Interpretation of Renal Transplant Biopsy

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The International Society of Nephrology

Vision

The International Society of Nephrology (ISN) aspires towards the elimination of kidney disease worldwide.

Mission

The International Society of Nephrology (ISN) promotes the global advancement of nephrology.

Values

The International Society of Nephrology (ISN):

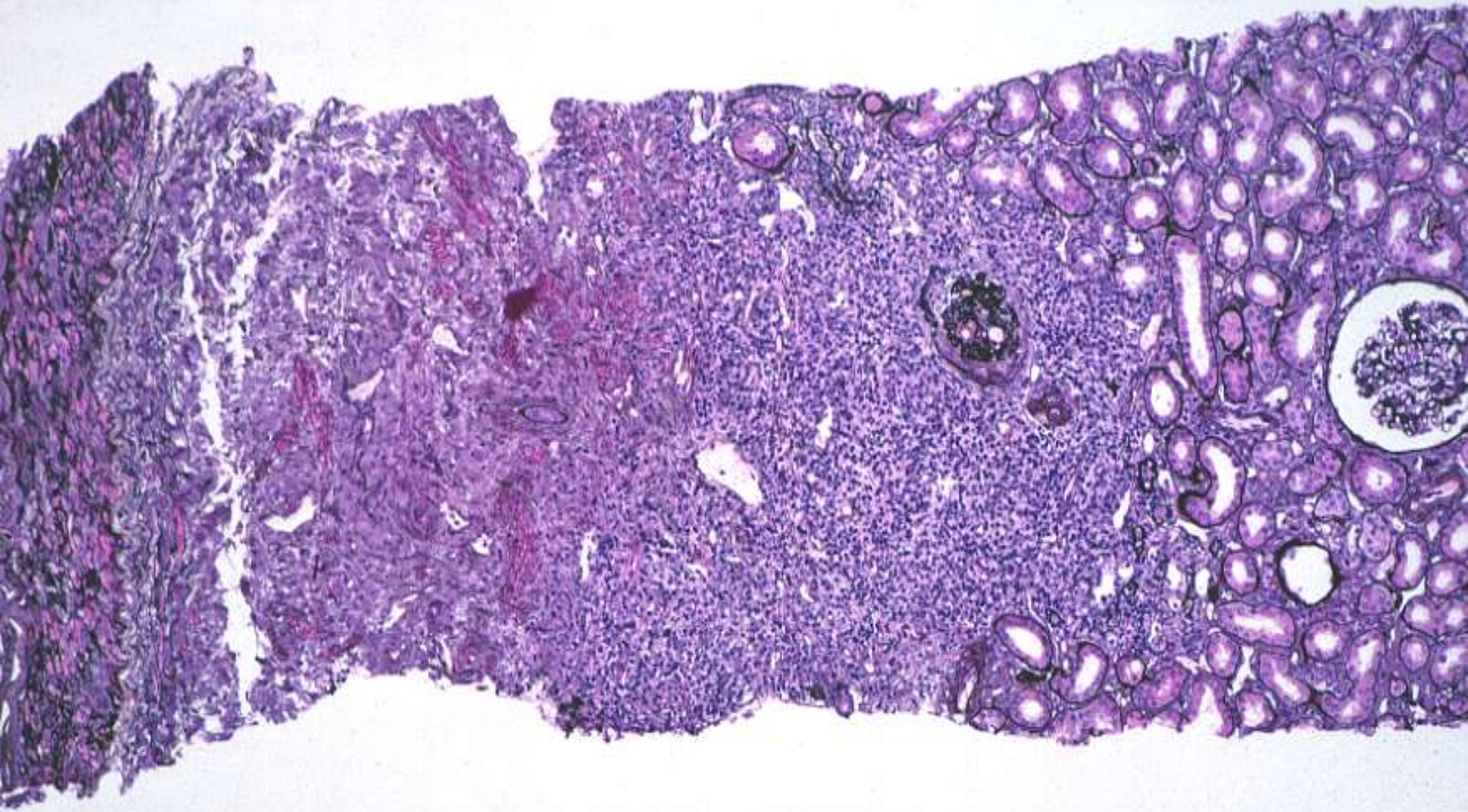
- Values new knowledge and is committed to excellence in research, education, and patient care in the developed and developing world.
- Will always act in a way that is politically neutral, humanitarian, and culturally diverse.
- Values collegiality and interaction and promotes diversity in membership, governance, committee structure, and programmatic activities.
- Is committed to the scientific basis of nephrology and to advancing knowledge across all geographic, national, racial, religious and economic conditions.
- Values the needs of nephrologists and their patients and is dedicated to achieving optimal standards of care worldwide.

Renal Transplant Biopsies Tissue Processing

- Ideal world – process as needed
- Realistic setting – as much as possible
  - LM, IF or Immunoperoxidase C4d (may be limited)
  - Try to collect tissue for EM and process if indicated

Renal transplant biopsy adequacy

- Cortex – **mandatory**
- At least 7 glomeruli
- At least 2 arterial cross sections
- Not exclusively subcapsular
- Two cores are better than one
  - Greater sensitivity



Approach to renal transplant biopsy interpretation

- Evaluation of all tissue components
  - Glomeruli
  - Interstitium
  - Tubules
  - Arteries and arterioles
- Determination if changes are acute and/or chronic

Structure	Acute	Chronic
Interstitium	Edema	Fibrosis
Tubules	T cell infiltrate Epithelial cell injury	Atrophy
Arteries	Inflammation Fibrin/necrosis	Fibrosis Inflammation
Glomeruli	T cell infiltrate	Capillary wall wrinkling Double contours

Acute lesions in transplant

- Acute rejection
- Acute drug toxicity
- Viral and other infections
- Acute ischemia
- Recurrent diseases (some)
- Vascular thromboses
- Posttransplant lymphoproliferative disorder
- Others

Assessment of transplant biopsy acute lesions

- Interstitium
  - Edema, infiltrate, extent
- Arteries
  - Inflammation, necrosis, thrombosis
- Tubules
  - Inflammation
  - Changes of injury
  - Viral changes
- Glomeruli
  - Cellular infiltration
  - Other

Chronic lesions in the graft

- Chronic rejection
- Chronic drug toxicity
- Chronic changes of indeterminate etiology
- Nephrosclerosis
- De novo, recurrent disease
- Obstruction
- Others

Assessment of transplant biopsy chronic lesions

- Interstitium
  - Fibrosis, inflammation
- Tubules
  - Atrophy, inflammation
- Glomeruli
  - Sclerosis, double contours
- Arteries
  - Fibrosis, inflammation
- Arterioles
  - Insudative lesions/hyalinosis

Transplant rejection

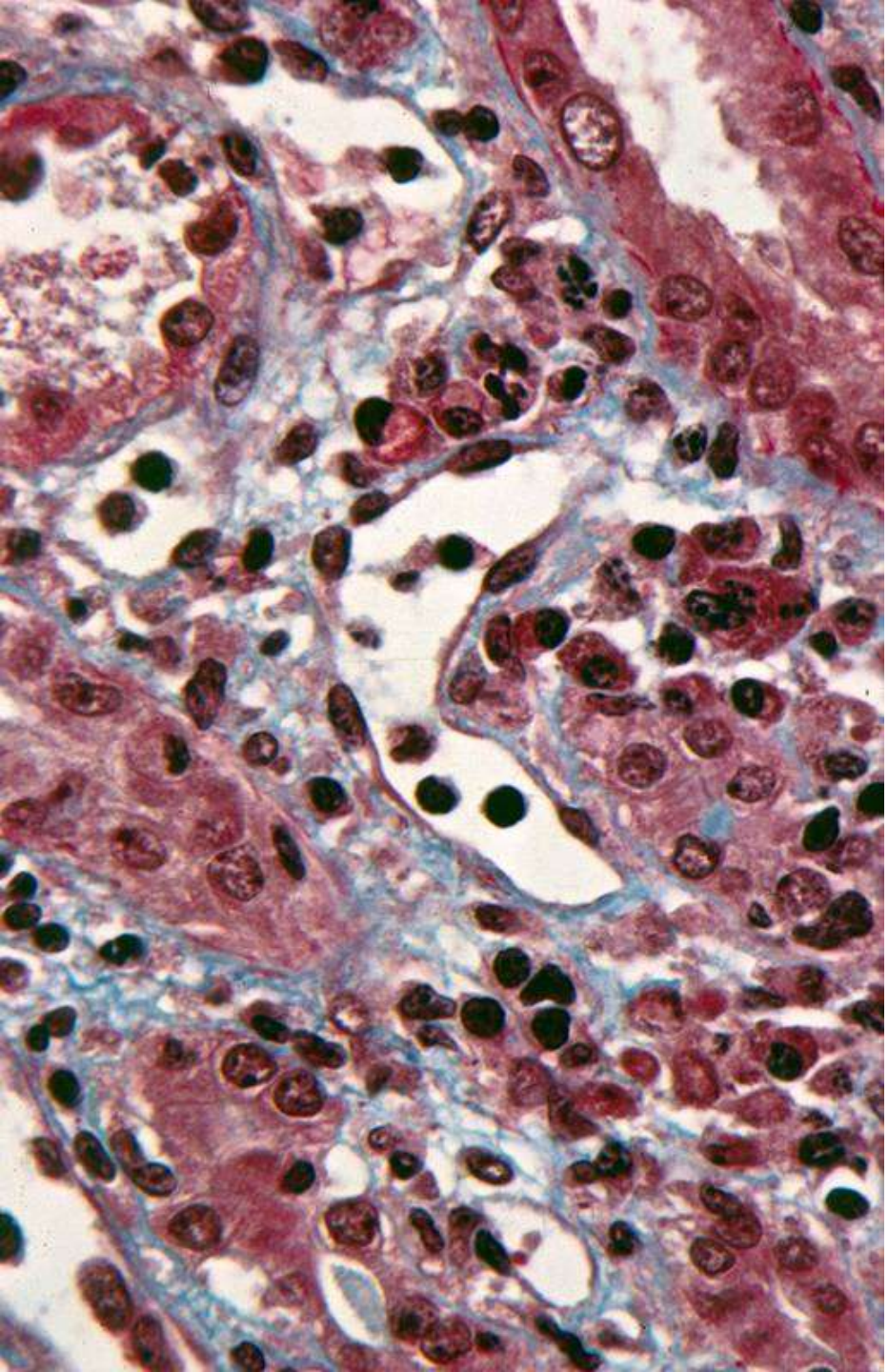
- Cell mediated
  - Various structures involved, including vessels
- Antibody mediated
  - Vascular
    - Different sizes, manifestations

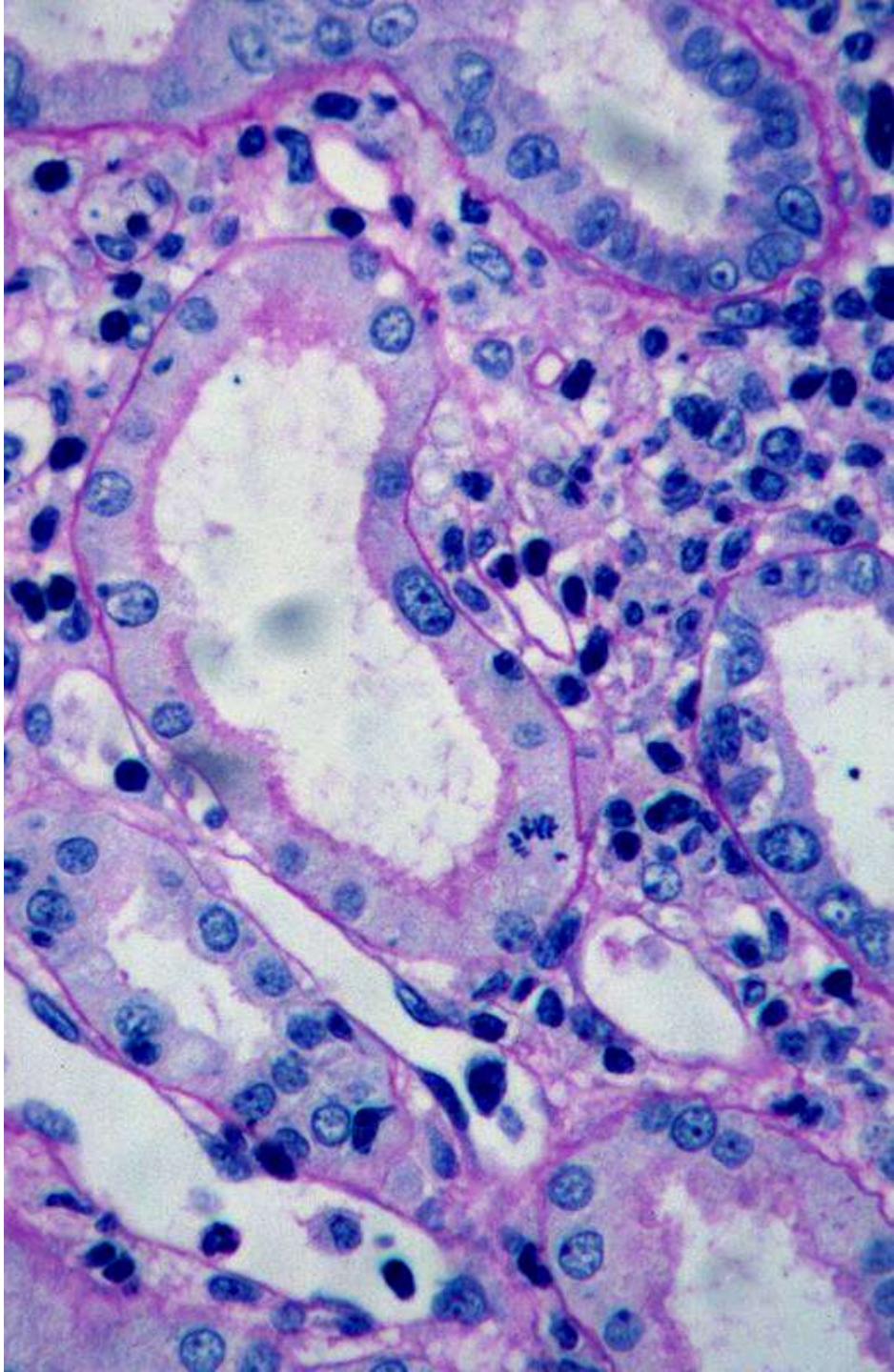
Acute cellular rejection

- Activated T lymphocyte and monocyte infiltrate
  - In edematous interstitium and tubules
  - Arterial intimas
  - Glomeruli
- Injury to target cells
  - Tubular cell apoptosis
  - Interstitial edema
  - Endothelial cell swelling and loss

Acute cellular rejection

- Tubulo-interstitial rejection (*Type I*)
  - T lymphocytes in peritubular capillaries, interstitium (edematous) and in tubules (tubulitis)
  - At least 5% of interstitium is involved
- Arterial rejection (endarteritis) (*TypeII*)
  - T cells *beneath* endothelium of arteries or arterioles
  - T cells *adherent* to surface of endothelium are suggestive but **not diagnostic**



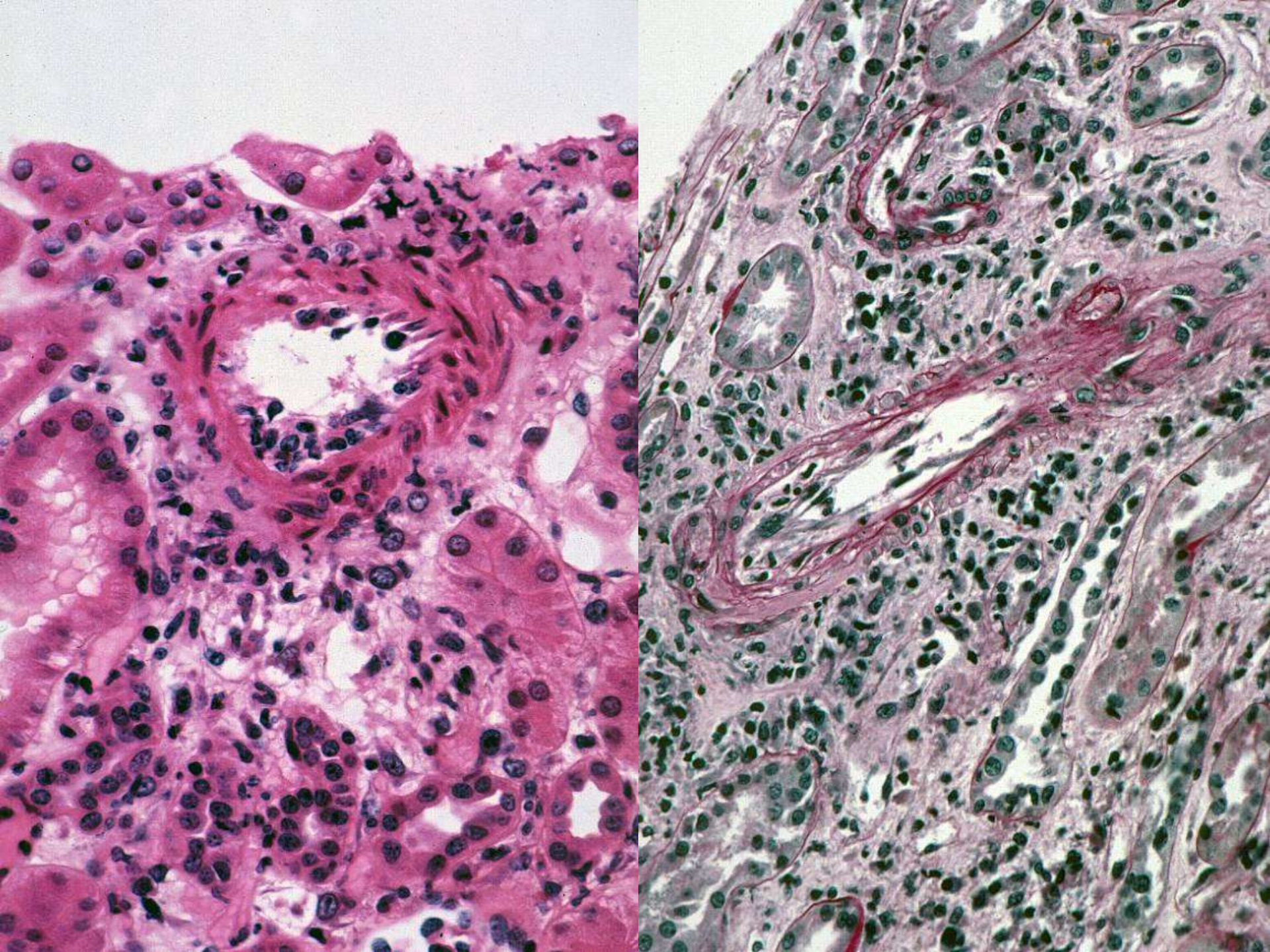


Acute cell mediated rejection Arterial

- Infiltration beneath endothelium of arteries and arterioles by T cells and macrophages
  - ***Endarteritis*** (best term)
  - Endothelialitis
  - Endothelitis
  - Endovasculitis
  - Intimal arteritis

Endarteritis

- Can occur with minimal or no interstitial or tubular infiltrate
  - Distinct pathogenic mechanism
- Can occur with or without glomerular infiltrate
- Reported in 10-60% of biopsies with cellular rejection
- Affects larger arteries preferentially, although all sizes can be affected; arterioles to arcuate and interlobar arteries



Antibody mediated rejection types

- Capillary
  - Peritubular – requires identification of C4d
  - Glomerular
- Arterial

Identification of tissue-bound C4d

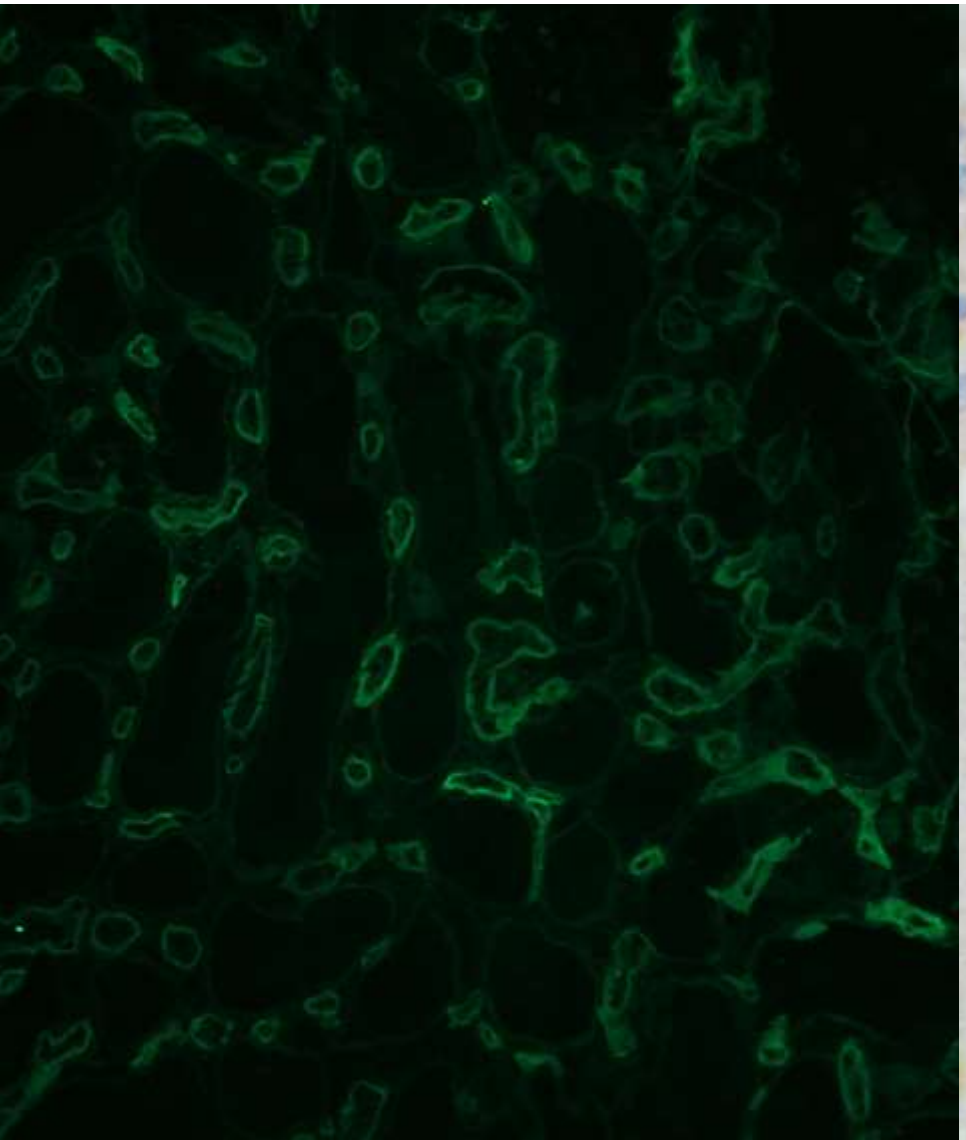
- Immunofluorescence – frozen sections
  - "False" negatives 5-10%
- Immunoperoxidase – fixed, paraffin embedded
  - "False" negatives 10-20%

Specimen adequacy
C4d evaluation

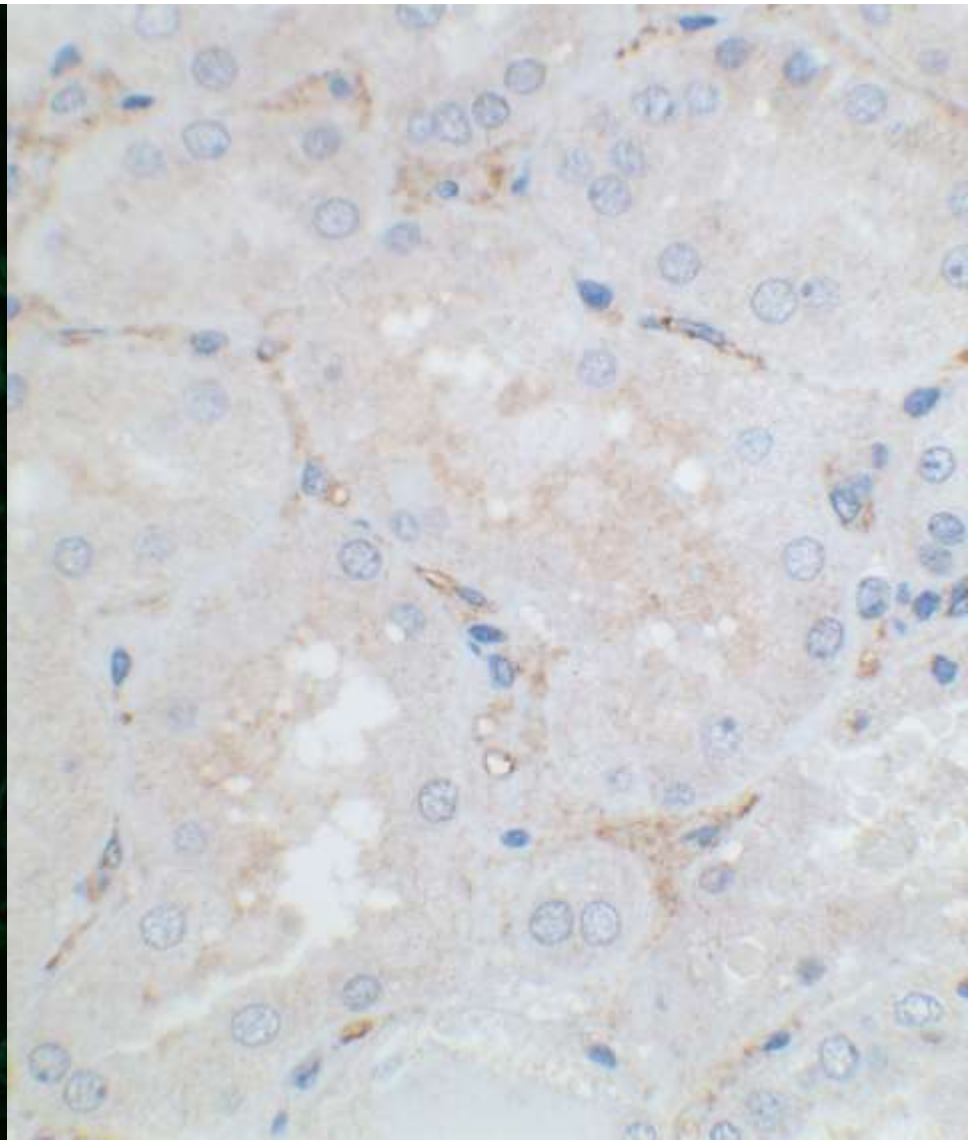
- Viable – cortex or medulla
  - Necrotic or scarred tissue is negative, even if C4d positive elsewhere
- Control
  - Frozen sections – glomeruli (mesangium mainly)
  - Paraffin embedded – no internal control
    - Related to fixation

C4d

frozen section IF

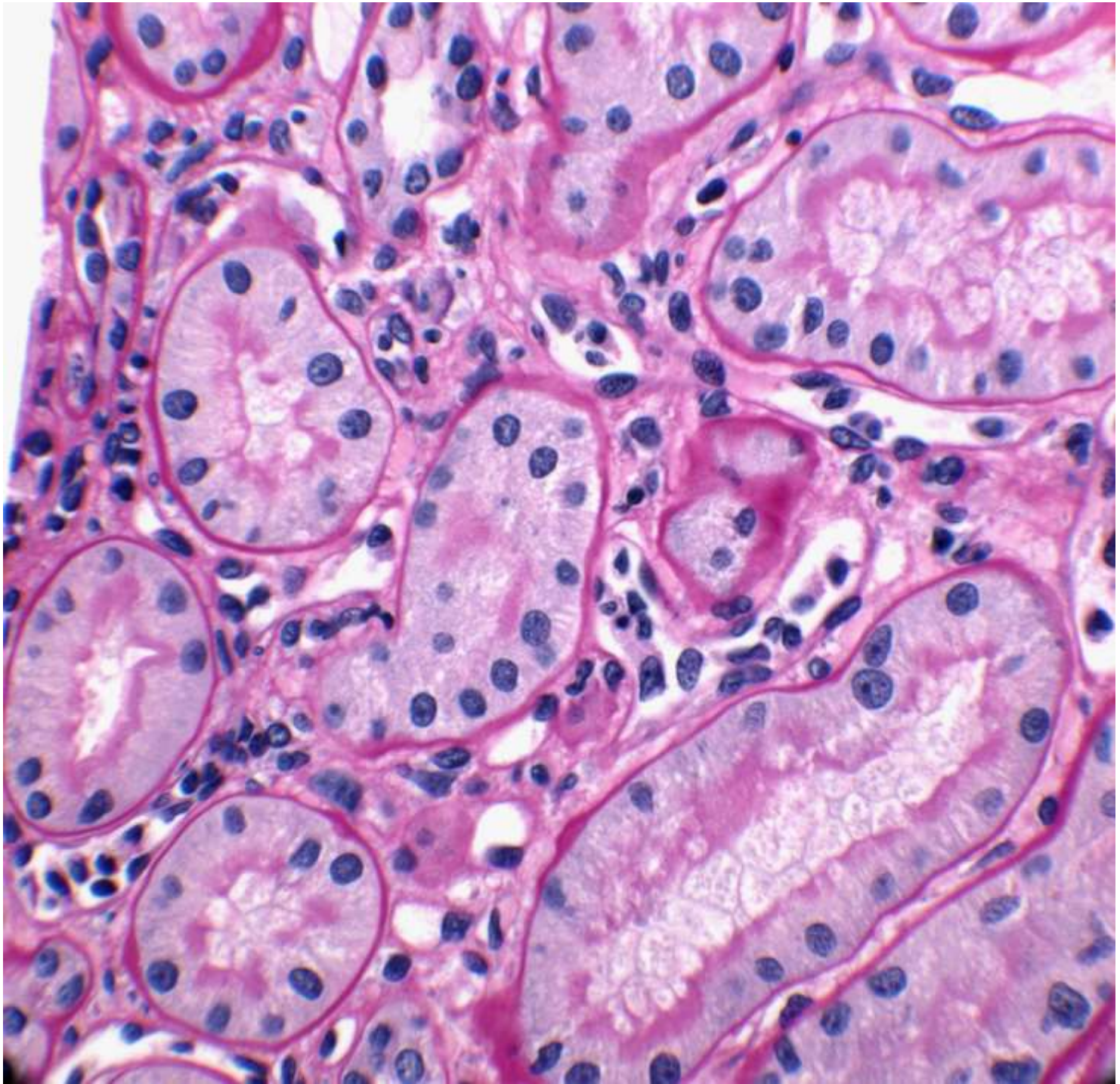


paraffin embedded IP



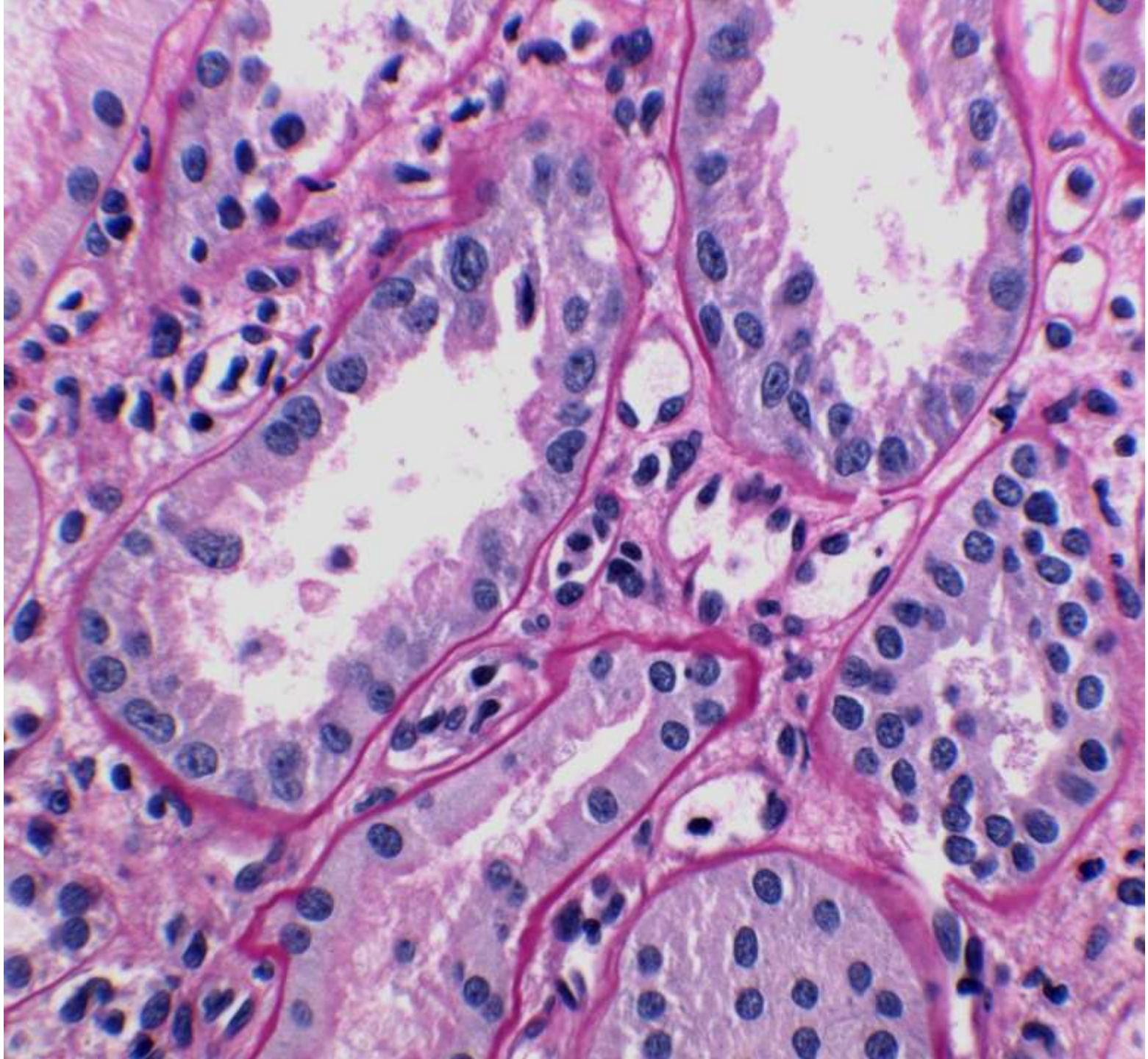
Acute humoral rejection peritubular capillaries - structure

- Leukocytes in lumina
  - Neutrophils
  - Mononuclear leukocytes
  - Platelets and fibrin
- Dilated lumina
- Endothelial cell swelling, detachment from basement membranes, lucent zone sometimes with entrapped erythrocytes
  - Lysis, apoptosis, fragmentation



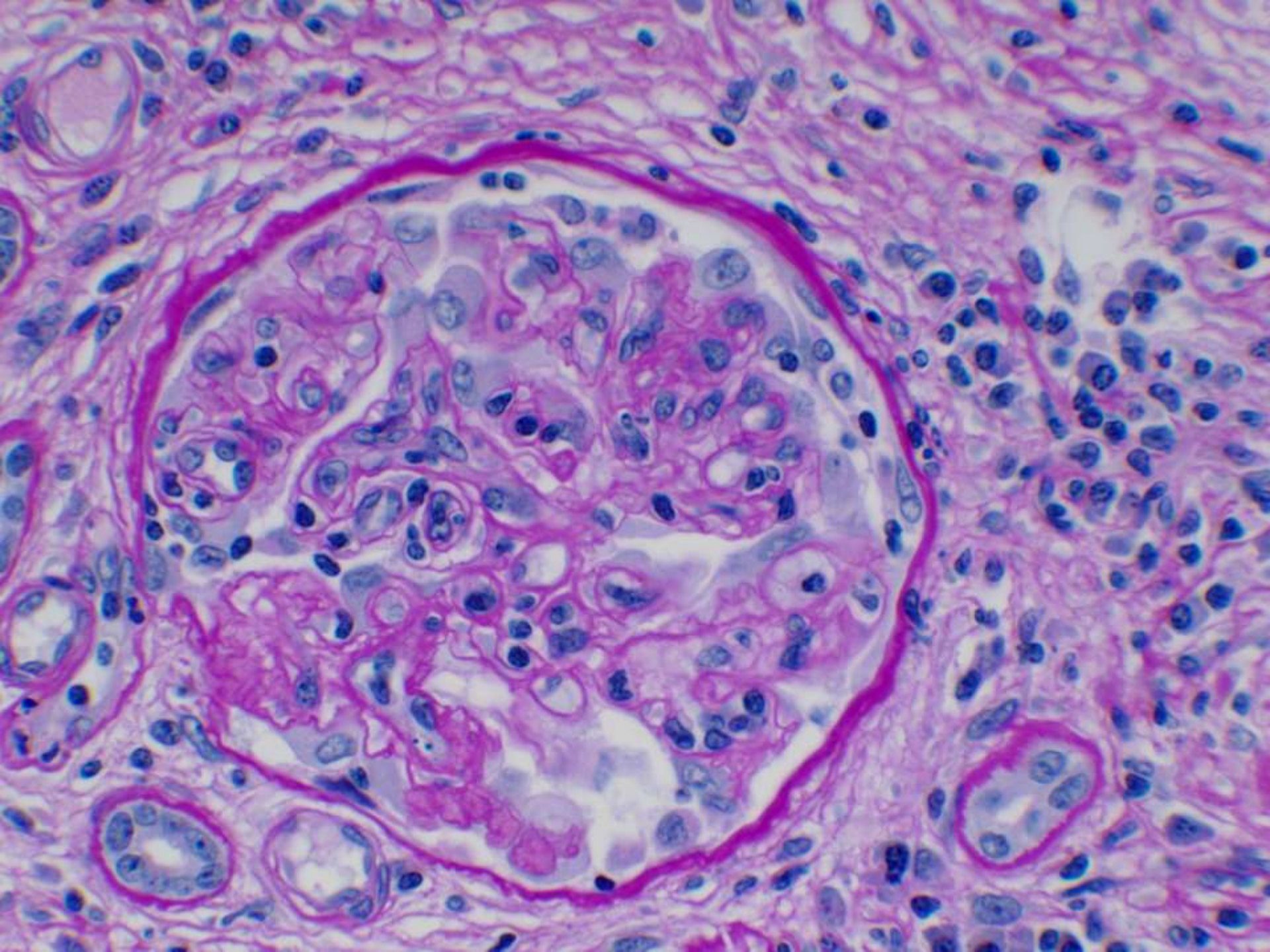
Acute humoral rejection tubules

- Acute tubular injury (ATN)
  - May be only structural manifestation
- Neutrophil infiltration
  - More common than in cell mediated rejection
- Mononuclear leukocyte tubulitis
  - Probably represents concomitant cell mediated rejection



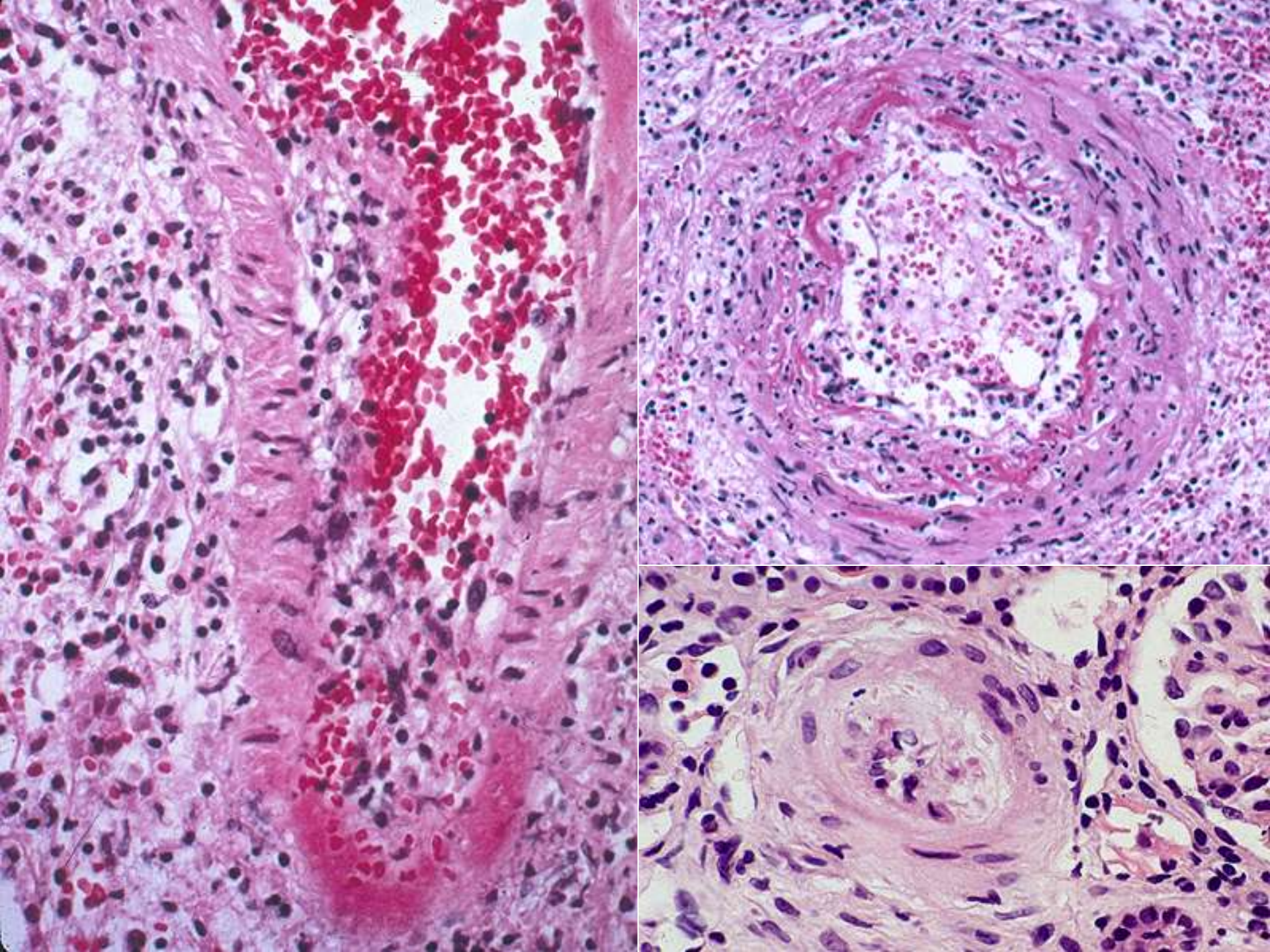
Acute humoral rejection light microscopy-glomeruli

- Capillary leukocytes
  - Neutrophils 10-55% of biopsies
  - Mononuclear leukocytes (monocytes/macrophages) 20-90%
  - Very few eosinophils
  - T cells
- Fibrin in capillary lumina
  - 20% of biopsies



Acute humoral rejection arteries

- Mural necrosis – myocytes, elastica fragmentation
- “Fibrinoid necrosis”
  - Little leukocyte infiltration
- Thrombosis
- Intimal mucoid thickening (similar to TMA)
- Neutrophils and/or eosinophils
- Fibrin, IgG and/IgM, C3, C4d



Criteria for acute antibody rejection

- 1. Immunopathologic evidence for antibody action
  - C4d (rarely Ig) in peritubular capillaries
  - Ig, C in arteries with “fibrinoid necrosis”
- 2. Morphologic evidence for acute tissue injury
  - Acute tubular injury
  - Inflammation in peritubular and/glomerular capillaries
  - Arterial necrosis/inflammation
- 3. Circulating anti-donor antibodies
- If 2 of 3, then *suspicious for acute humoral rejection*

C4d cautions!

- ABO incompatible transplants
  - C4d positive (almost always) – does not indicate rejection
- C4d negative antibody mediated rejection is possible – that is, negative C4d does not preclude antibody rejection

Chronic changes in the graft

- Chronic rejection
- Chronic calcineurin inhibitor toxicity
- Nephrosclerosis
- Recurrent diseases
- De novo diseases
- Renal artery stenosis
- Obstruction

Chronic allograft nephropathy

First introduced in Banff 1993 to indicate chronic changes in graft which cannot be assigned an etiology based on morphology: these included chronic rejection, chronic calcineurin toxicity, nephrosclerosis, chronic infection/reflux.

These entities can be frequently distinguished from one another; *chronic allograft nephropathy should no longer be used when that is the case.*

Chronic rejection

- Ongoing immunologic injury
  - T cell mediated
  - **Antibody mediated**
- Slowly progressive decline in renal function beginning at about 3 months post-transplant
- Reasonably characteristic pathology

Chronic rejection

- Arteries and arterioles
- Tubules and interstitium
- Glomeruli

Chronic rejection glomeruli
Transplant Glomerulopathy

Double contoured capillary walls – segmental or global (hallmark)

Variable mesangial expansion

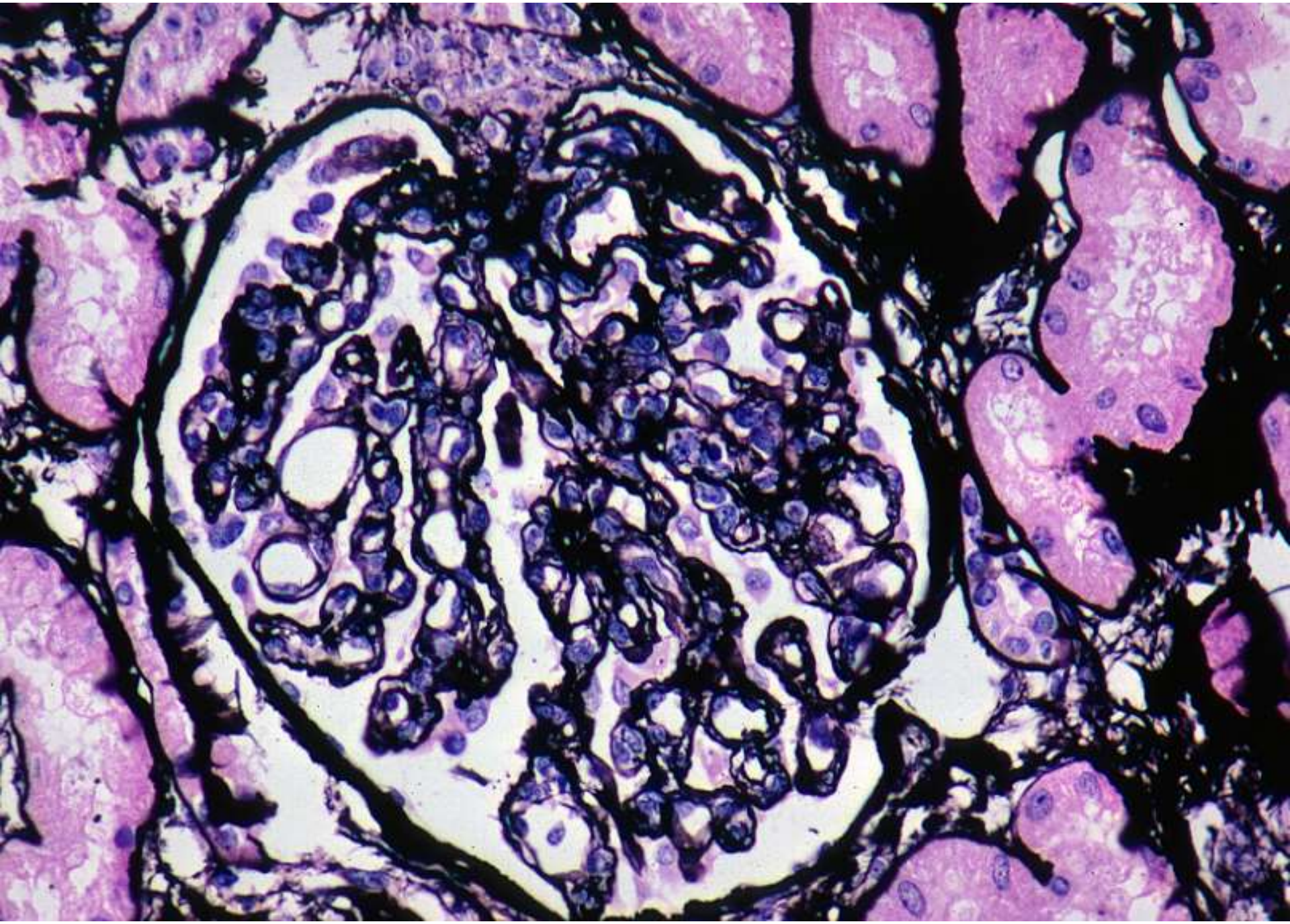
increased mesangial matrix, cells

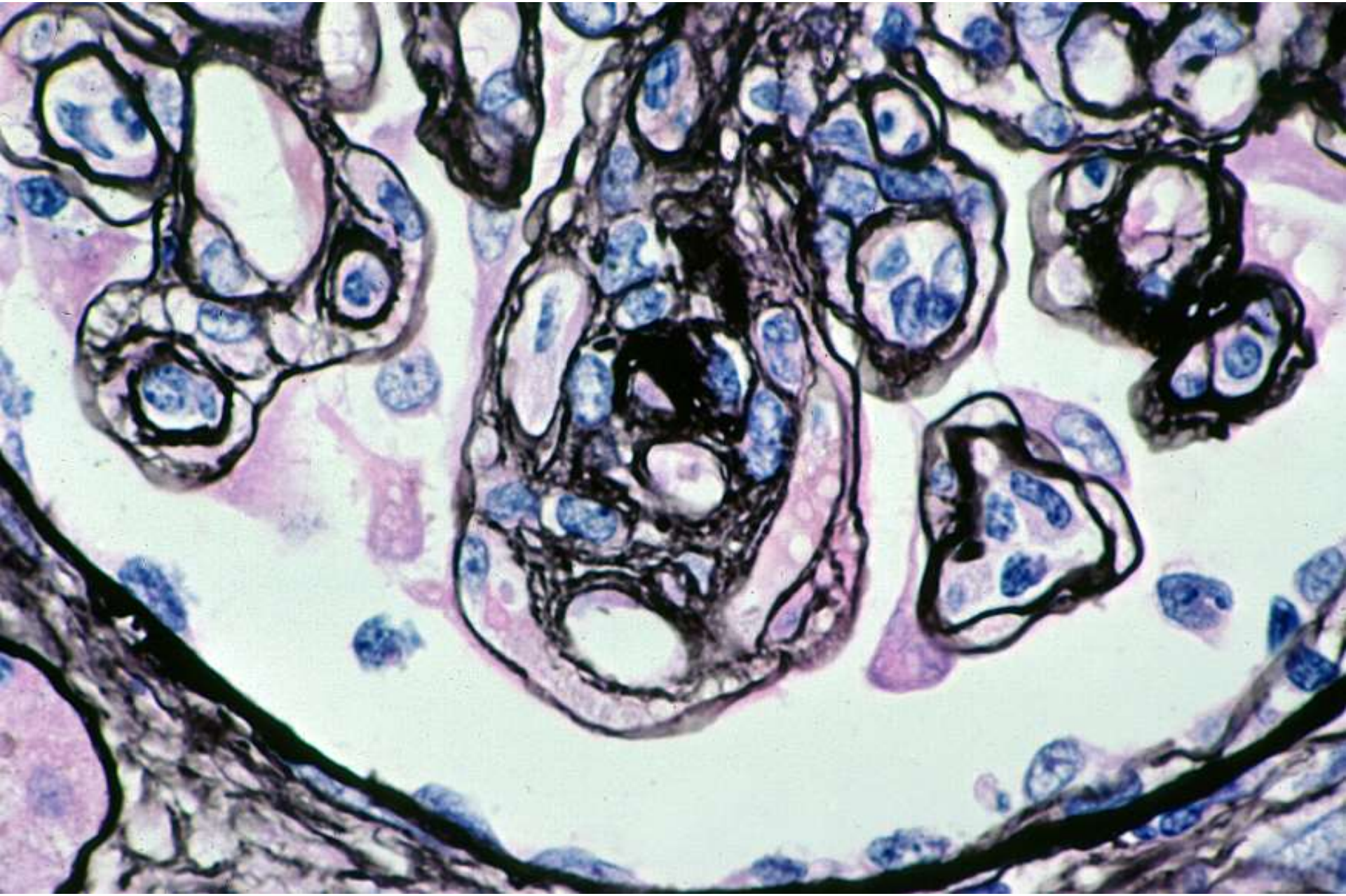
Lobular architecture

Active rejection

mononuclear leukocytes in capillaries

endothelial swelling





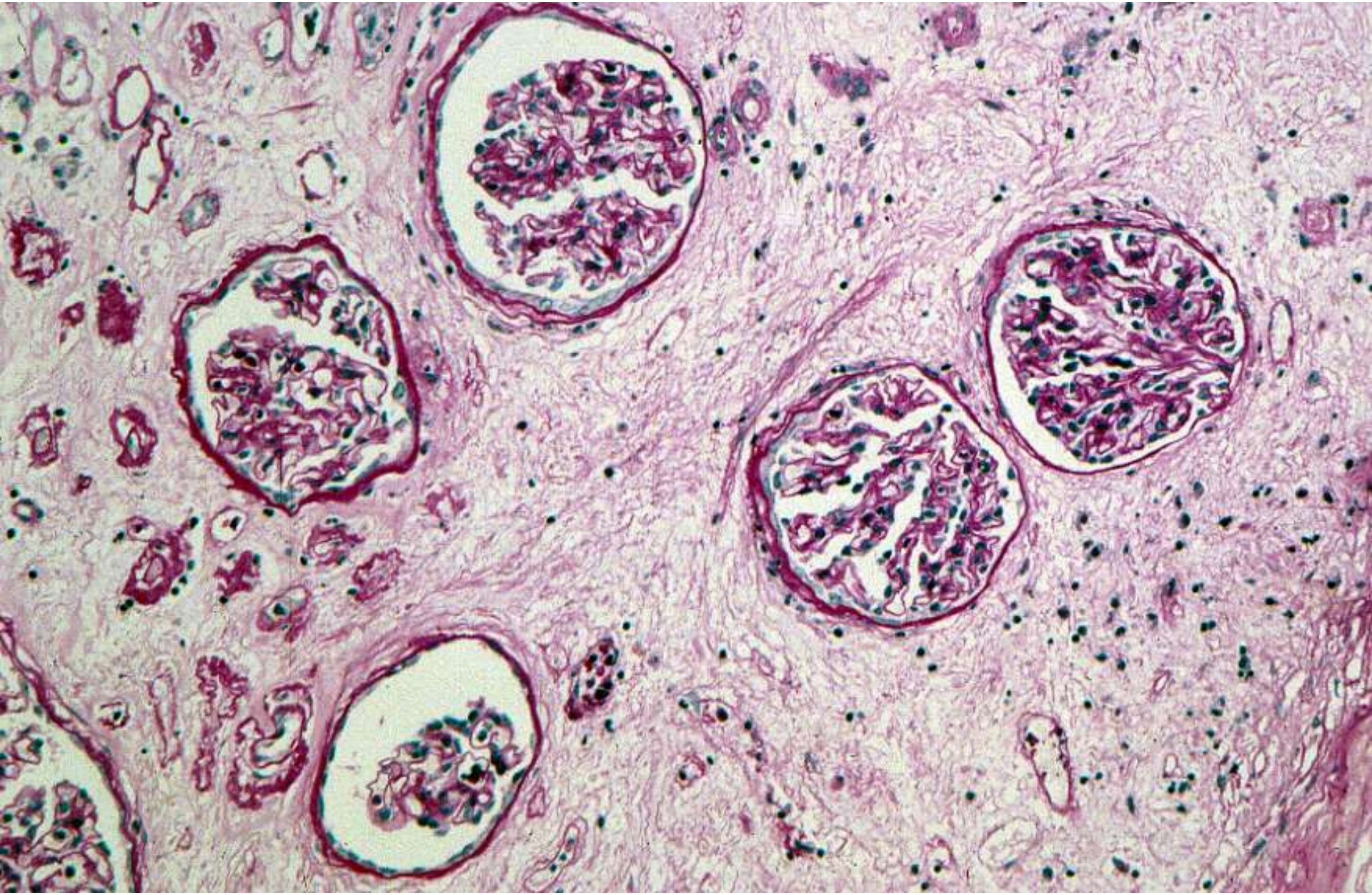
Chronic rejection tubules

Atrophy

- few mononuclear leukocytes

Thick tubular basement membranes

Tubular dropout



Chronic rejection interstitium

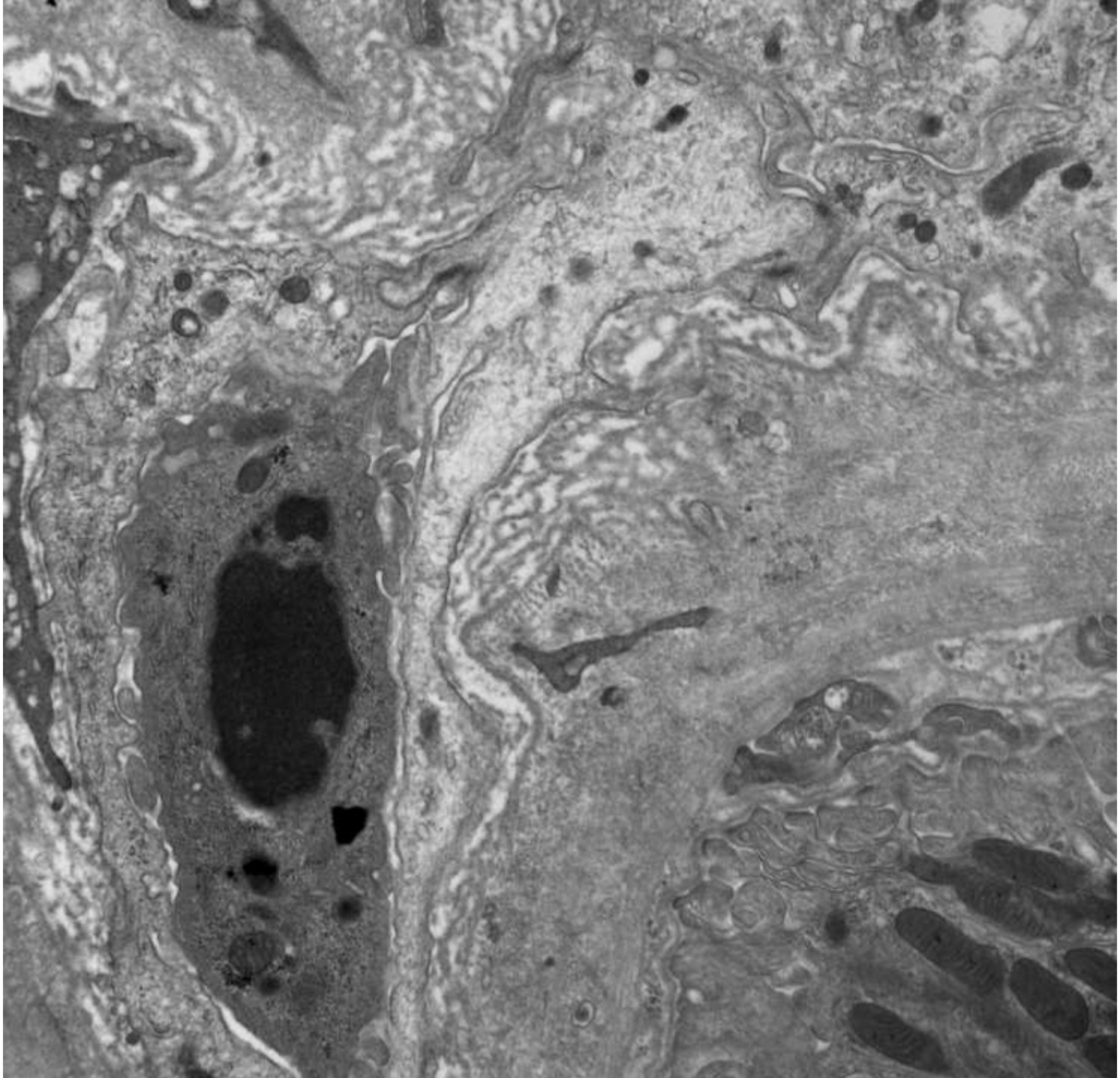
Fibrosis

- many different patterns
- variable leukocyte infiltration – lymphocytes, plasma cells, mast cells
- no cellular activation

Nodular aggregates of lymphocytes, often around vessels, especially at cortico-medullary junction

Chronic rejection peritubular capillaries

- Decreased numbers
- C4d positive in approximately 50% of grafts with glomerulopathy or arteriopathy
  - Usually fewer positive than in acute humoral rejection
- Multilayered basement membranes (EM)
  - Represents repetitive endothelial injury
  - Correlation with transplant glomerulopathy,
  - C4d deposits, loss of peritubular capillaries



Chronic rejection arteries – transplant arteriopathy

Intimal proliferation – may begin as early as one month post-transplant

Most prominent in larger arteries, but affects all sizes

Intimal fibrous thickening, often concentric

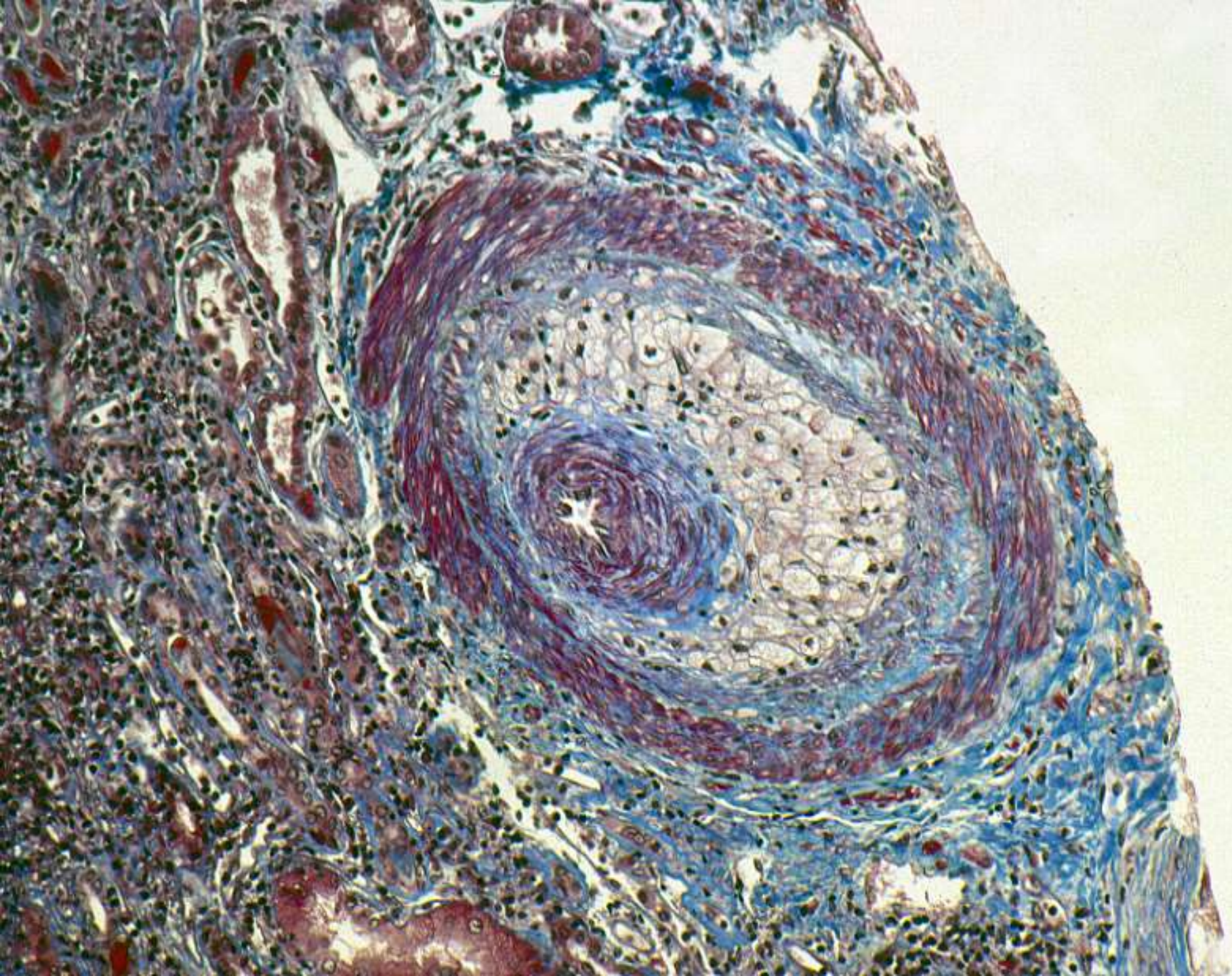
- intact internal elastic lamina
- no medial involvement

Loose matrix, with myofibroblasts

“Double media”, with concentric neo-media including smooth muscle cells and elastic lamina beneath endothelium

T cells and macrophages – sign of continuing activity

Foam cells may be prominent



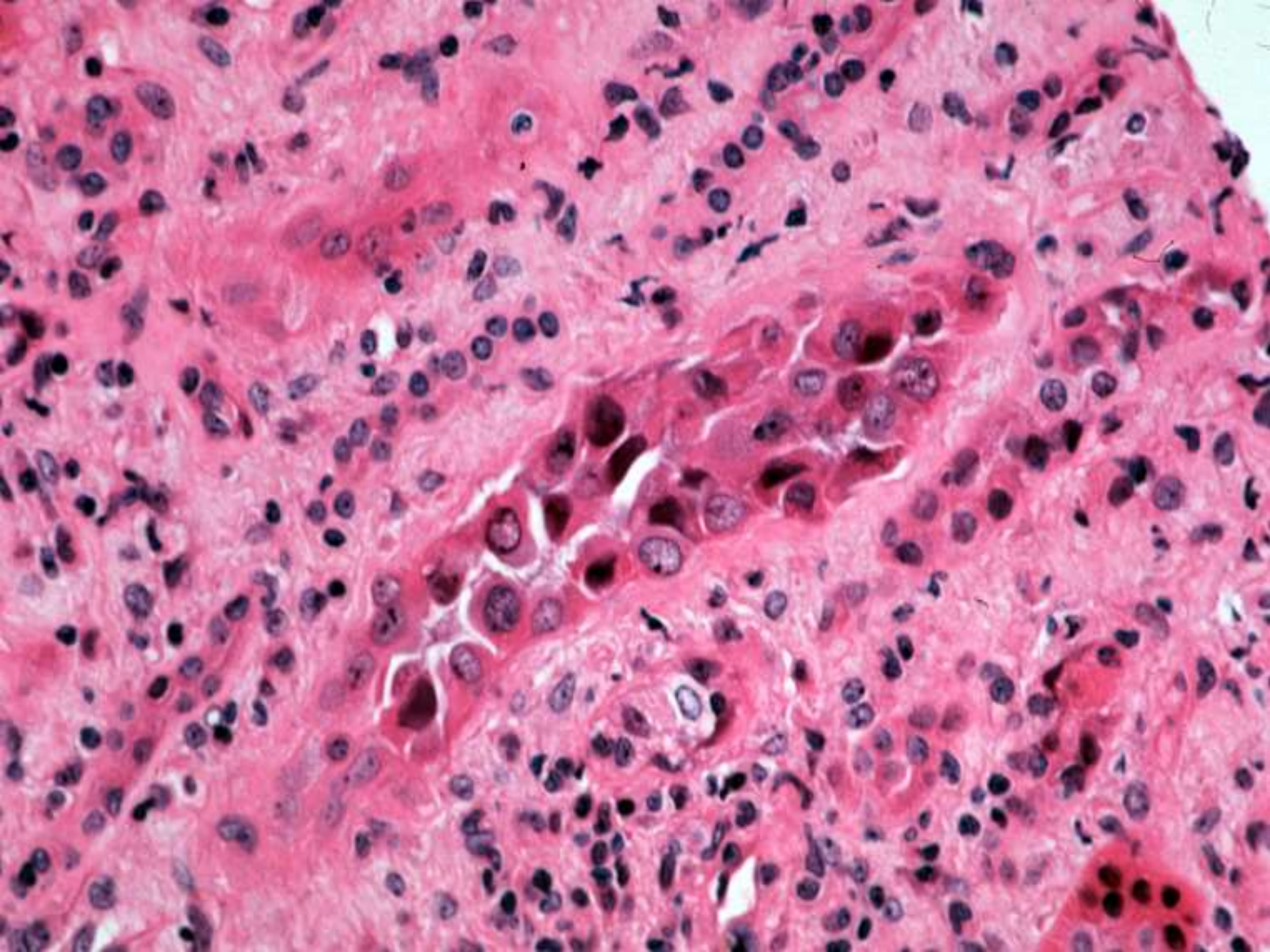
Infections

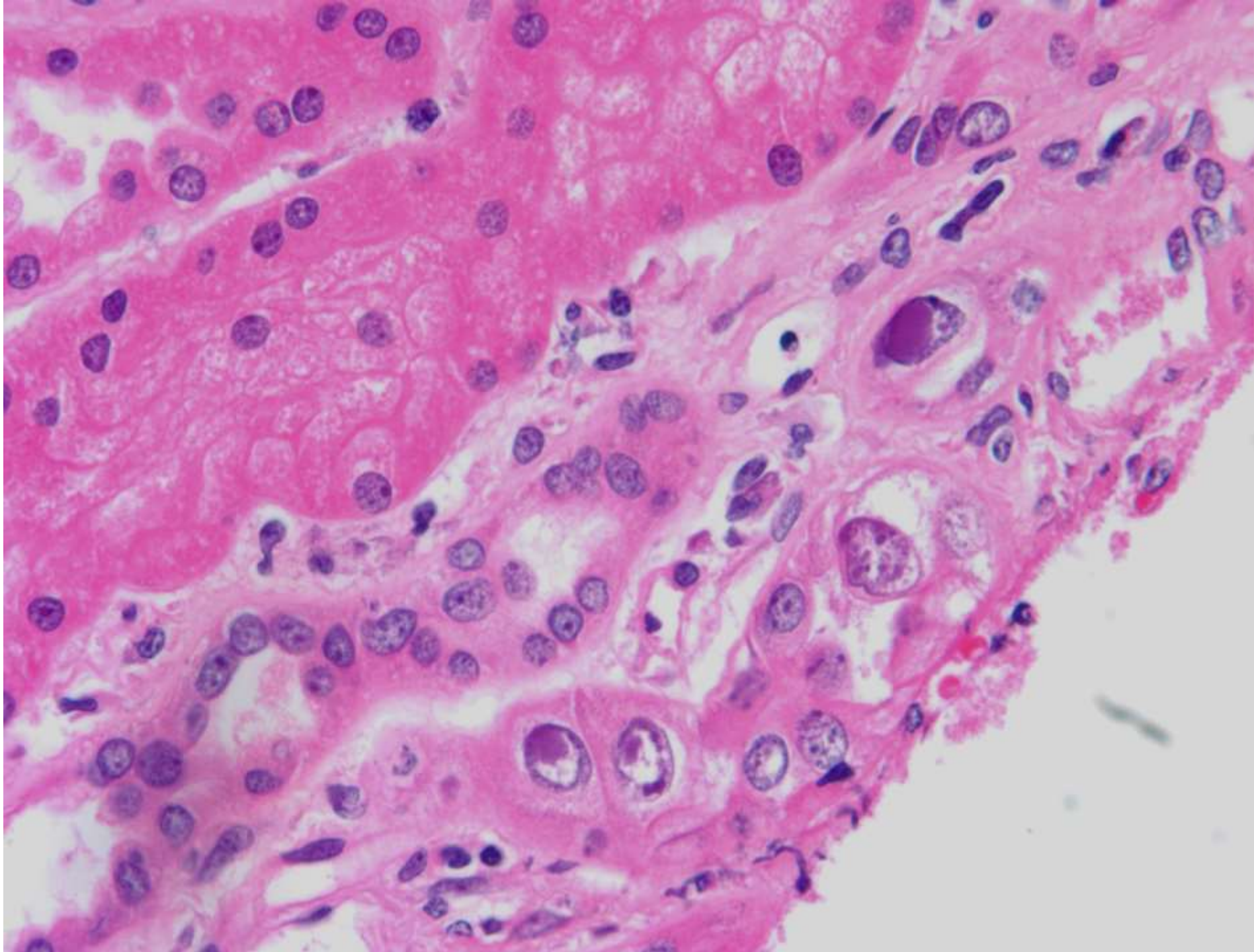
- MAJOR
  - **Polyomavirus**
  - Cytomegalovirus (CMV)
  - Adenovirus
  - Epstein-Barr virus (EBV)

  - Parvovirus
- Minor
  - Fungal
  - Bacterial (pyelonephritis)

Polyomavirus nephropathy

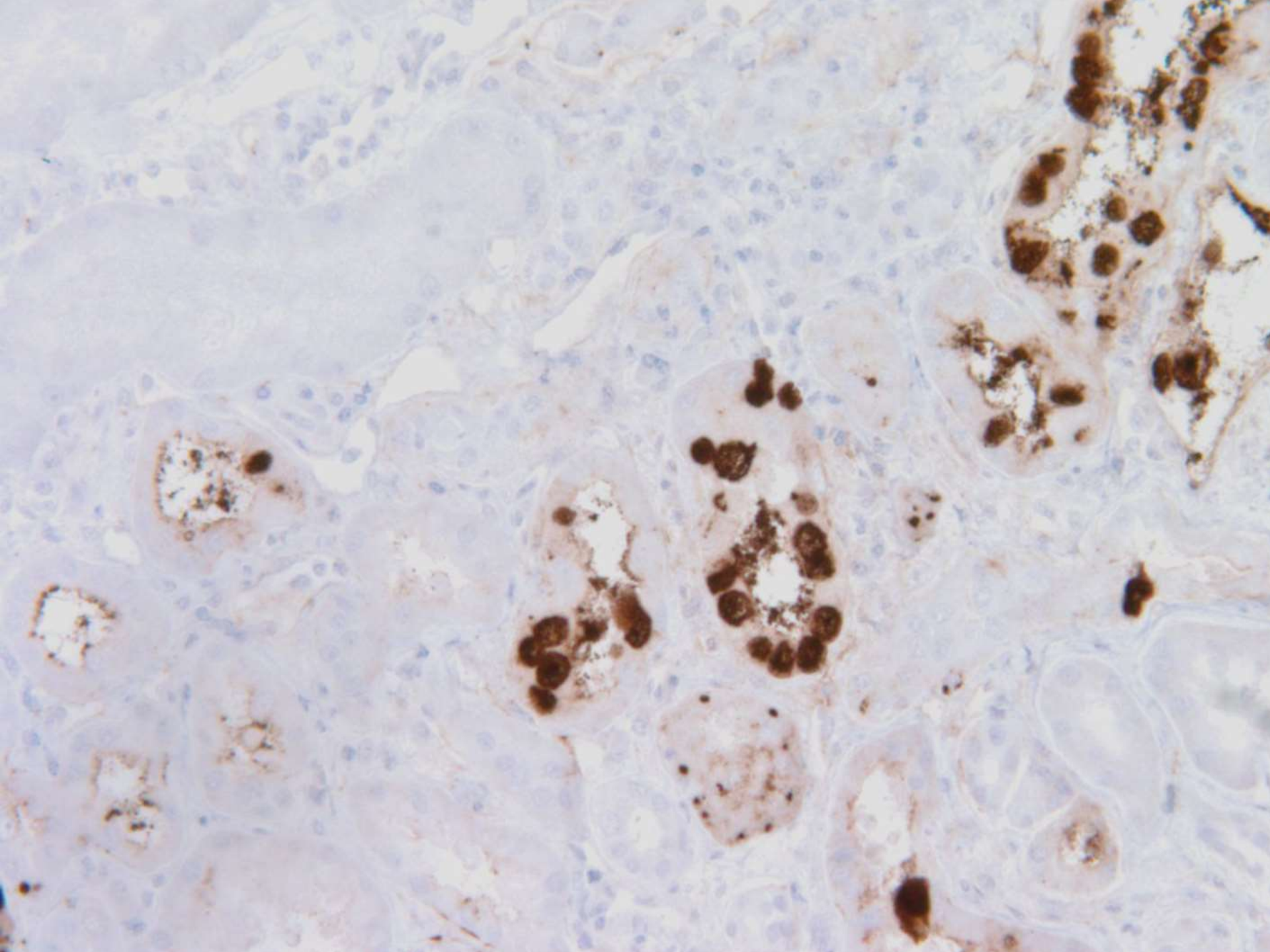
- Tubular cell involvement
  - Medulla
  - Deep cortex
  - Rest of cortex
- Tubular nuclear inclusions
- Tubular cellular damage
- Interstitial inflammation, fibrosis
- Little glomerular involvement





Immunofluorescence immunohistochemistry

- Commercially antibodies to SV40 T antigen (IHC)
  - Crossreacts with BK, JC and SV40 antigens which are in polyomaviruses which are pathogeneic in humans
  - Positive in early stages of viral replication, even before inclusions are present
- Granular deposits of IgG and C3 along tubular basement membranes (IF)
  - Immune complex deposits
  - EM dense deposits

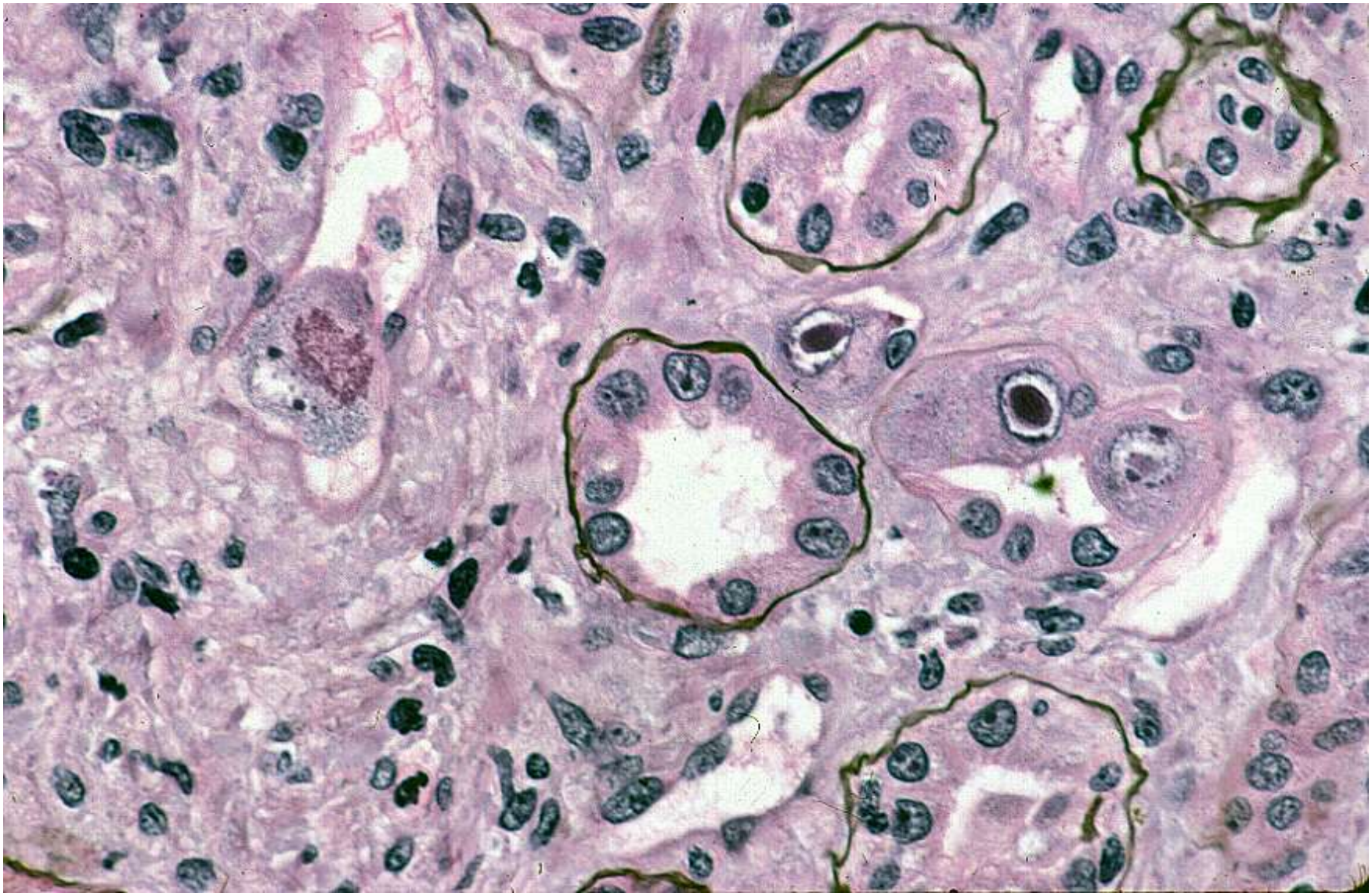


CMV - Pathology

- Enlarged cells with nuclei containing central round inclusion surrounded by a clear zone
- Small cytoplasmic inclusions
- Affects tubular cells predominantly; also endothelial cells (glomeruli, peritubular capillaries), occasionally mononuclear leukocytes
- Focal interstitial inflammation inconstant

Cytomegalovirus (CMV)

nuclear and cytoplasmic inclusions

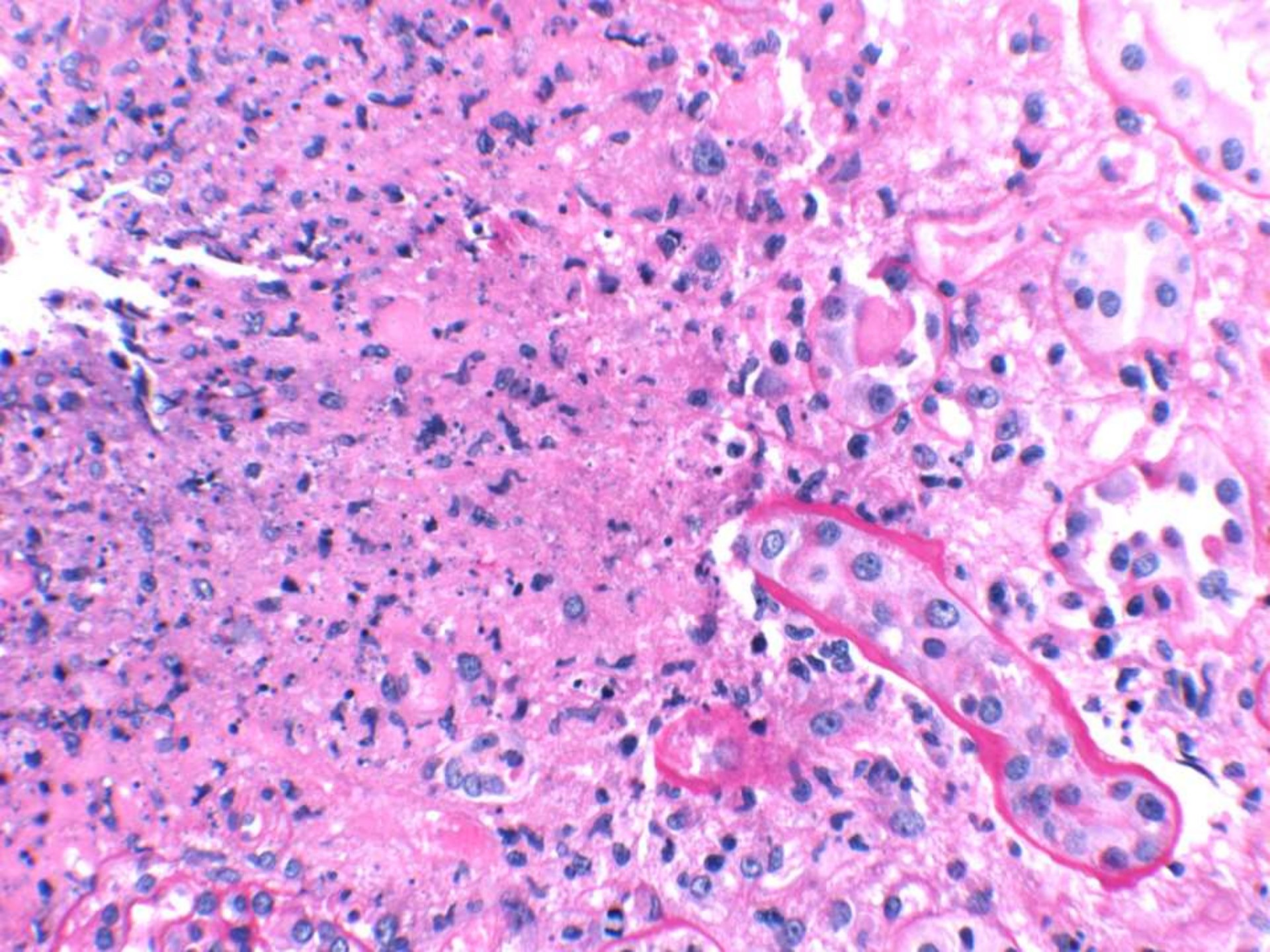


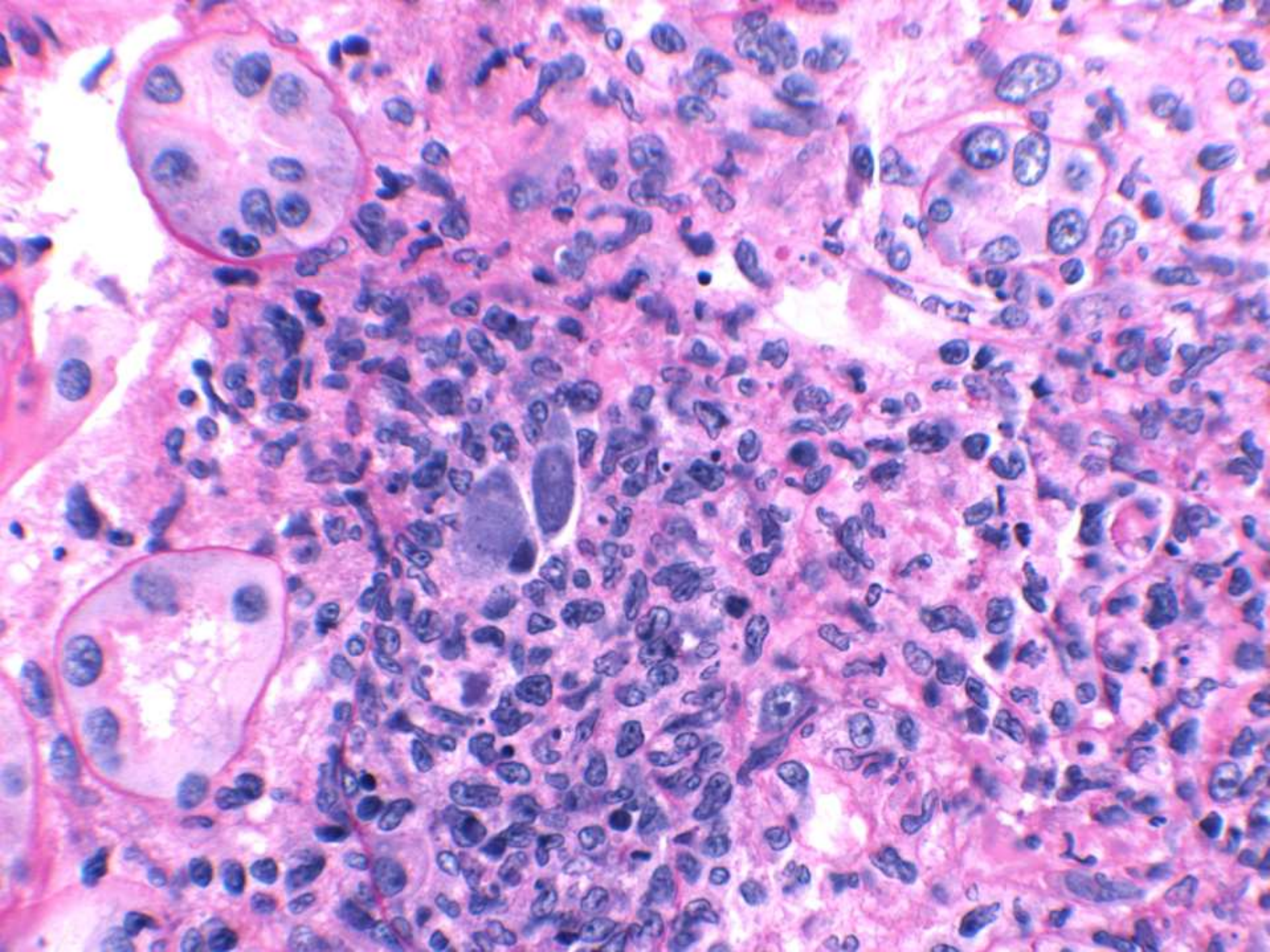
Adenovirus

- Very rare; incidence may be increasing
- Good response to therapy
- Good prognosis

Pathology

- Focal necrotizing tubulointerstitial nephritis
- Tubular cell intranuclear inclusions
  - Ground glass type
- Tubular cell destruction/ necrosis with neutrophils
  - Interstitial mononuclear and plasma cell infiltrate; nodular, granulomatous
  - Interstitial hemorrhage
- EM virions 75-80 nm in nuclei and cytoplasm





Calcineurin inhibitor nephrotoxicity pathology acute and chronic

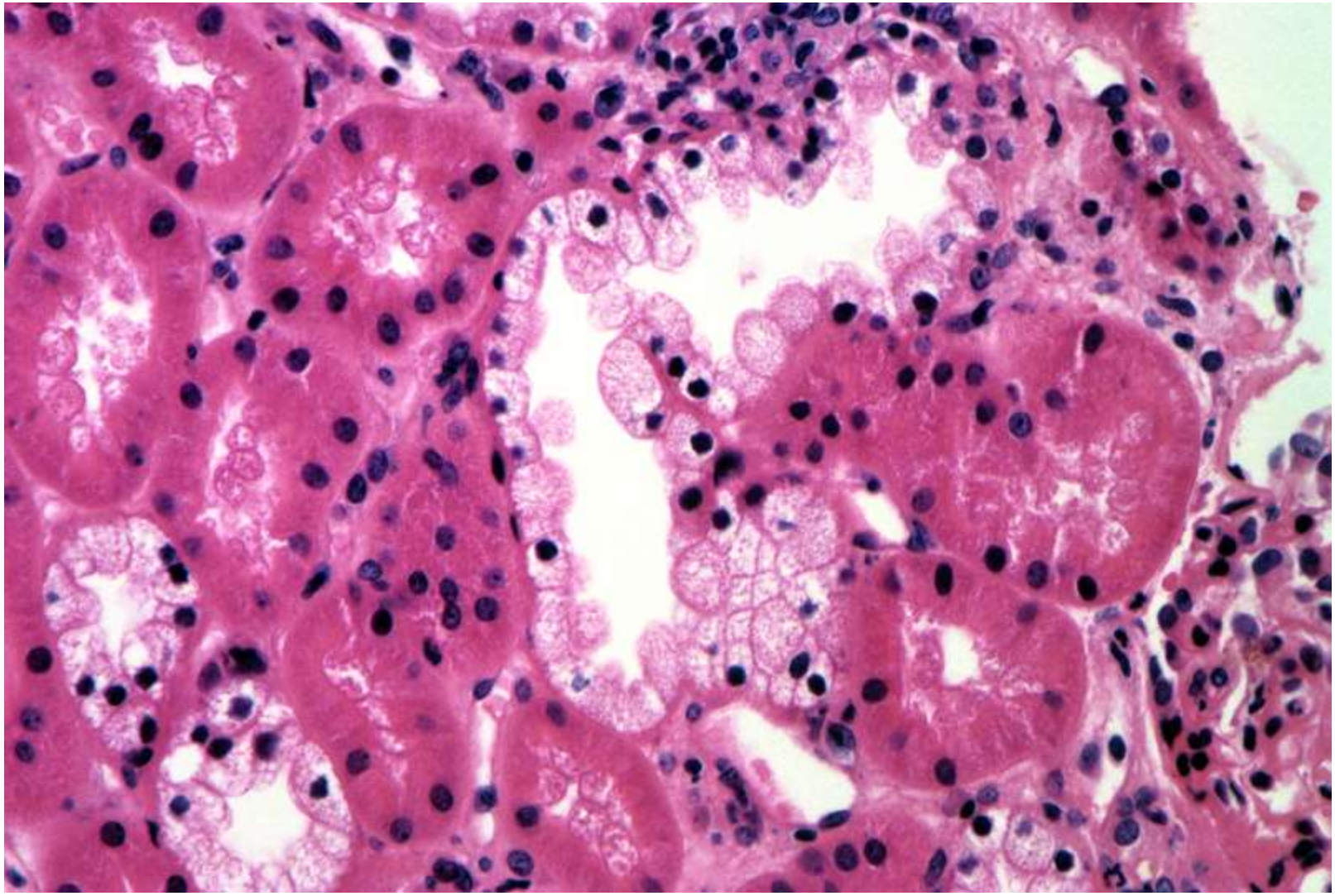
- Tubular lesions
- Interstitial abnormalities
- Arteriolopathy
- Glomerulopathy

Calcineurin inhibitor tubular lesions

- Vacuolization of cells
  - Isometric vacuoles
- Acute tubular injury/necrosis
- No changes

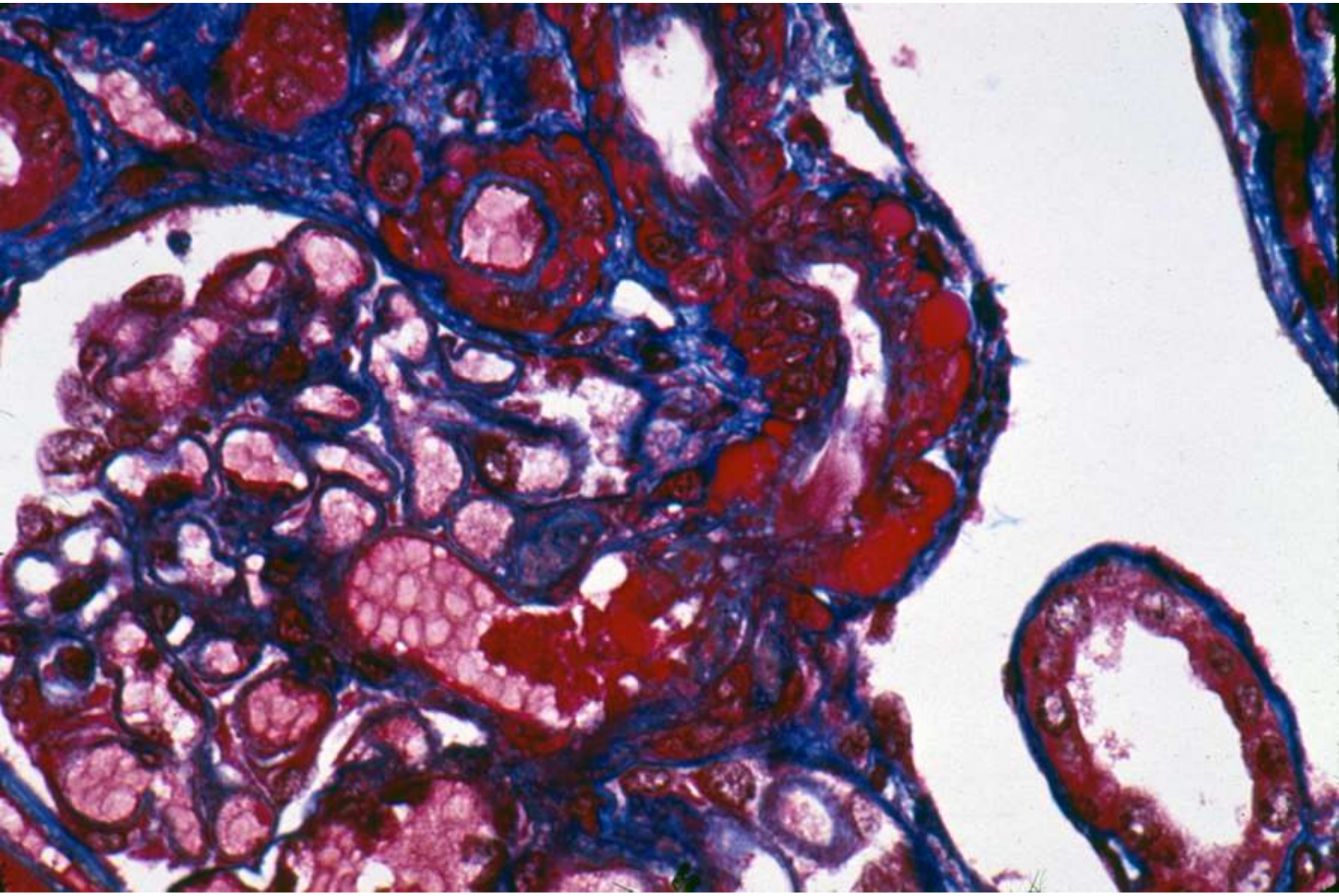
Differential diagnoses tubular changes

- Acute tubular injury (necrosis)
  - Any cause; no distinctive changes
- Isometric vacuoles
  - Lipid
  - Osmotic nephrosis
    - Following IVIG, for example
  - Major distinction by *light microscopy* is focal nature of CI lesion
  - Major distinction by *electron microscopy* is osmotic lesion results in dilated phagolysosomes; CI dilated smooth endoplasmic reticulum



Arteriolar lesions

- Insudative lesions
  - Nodular, subadventitial
- Hypertrophy
- Juxtaglomerular apparatus enlargement



Differential diagnoses arteriolar hyalinosis

Hypertension

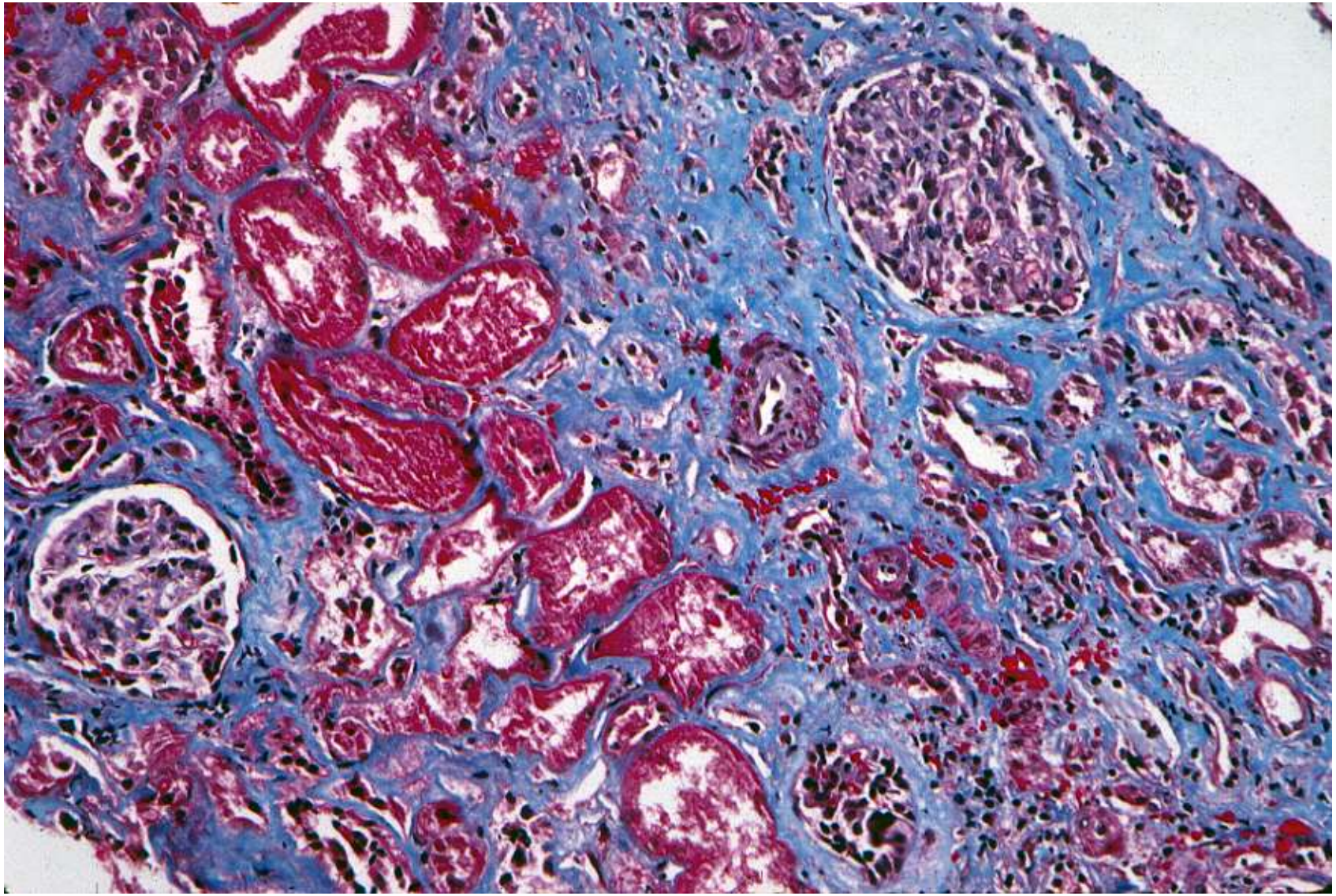
- Insudates are subendothelial over intact medial smooth muscle, sometimes atrophied
- Transmural insudates less common
- Concomitant arterial changes

Diabetes mellitus

- Involvement of both afferent and afferent arterioles
- Glomerular lesions

Interstitial lesions

- Fibrosis
  - Patchy/”striped”
  - Tubular atrophy



Differential diagnosis
tubular atrophy/interstitial fibrosis

- Chronic ischemia
  - Nephrosclerosis
    - Arterial intimal fibrosis
  - Chronic rejection
    - Arterial fibrosis, interstitial WBCs, glomerulopathy
  - Chronic interstitial nephritis
    - Interstitial WBCs

Nephrotoxicity of other therapies

- Antibiotics, etc
  - Acute tubulo-interstitial nephritis
- Many other drugs
  - Acute tubular injury/necrosis
- Anti-CD3 monoclonal antibody (OKT3)
  - Thrombotic microangiopathy/large vessel thrombosis
- Sirolimus/rapamycin
  - Acute tubular injury
  - Casts with morphological features similar to Bence Jones cast nephropathy
- IVIG, with diluent
  - Acute tubular injury with widespread tubular vacuolization

Drug induced interstitial nephritis: distinguish from acute cellular rejection

- **REJECTION**
- Few eosinophils, BUT
- Plasma cells
- Cortex
- No granulomata
- Arterial, glomerular inflammation

- **DRUG INDUCED**
- Eosinophils, BUT..
- Plasma cells
- Deep cortex, medulla
- Poorly formed granulomata
- No arterial, glomerular inflammation