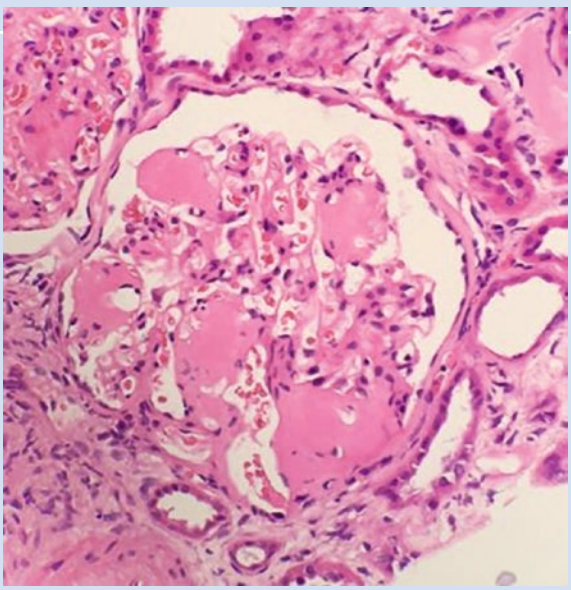


Fig. 7.50 Case 1 H&E. x400

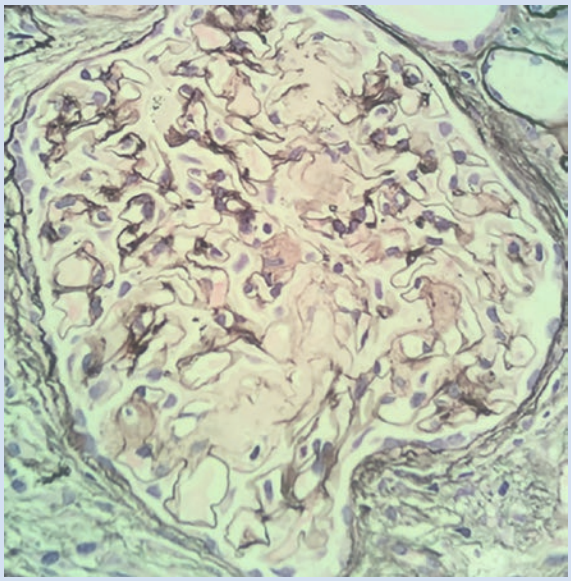


Fig. 7.51 Case 1. PAMS X400

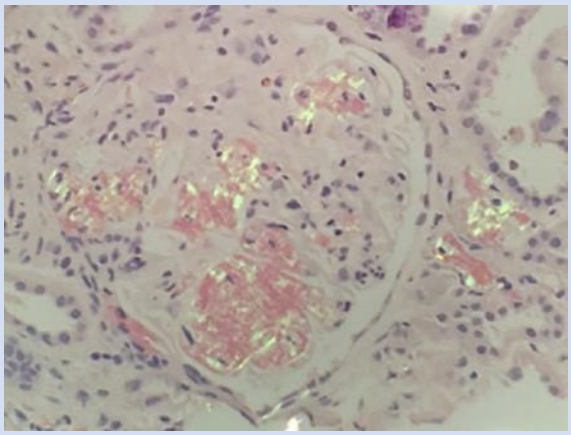


Fig. 7.52 Case 1. Photographed under polarised light. Congo red. X400

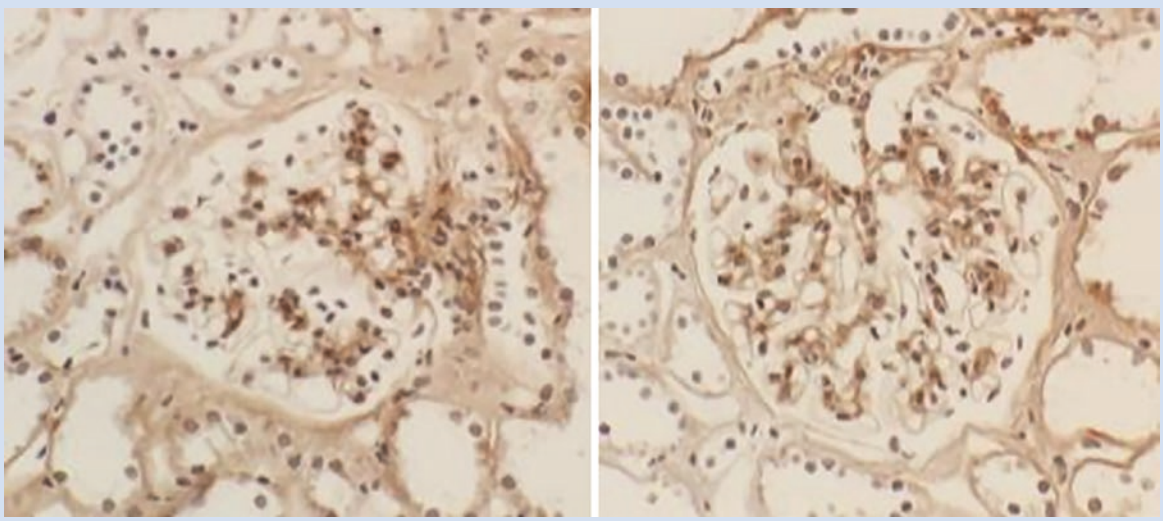


Fig. 7.53 Case 1. Kappa (right) and lambda (left) light chain immunohistochemical stains. X400

Case 2

Clinical Scenario.

A 25-year-old lady with increasing proteinuria and low serum complement levels.

The initial H&E (Fig. 7.54) shows a glomerulus with a segment of necrosis and a small cellular crescent. At this point, it is apparent that there is an active glomerular process (necrosis and cellular crescent).

The PAMS stain (Fig. 7.55) demonstrates diffuse, global spike formation along the capillary walls indicating that there are subepithelial deposits producing a membranous pattern.

Immunostains (Figs. 7.56 and 7.57) show granular capillary wall and mesangial positivity for immunoglobulins G, A and M and complement component C3 and C1q. This implies that immune deposits are present both in the capillary walls and the mesangium. The capillary wall deposits are predominantly subepithelial, with occasional subendothelial deposits.

Electron microscopy (Fig. 7.58) shows subepithelial (green arrow), mesangial (black arrow) and occasional subendothelial (blue arrow) electron dense deposits. Tubuloreticular inclusions are seen within endothelial cells (Fig. 7.59).

Diagnosis: Lupus nephritis, class III + V.

Clinical correlation – this lady had positive ANA and dsDNA antibodies and fulfilled clinical criteria for a diagnosis of SLE.

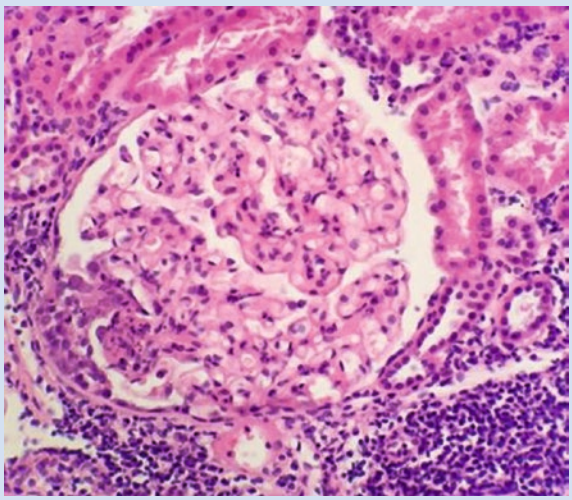


Fig. 7.54 Case 2. H&E x200

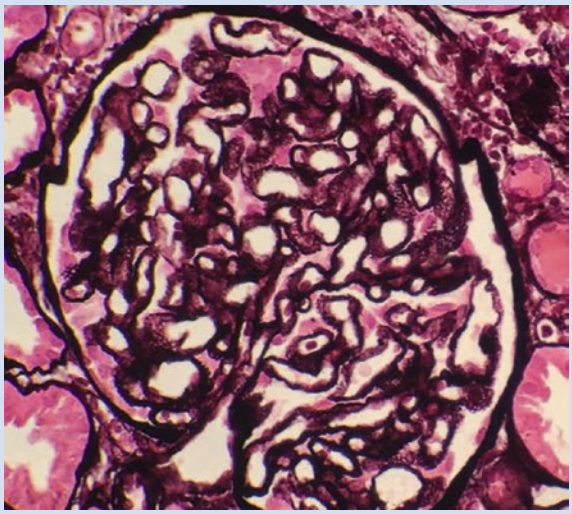


Fig. 7.55 Case 2. PAMS x400

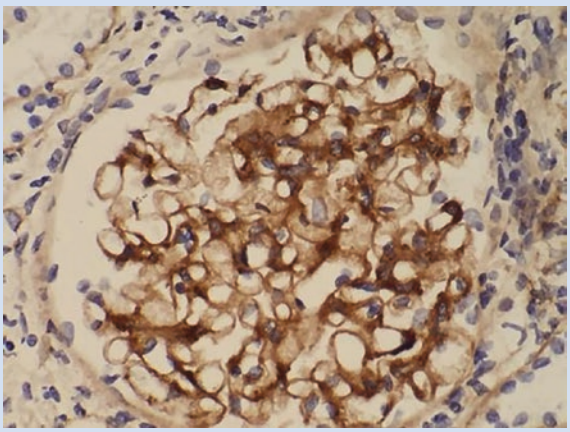


Fig. 7.56 Case 2. Immunohistochemical stain for IgG. IgA and IgM showed similar patterns of staining. X400

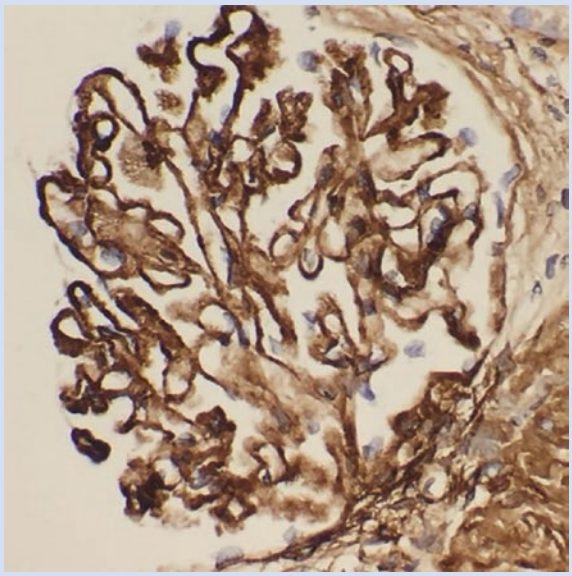


Fig. 7.57 Case 2. Immunohistochemical stain for C3. C1q showed a similar pattern of staining. X400

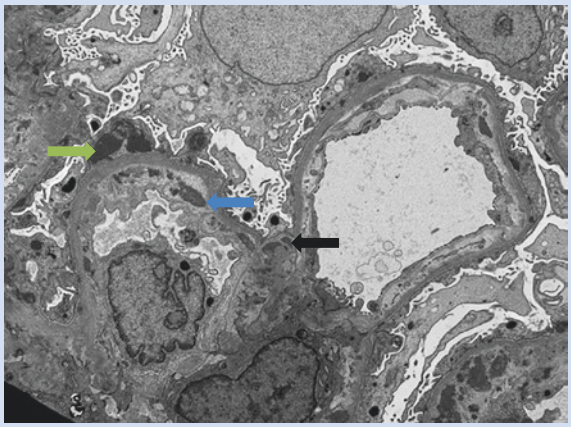


Fig. 7.58 Case 2. Electron micrograph X1200. (Image courtesy of Leicester Royal Infirmary electron microscopy department)

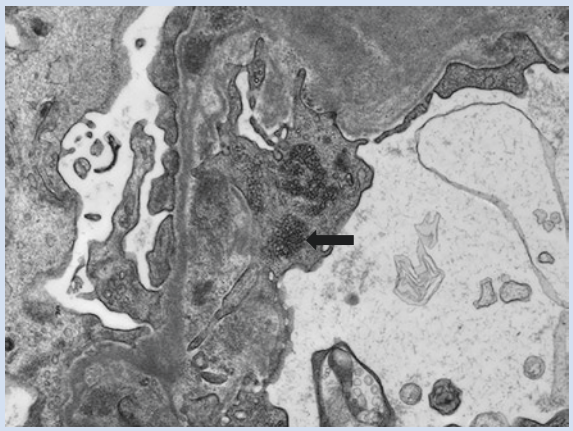


Fig. 7.59 Case 2. Electron micrograph. X5000. (Image courtesy of Leicester Royal Infirmary electron microscopy department)

Case 3

Clinical Scenario.

A 44-year-old man, recipient of a DBD renal transplant 16 months ago. Best creatinine 170, now risen to 236.

Initial H&E sections (Fig. 7.60) show acute tubular injury with some nuclear pleomorphism and focal tubulitis. No acute vascular rejection is seen. There is moderate chronic damage. Some glomeruli (not shown) are poorly perfused but show no other acute changes. At this point, the differential lies between acute cellular rejection and BK nephropathy. Without confirmatory tests, it can be very difficult to reliably distinguish these possibilities. In this case, viral cytopathic changes are present, and further clinical history was sought, making the diagnosis straightforward.

Further tests show positive tubular nuclei on BK immunohistology (Fig. 7.61).

Diagnosis: BK nephropathy.

Clinical correlation – this patient was compliant with immunosuppressive therapy, and a serum BK viraemia was identified.

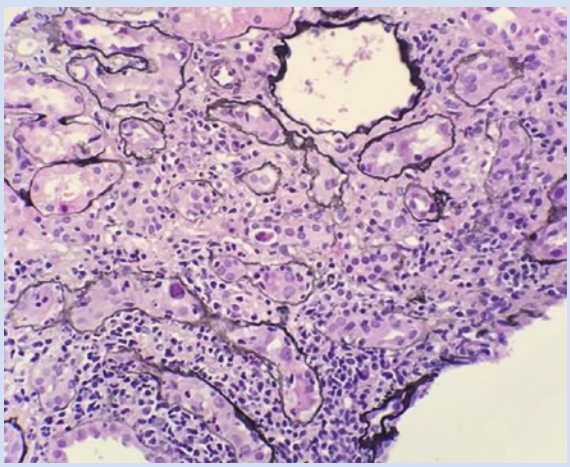


Fig. 7.60 Case 3. PAMS x200

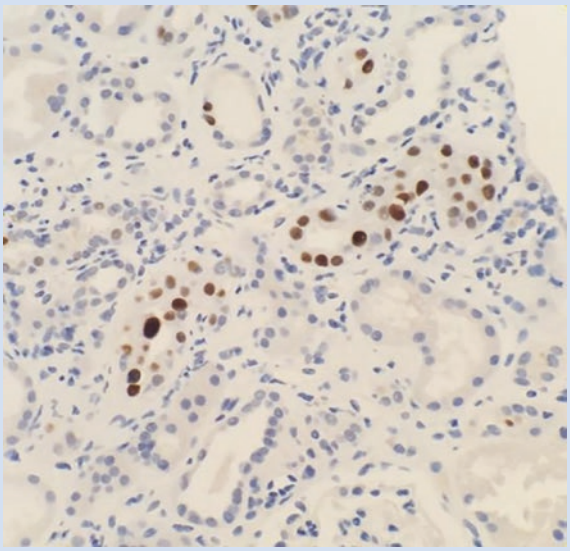


Fig. 7.61 Case 3. Immunohistochemical stain for SV40. X200

Questions

The answers may be found within the text.

1. What is the differential diagnosis for a nodular glomerulopathy?
2. What is the differential diagnosis for glomerular IgA deposition?
3. What is the differential diagnosis for a glomerulopathy with negative IHC/IF?
4. Which feature on a biopsy is generally considered to be the best predictor of long-term outcome?
5. What are the Banff criteria for a diagnosis of acute cellular rejection?

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