Thrombotic Microangiopathy. The Pathologist's Perspective

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Case Study

Clinical History

- 32-year-old Hispanic male presents with headache and severe HTN (230/120 mmHg)
- Hew was told that he has HTN a year ago but was not followed-up; otherwise no relevant previous medical history
- Patient weighs 81 Kg and is 178 cm tall.
- Heart rate 82/min, No edema
- Family history is unclear

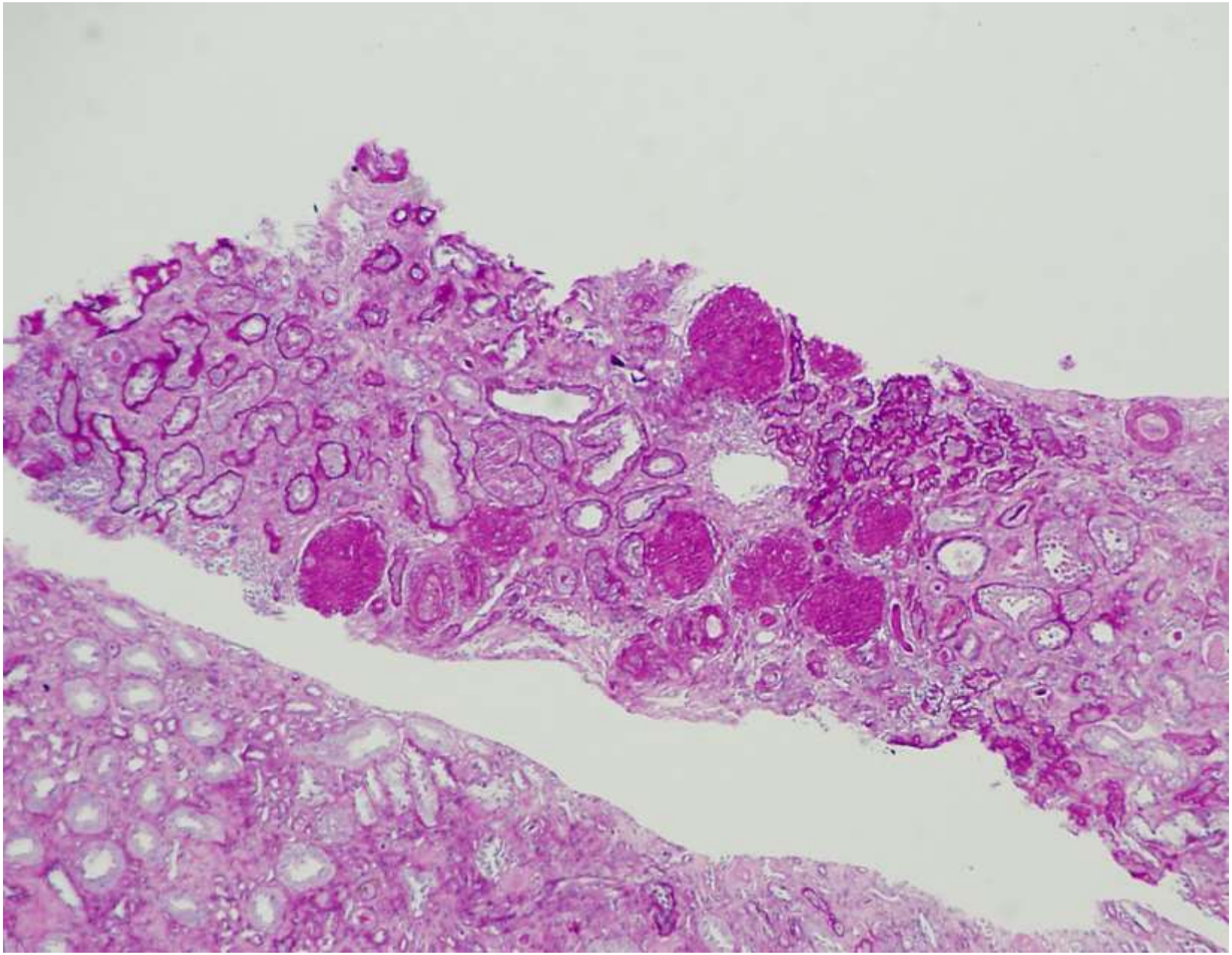
Laboratory Findings

- Serum Cr. 15.7 mg/dl (1387.8 µmol/L)
- LDH 782 U/L
- Platelets 97,000, Hgb: 7.5 g/dL, HCT: 22
- C3: 54 mg/dL (0.54 g/L), C4: 25 mg/dL (0.25 g/L)
- Serum complement factors D, B, C5A, C4d, sMAC (sC5B-9) all elevated
- ADAMTS13 activity 91%
- No obvious schistocytes
- Haptoglobin normal

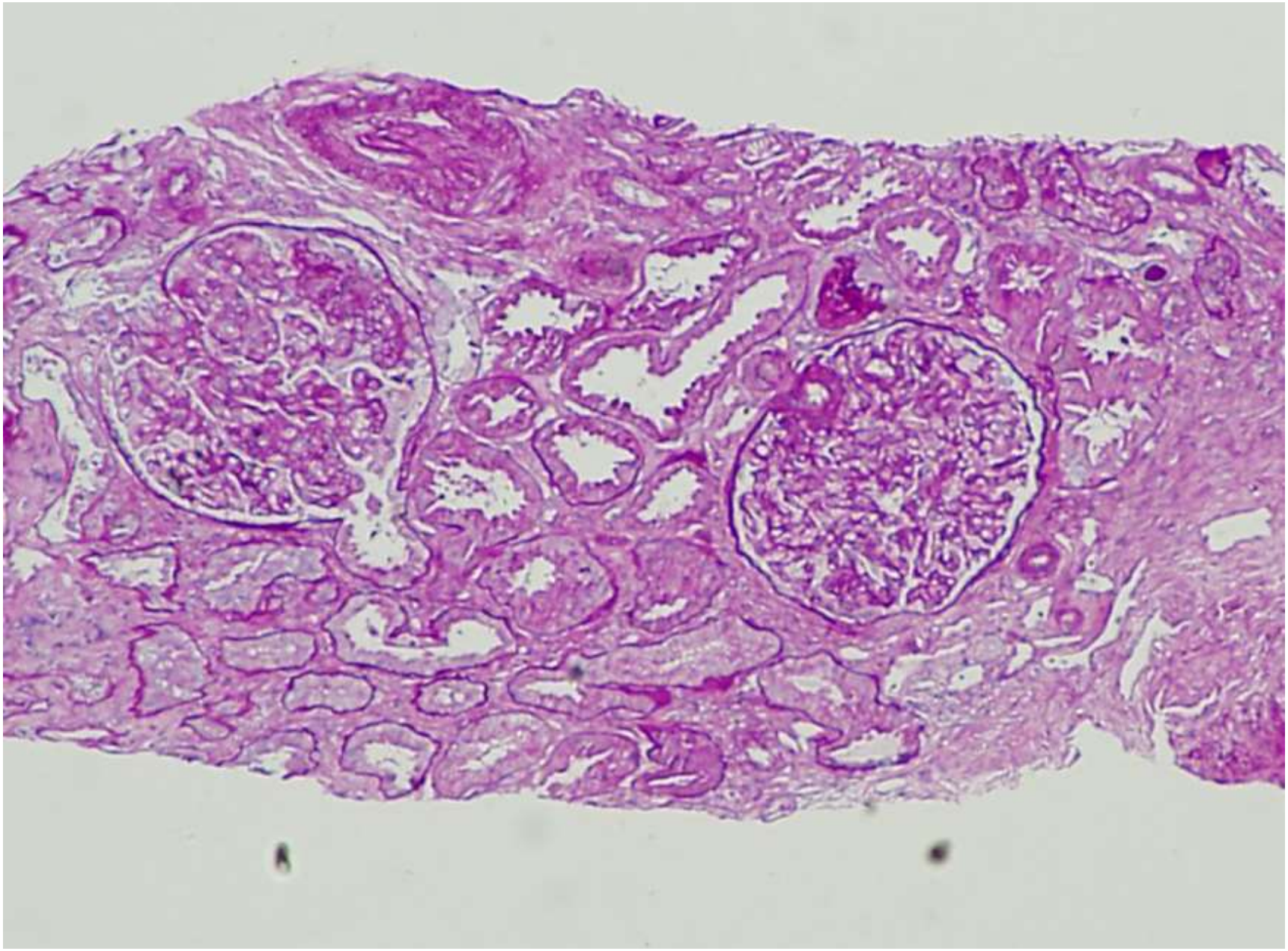
Labortory Findings

- 5.3 g/24h proteinuria
- No monoclonal protein in serum or urine
- Microscopic hematuria with 6-9 RBC/HPF
- Serum albumin 3.5
- WBC: 7,500
- PTH: 195

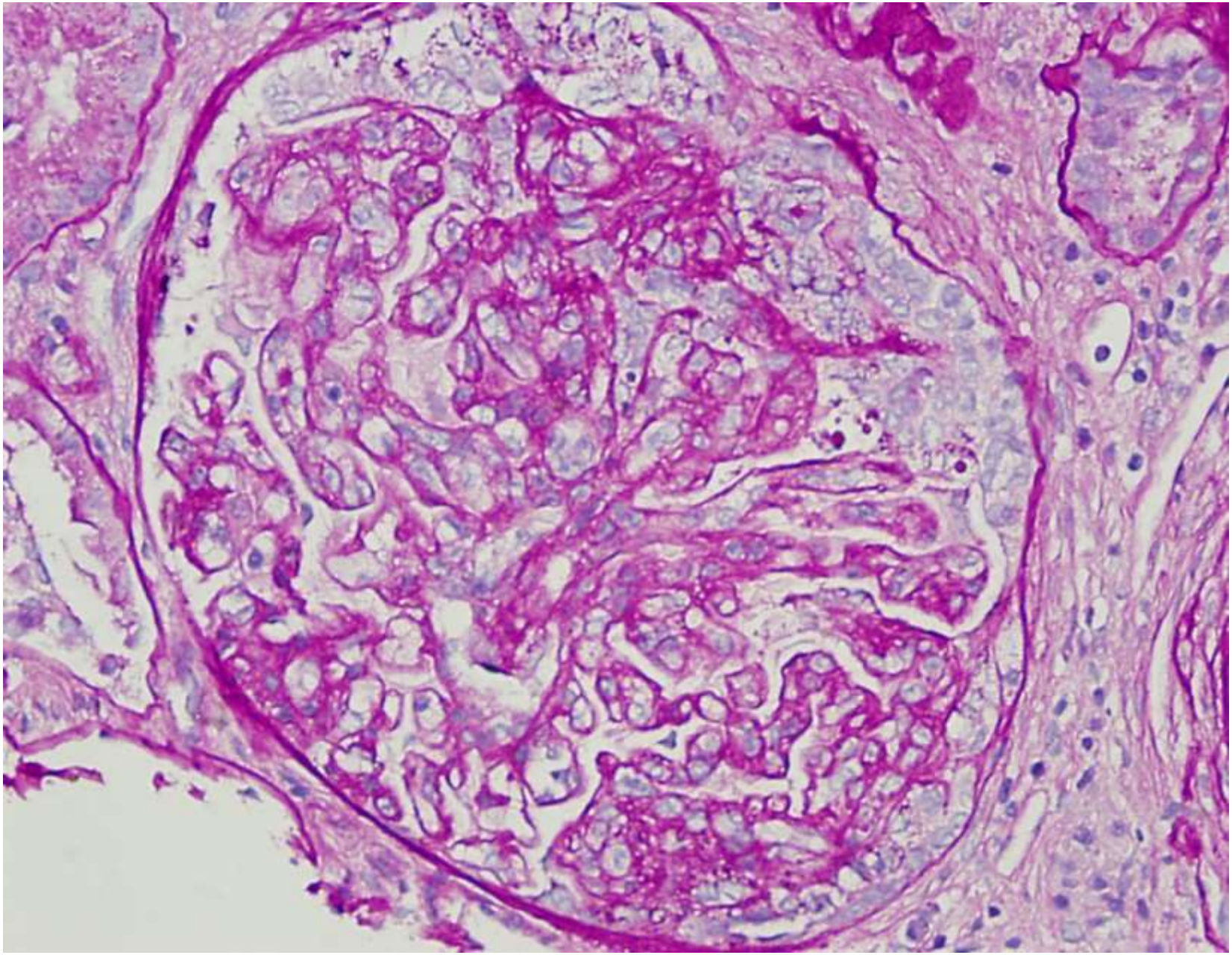
Hemodialysis was stared and a renal biopsy was performed



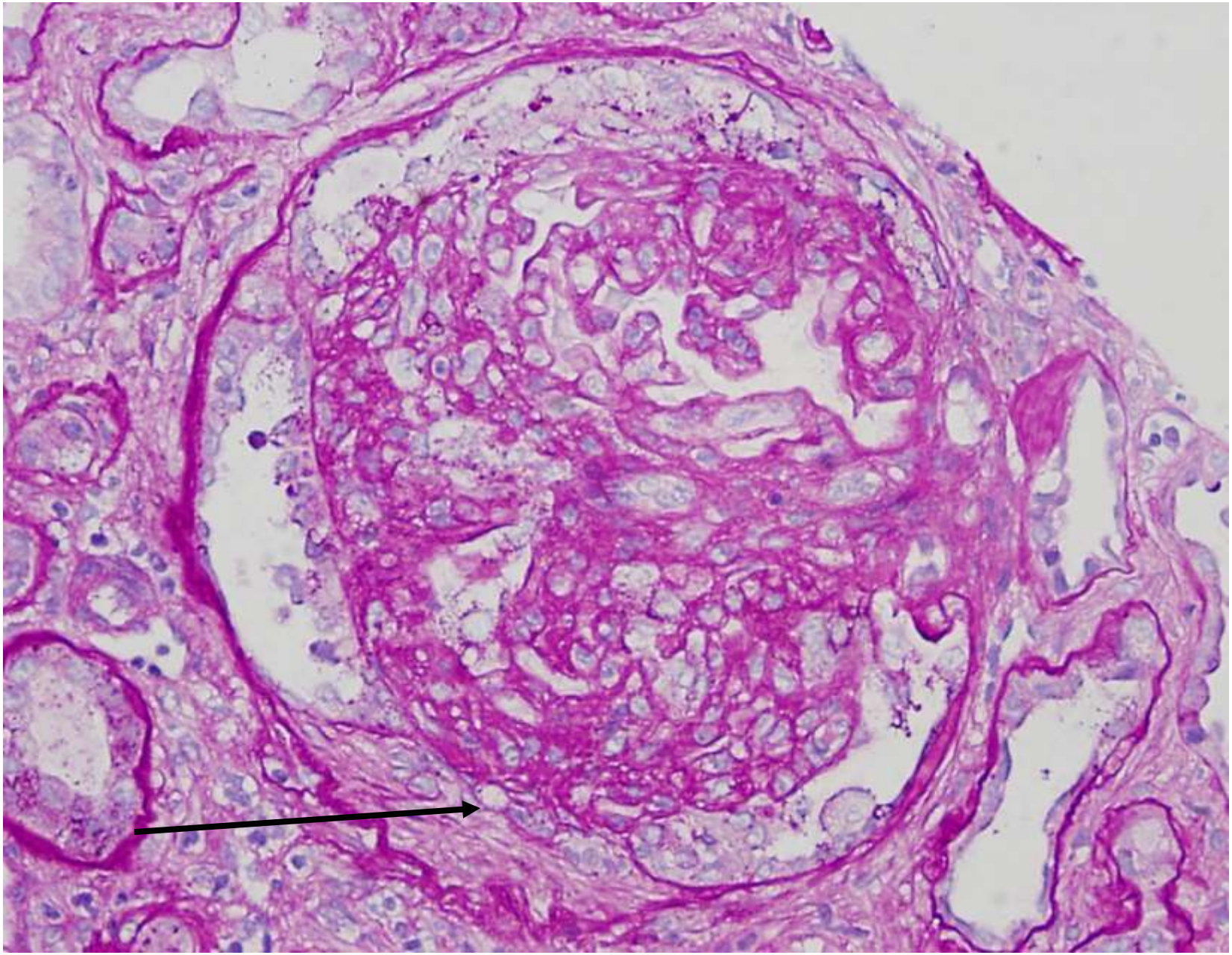
Advanced chronic injury with many sclerotic glomeruli. PAS



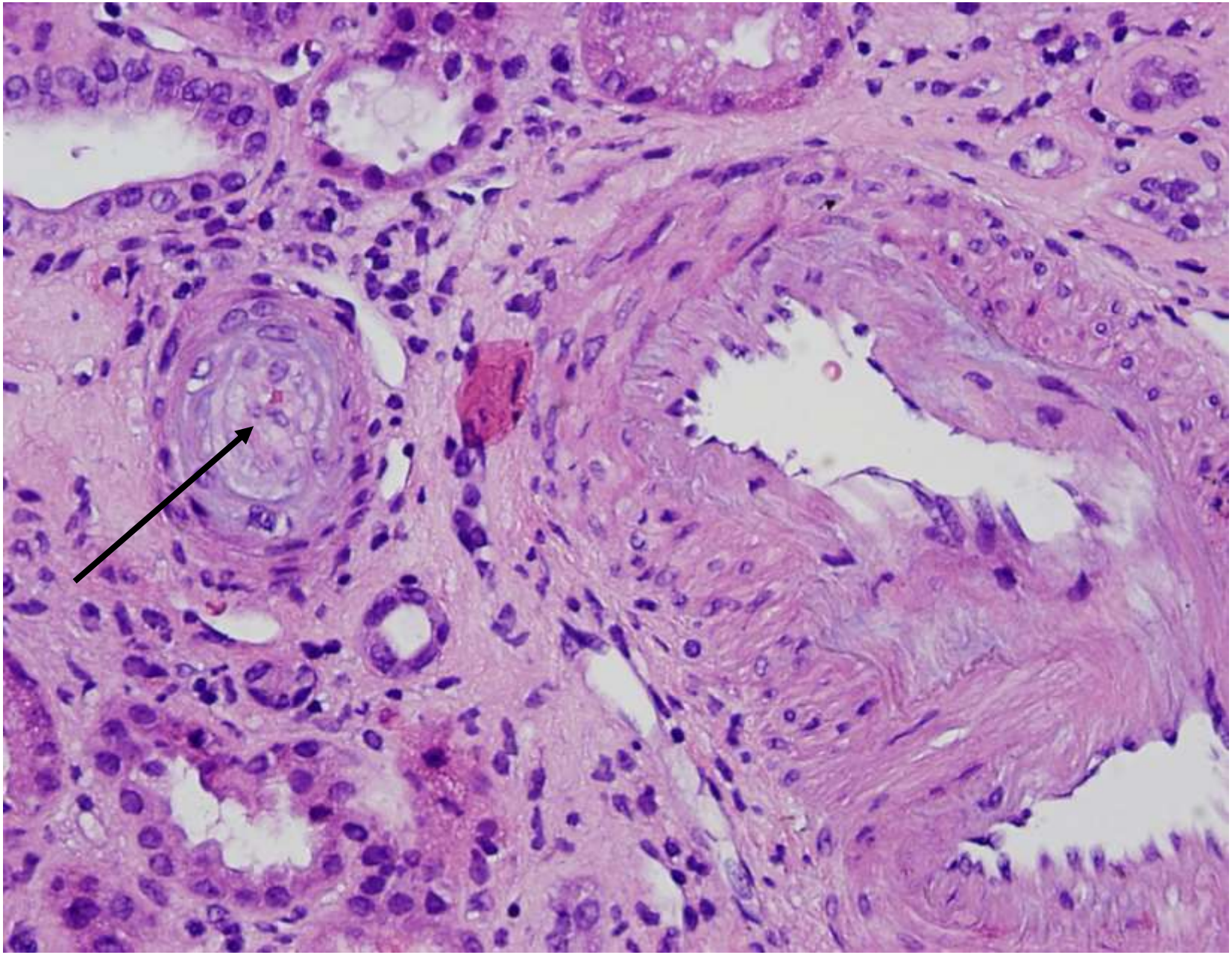
Large glomeruli with open capillaries. PAS



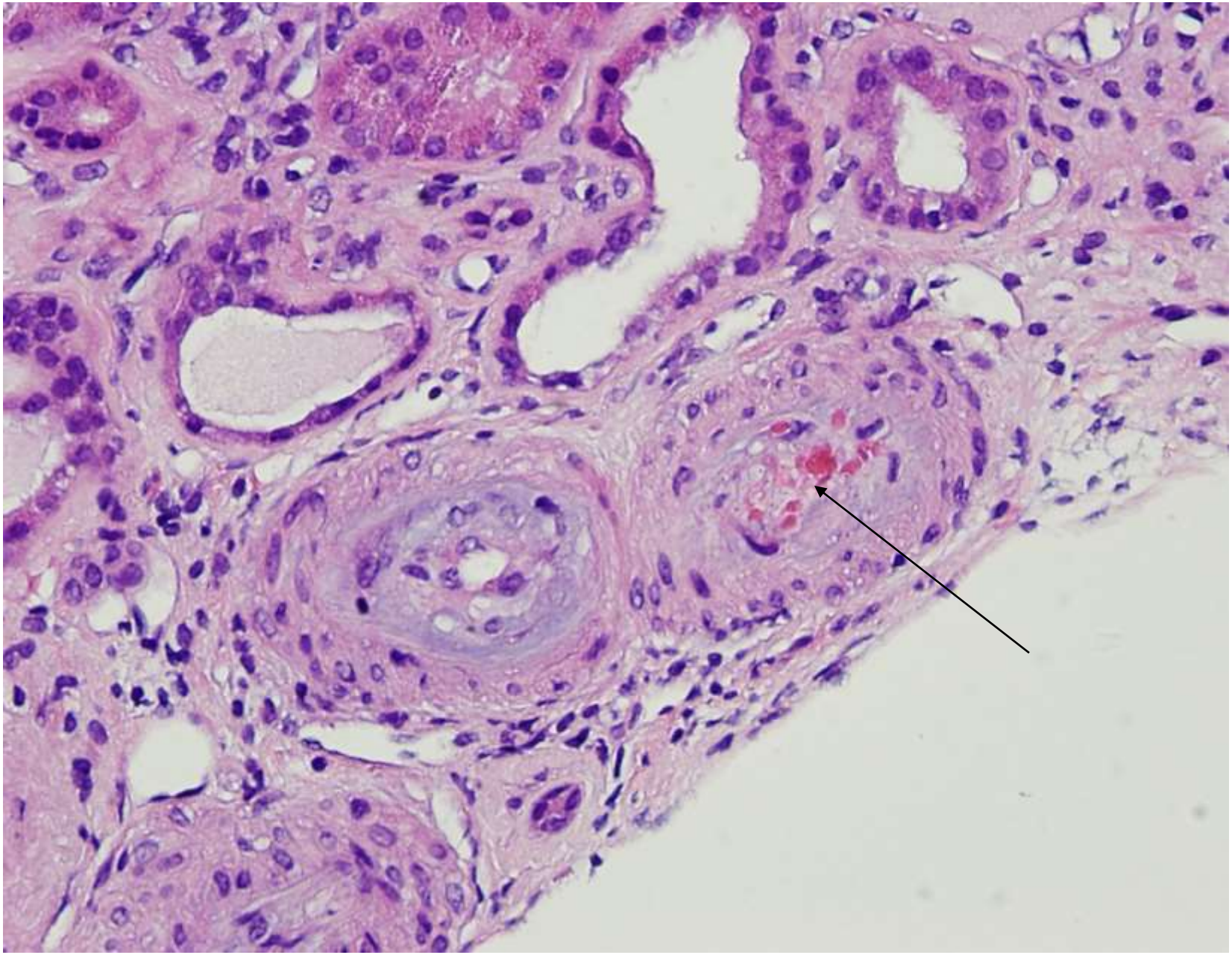
Segmental sclerosis with hypertrophic glomerular epithelial cells in the Bowman’s capsule



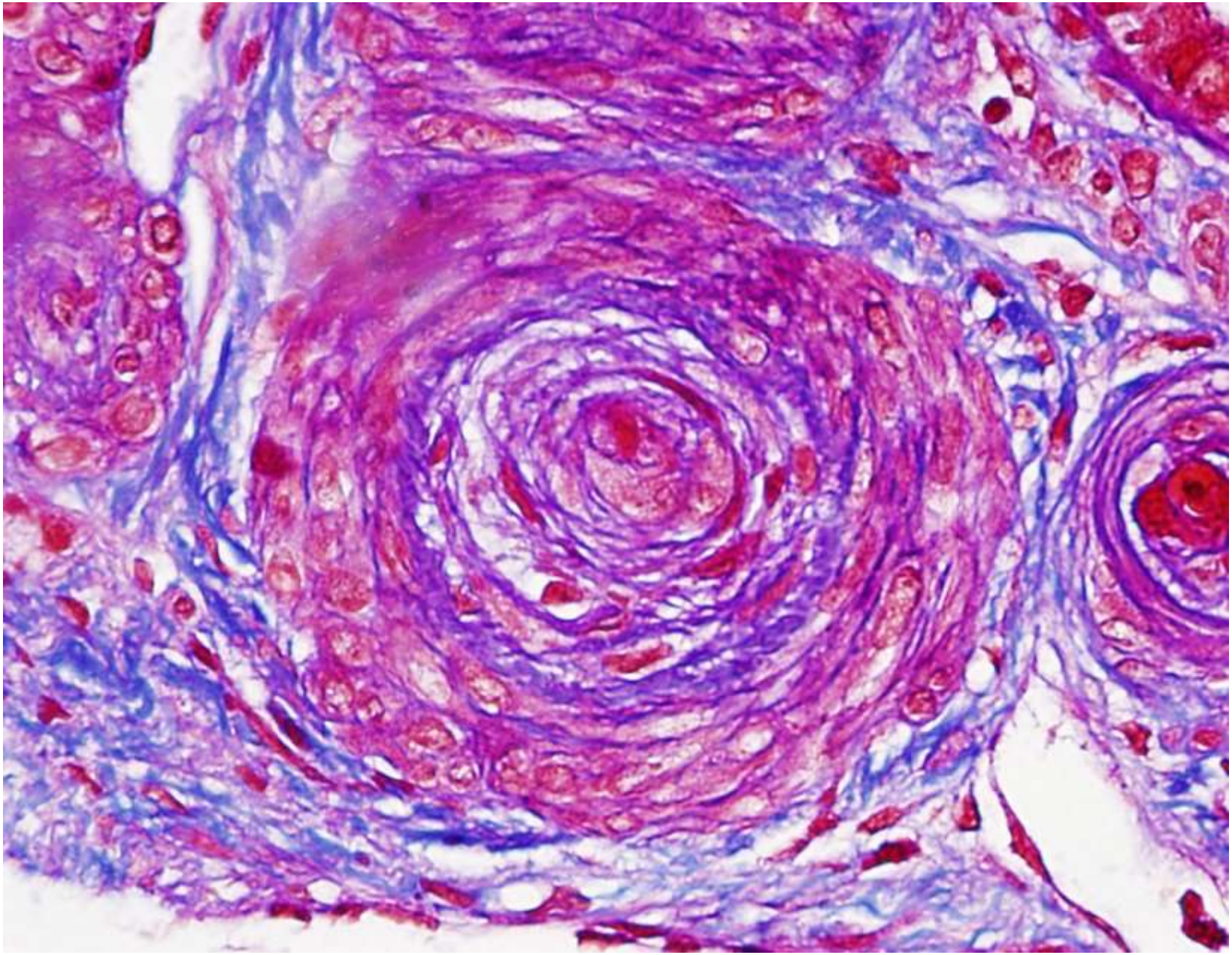
Another sclerosing glomerulus with an adhesion (arrow). PAS



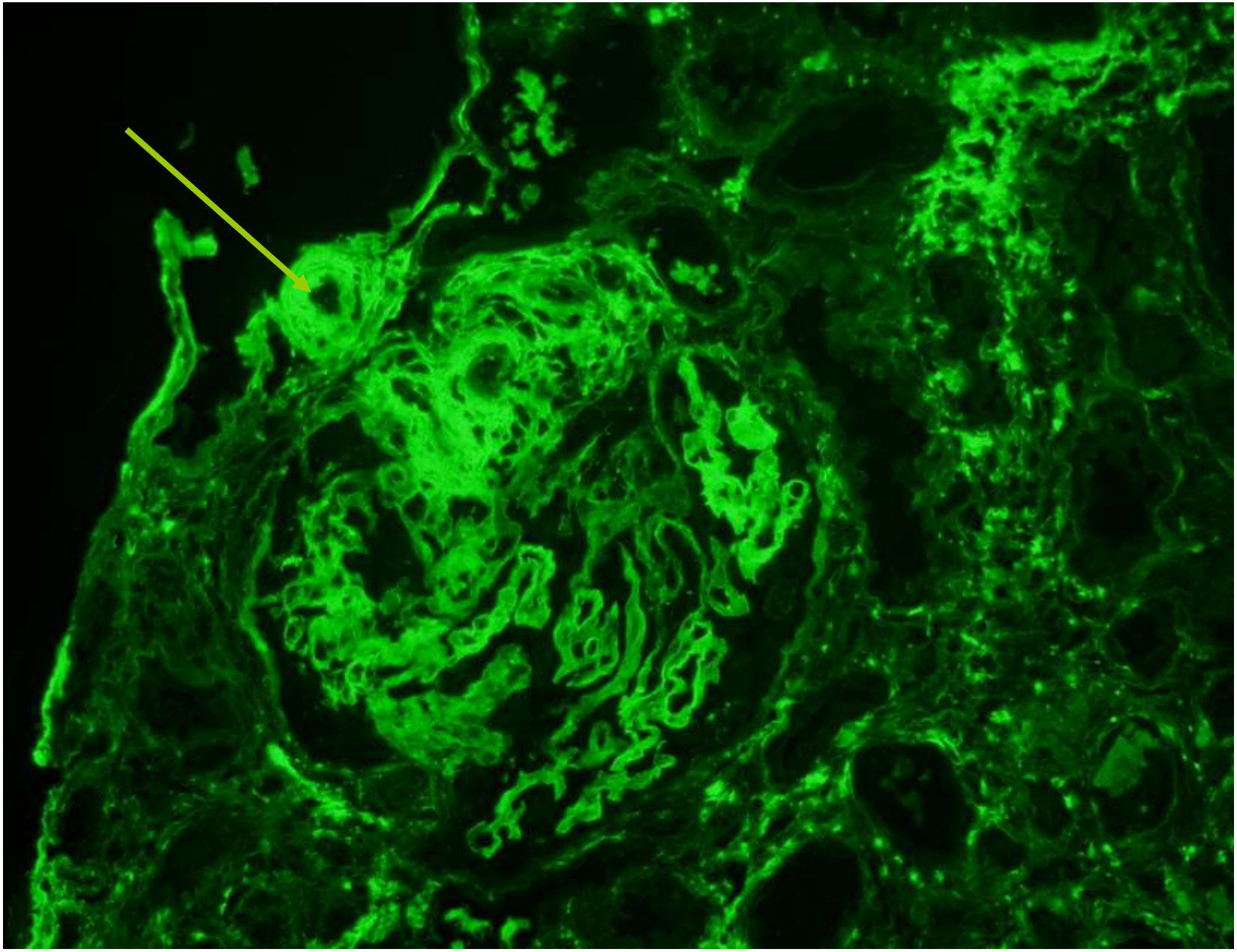
Obliterative microvascular damage (arrow). The larger arteries (right side of image) were less involved



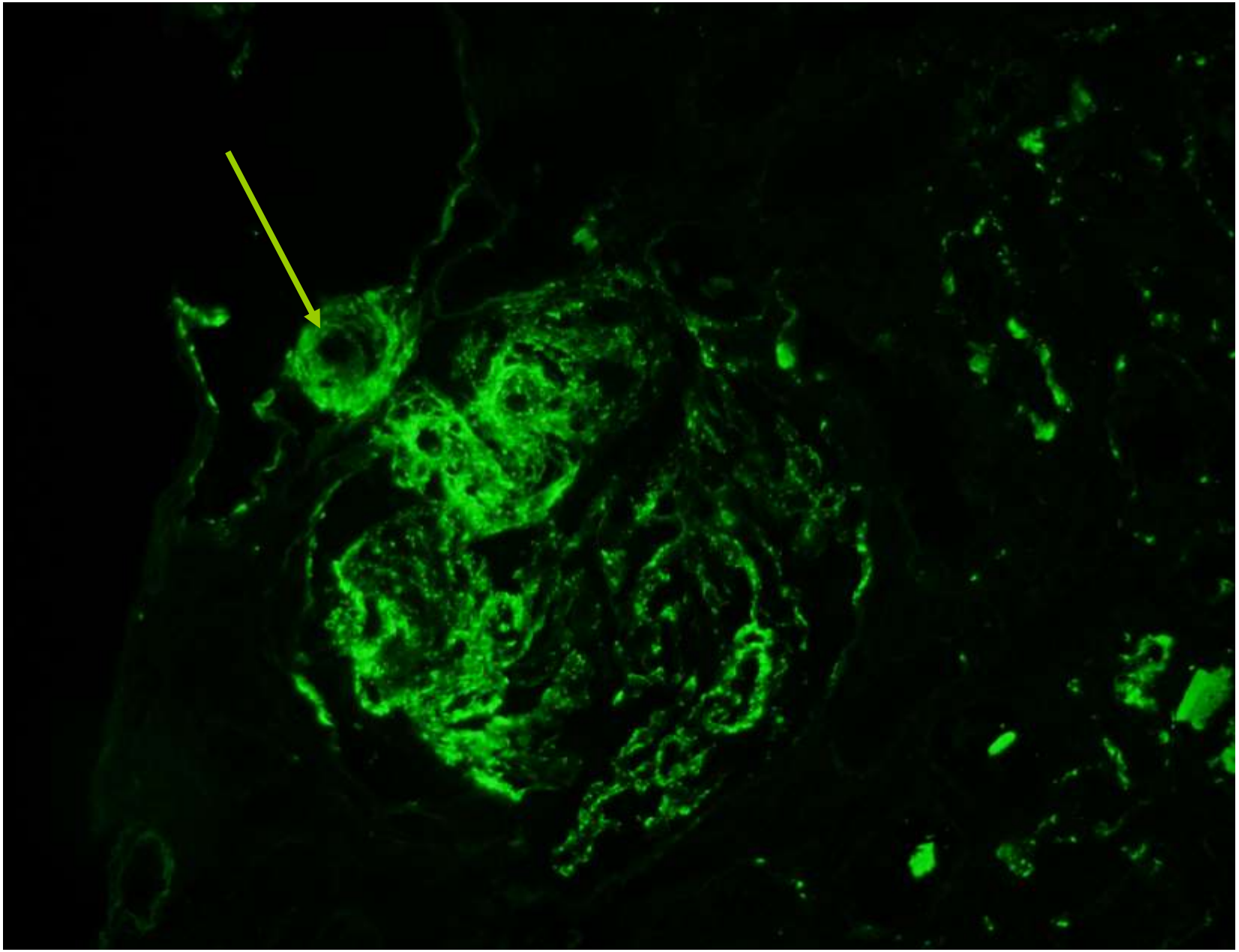
Mucoid thickening and obliteration of the lumina in small arteries. Note the fragmented RBCs in the thickened arterial wall. H&E



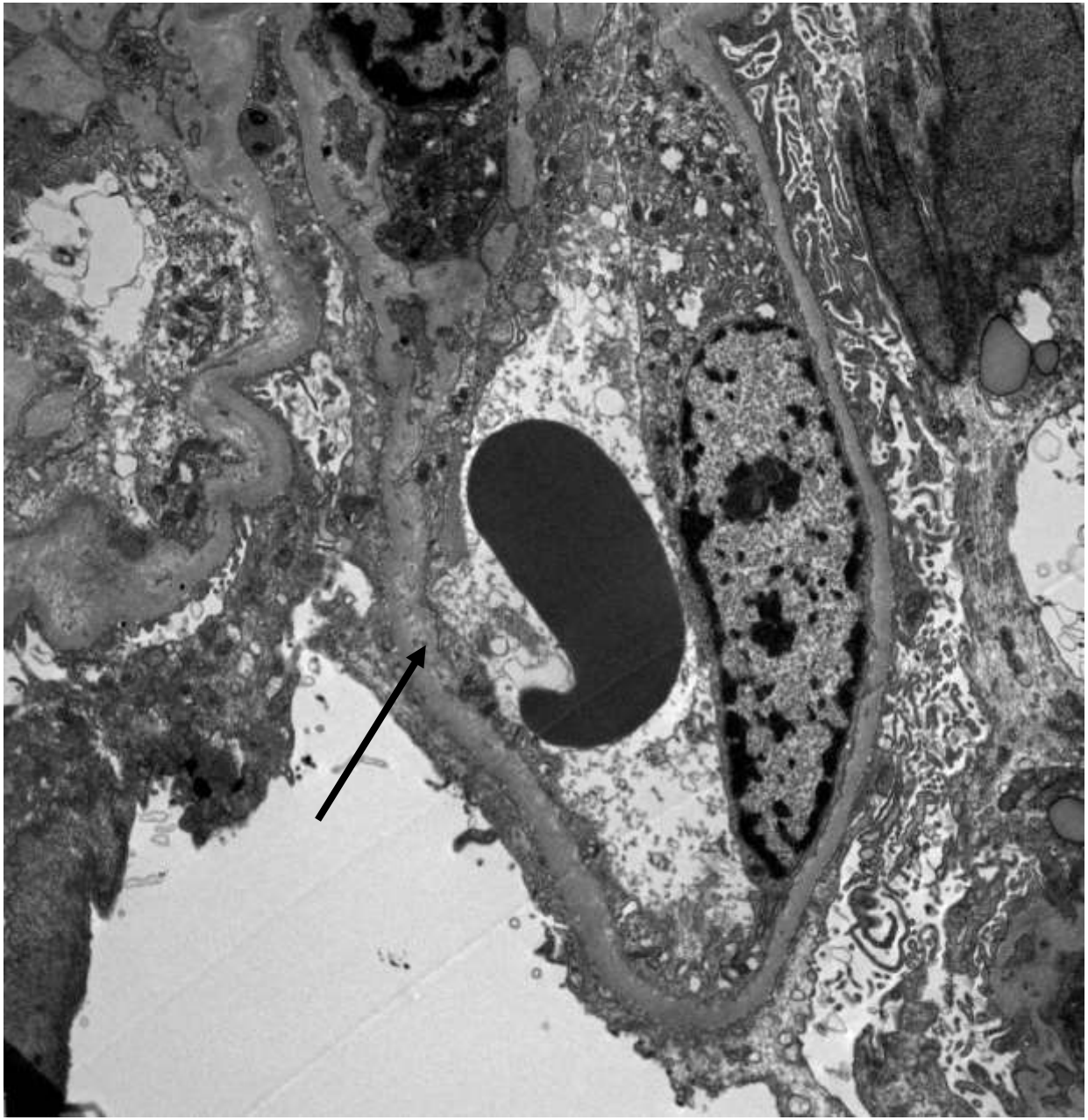
Concentric thickening of the wall of a small artery (“onion skinning”)



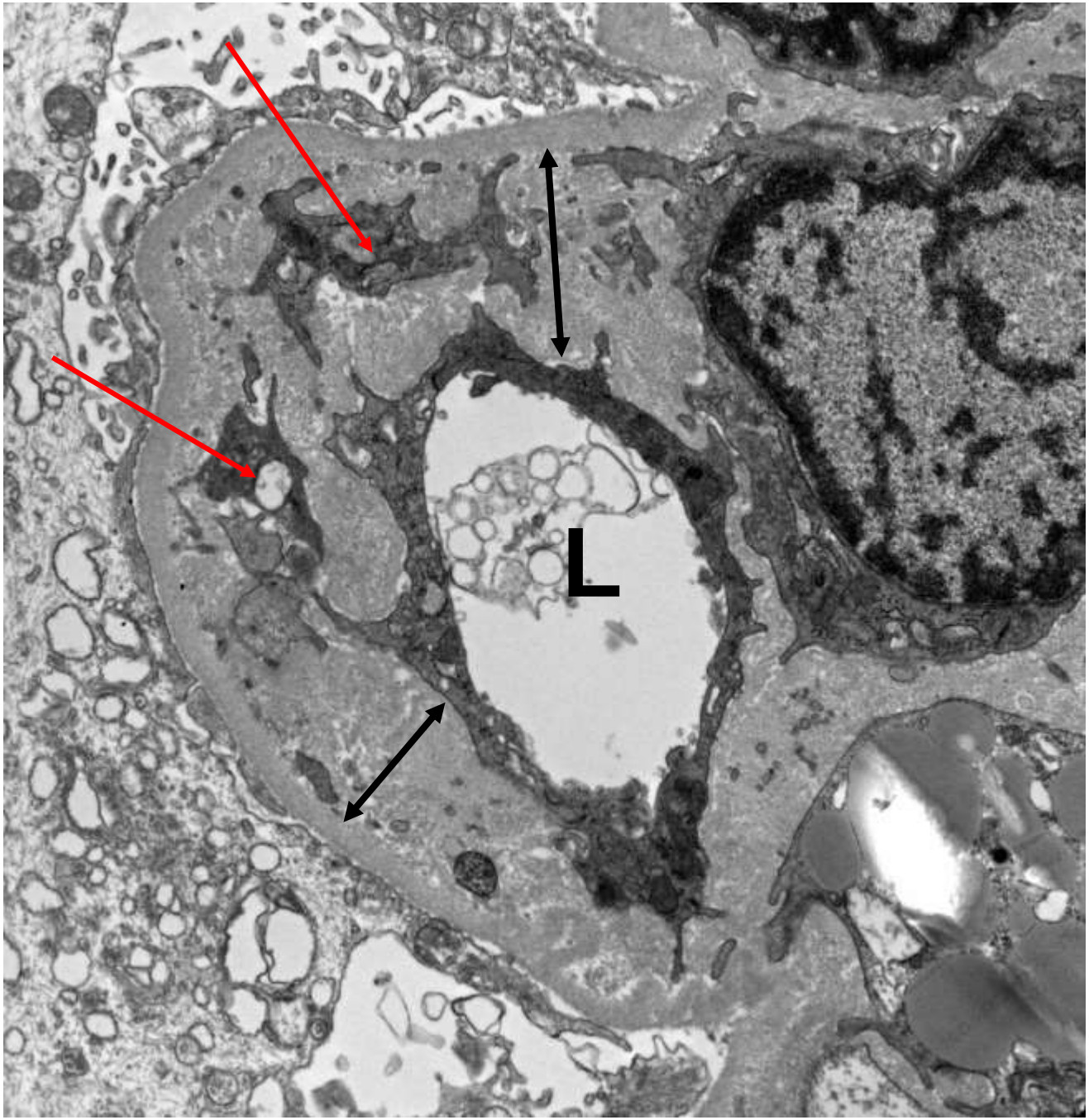
Fibrinogen staining in the afferent arteriole (arrow), in the glomerulus and focally in the interstitium



C3 with arteriolar (arrow) and glomerular staining



Glomerular capillary with only mild segmental subendothelial widening (arrow)



Prominent subendothelial amorphous widening (spanning arrows) with mesangial cell interposition (red arrows) and endothelial swelling. L: glomerular capillary lumen

Diagnsosis

- Thrombotic microangiopathy (TMA)
- Focal segmental glomerular sclerosis
- Prominent interstitial fibrosis and tubular atrophy

What happened (scenario #1)?

FSGS

↓

Worsening/malignant HTN

↓

TMA, fibrosis

What happened (Scenario #2)?

Severe (malignant) hypertension

TMA, FSGS, fibrosis

What happened (scenario #3)?

TMA

↓

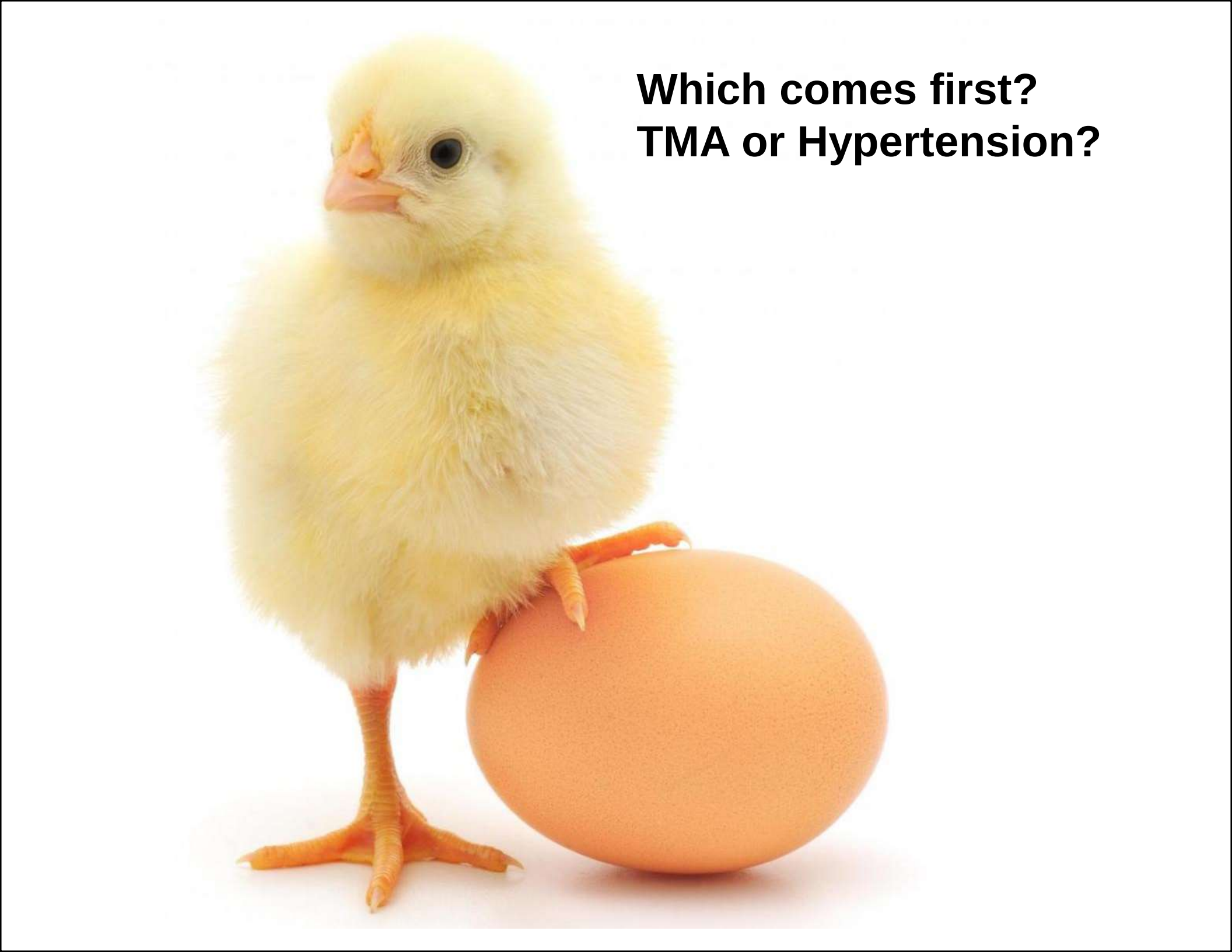
Worsening/malignant HTN

↓

FSGS fibrosis

Thrombotic Microangiopathy (TMA)

- ***Acute and/or chronic microvascular damage secondary to endothelial injury***
  - Typical, diarrhoea+ HUS
    - Shiga toxin-mediated (mostly E-coli)
  - Atypical (diarrhoea negative) HUS (aHUS)
    - Long list of disorders, frequently alternate complement pathway dysregulation
  - TTP
    - Hereditary or acquired ADAMTS13 deficiency
  - Preeclampsia/eclampsia/HELLP syndrome
  - Organ and bone marrow/hematopoietic stem cell transplantation
  - Malignant essential hypertension (not secondary to TMA but causing the TMA)


Which comes first?
TMA or Hypertension?

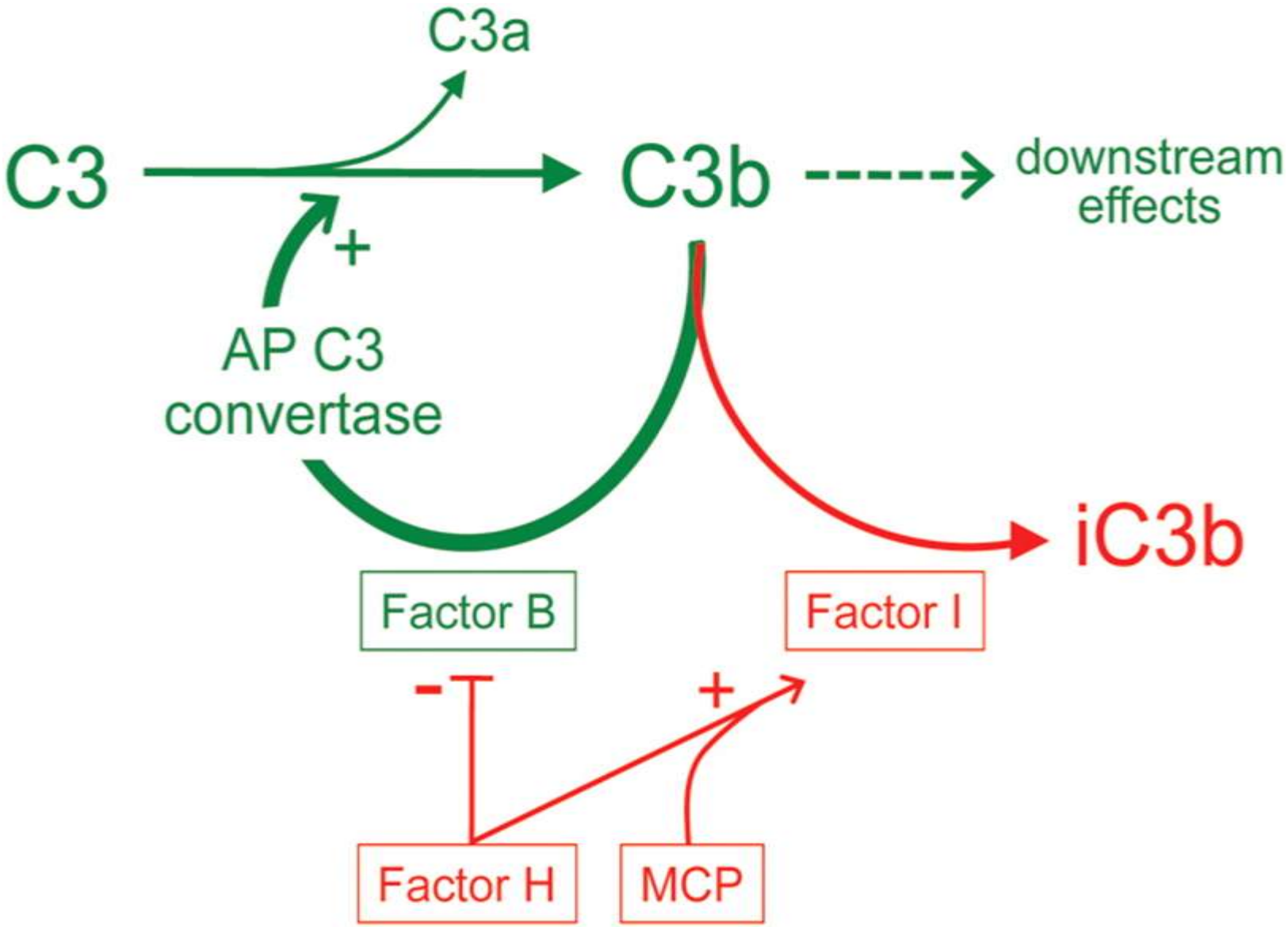
Severe (malignant) essential hypertension or TMA?

- Essential HTN is a diagnosis of exclusion
- Differentiating primary TMA from TMA secondary to malignant hypertension is very difficult
  - Genetic, autoimmune and coagulation workup careful clinical history, review of medications may find an underlying cause for TMA
- Symptoms of microangiopathic hemolytic anemia (MHA) may be less common in malignant HTN associated TMA than in TMA not secondary to hypertension (but MHA may be subtle or absent in chronic forms of TMA as well)
- Sometimes primary TMA is not associated with severe malignant hypertension at the time of diagnosis (e.g., sometimes in scleroderma)

Causes of atypical HUS

- Complement abnormalities (alternate pathway)
  - Most common form of aHUS
  - Genetic (over 120 mutations in complement regulatory proteins described. Most common in aHUS: Factor H, Factor I, MCP, factor B, C3
  - Autoantibodies to factor H (rarely factor I)
- Autoimmune diseases
  - SLE, antiphospholipid antibody syndrome, scleroderma
- Drugs
  - E.g., VEGF inhibitors, gemcitabine, calcineurin inhibitors, quinine
- Malignancies
- Infections, such as:
  - HIV
  - Invasive pneumococcus infection in children
  - Rarely, other infections (e.g., c. difficile)
- Other genetic mutation (mostly coagulation related)
  - Thrombomodulin, diacylglycerol kinase, plasminogen, MMACHC (cobalamine deficiency)

Regulation of the AP of complement.



Barbour T et al. Nephrol. Dial. Transplant. 2012;27:2673-2685

NDT Nephrology Dialysis Transplantation

Onset of TMA/aHUS

- Mostly in children and young adults but can happen at any age
  - It is likely that a second hit, such as an infection (that brings the complement regulatory abnormality on the surface) is necessary to trigger the pathogenetic processes
- Rare genetic forms of aHUS manifest already during early childhood
  - MMACHC, DGKE
- Variable inheritance pattern with variable penetrance
  - In the past many patients were diagnosed as “hypertensive nephrosclerosis” (hypertensive nephrosclerosis affecting young people, particularly if not of African ancestry, – unlikely). Consider TMA

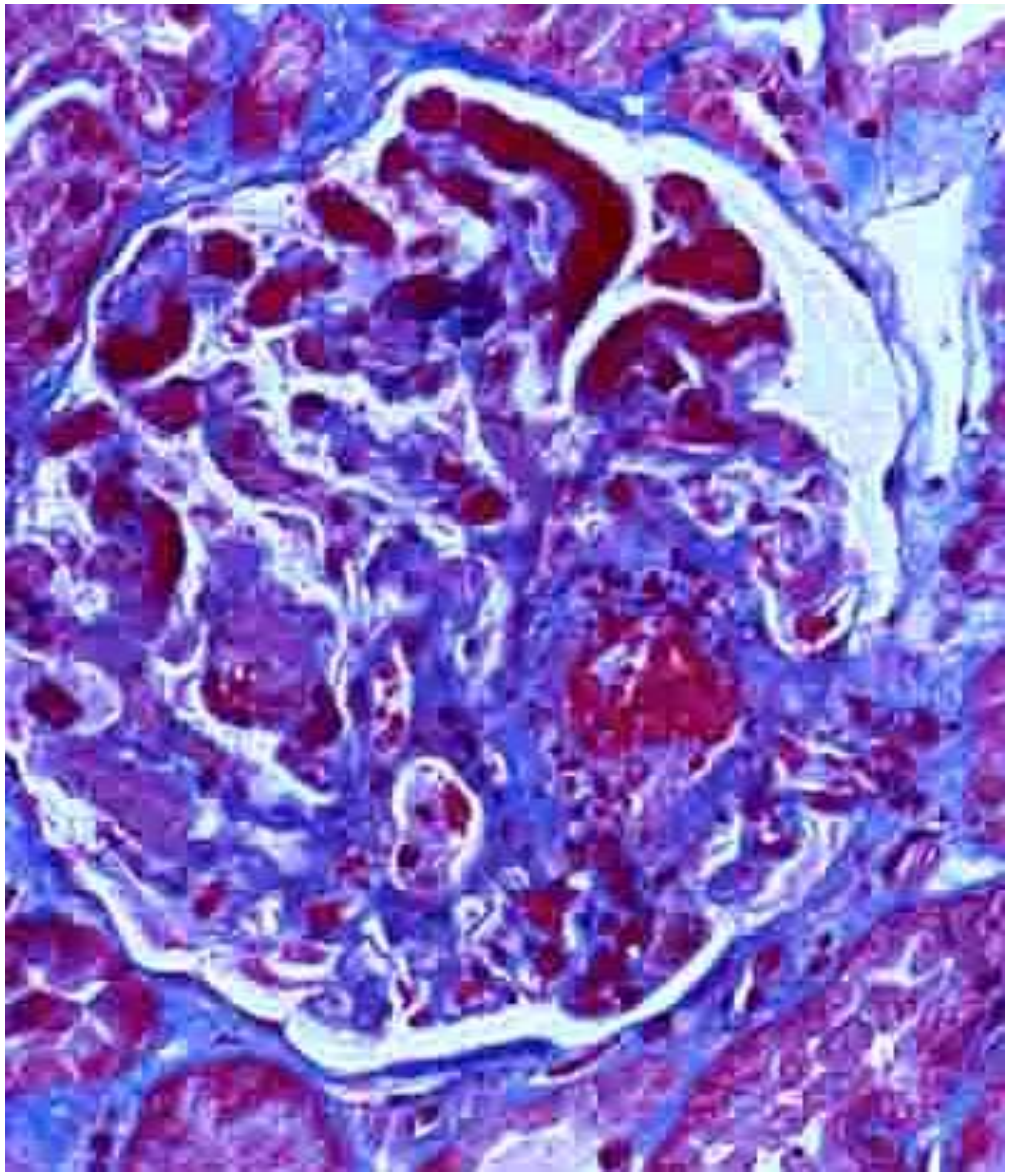
Clinical symptoms of aHUS

- Acute onset active disease
  - AKI
  - Microangiopathic hemolytic anemia (may be subtle or absent in some chronic cases)
  - Severe hypertension (not always, may be normal initially)
  - Sometimes, in severe cases, multiorgan failure
  - Serum C3 may be low (but is frequently normal) in alternate complement pathway dysregulation-related TMA
- Chronic, insidious aHUS
  - Progressive CKD
  - Worsening HTN
  - Symptoms of microangiopathic hemolytic anemia frequently subtle
  - Can have exacerbation with acute/active disease
  - Can be misdiagnosed as hypertensive nephrosclerosis

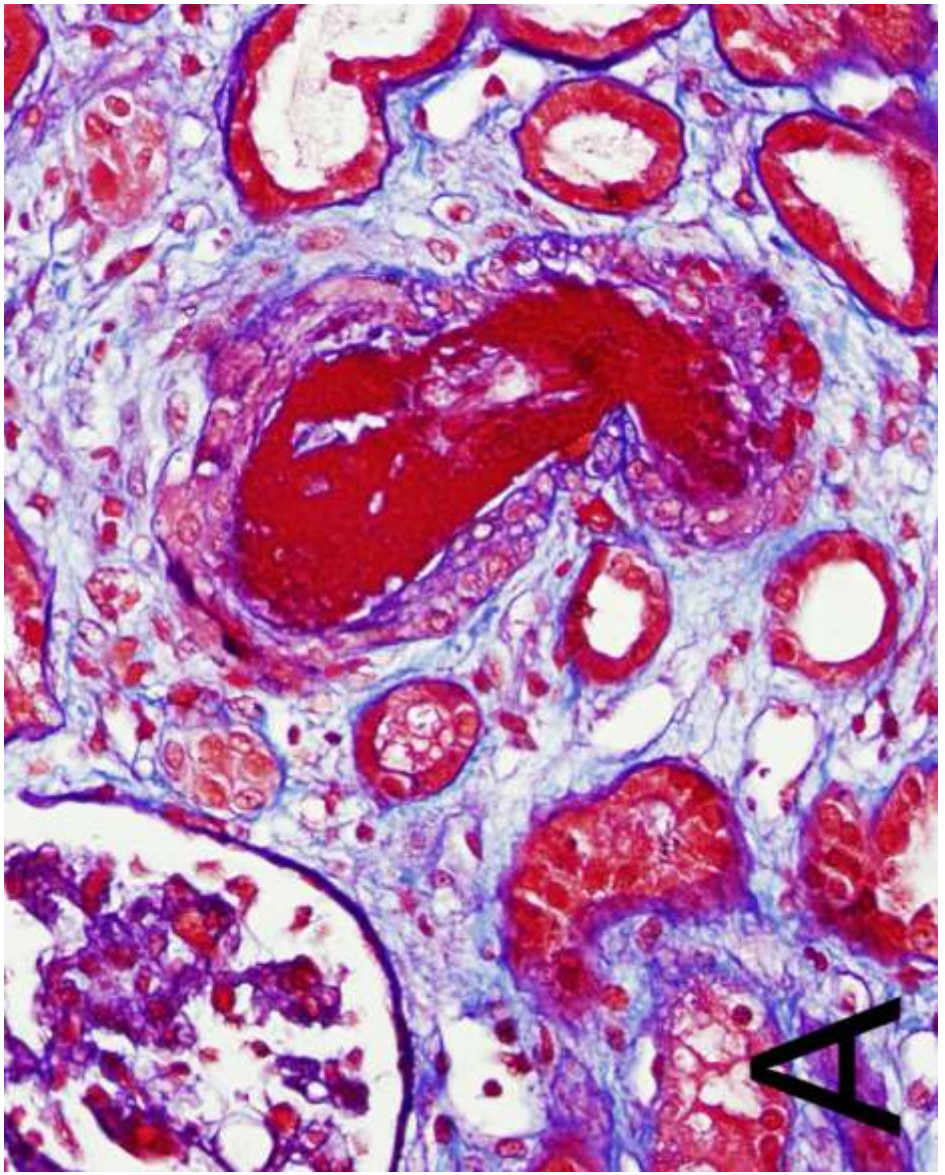
Pathologic (kidney) findings in TMA/aHUS

- In acute, active cases arterial, arteriolar and glomerular fibrin thrombi
  - In d+ HUS there is mostly glomerular involvement; only mild vascular changes – usually reversible)
  - In aHUS, arteries/arterioles are also prominently involved
- In rare very severe acute forms renal cortical necrosis may develop
- In chronic cases
  - concentric mucoid thickening of small arteries, arterioles
  - Glomerular capillary ischemia and/or
  - Glomerular capillary thickening frequently with double contours on Jones stain due to subendothelial widening of the glomerular capillary loops

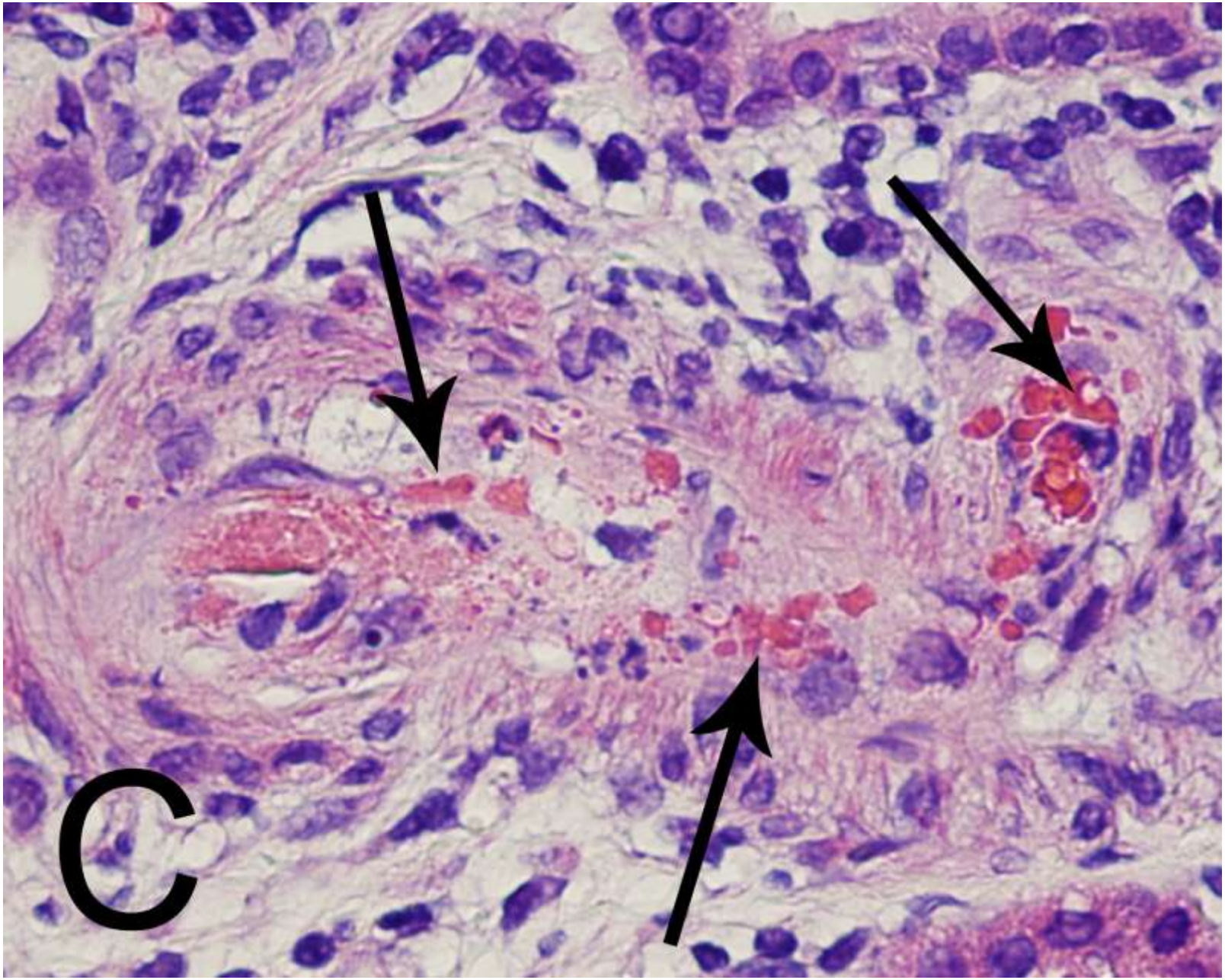
Renal changes in acute/active TMA



Glomerular thrombi

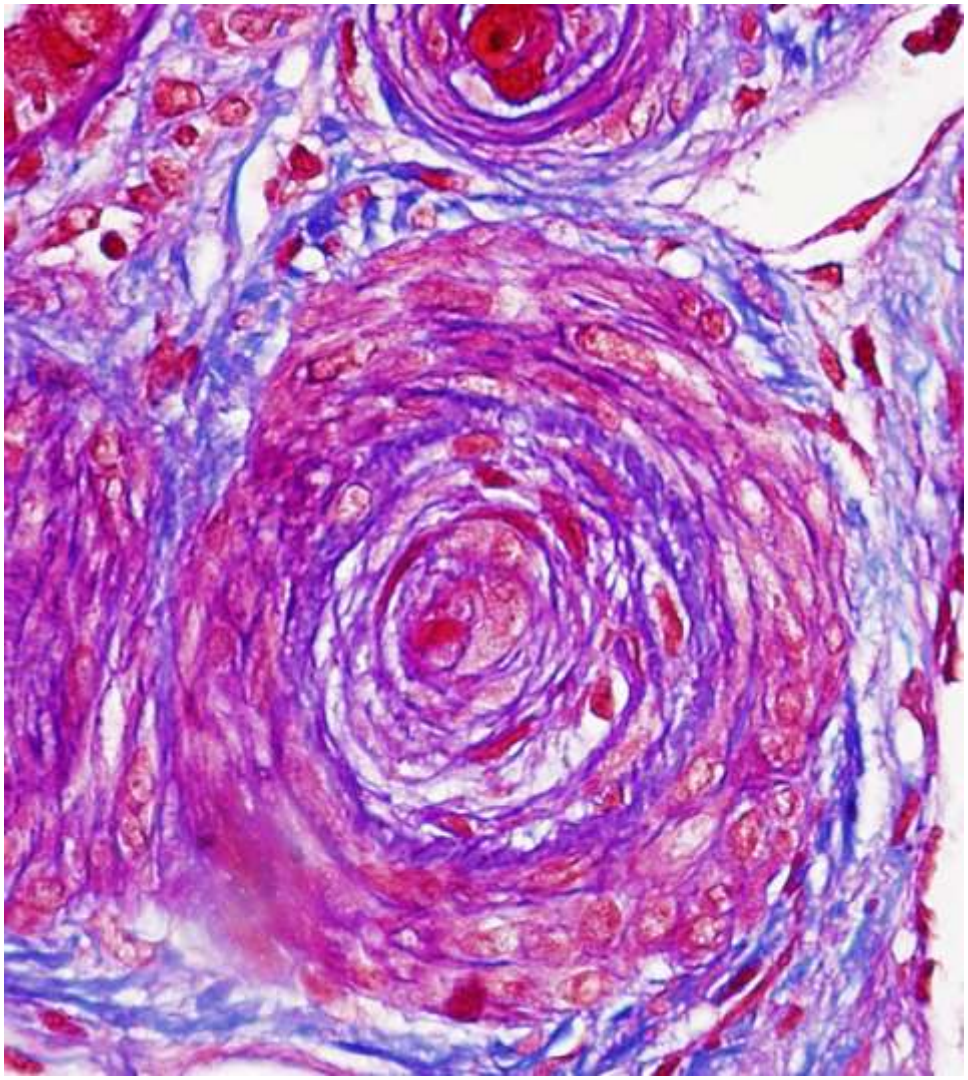


Arterial thrombi

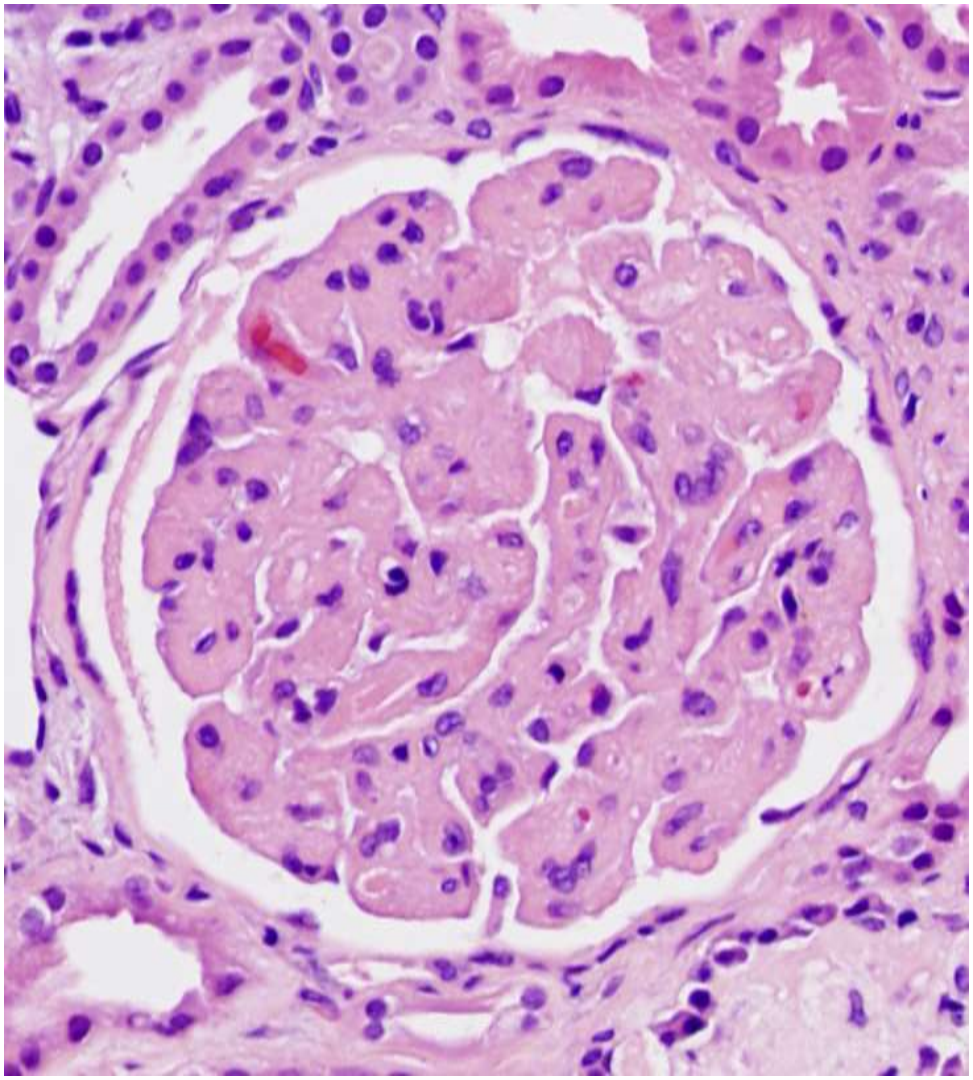


Fragmented RBCs in the wall of a thickened artery

Renal changes in chronic stage TMA

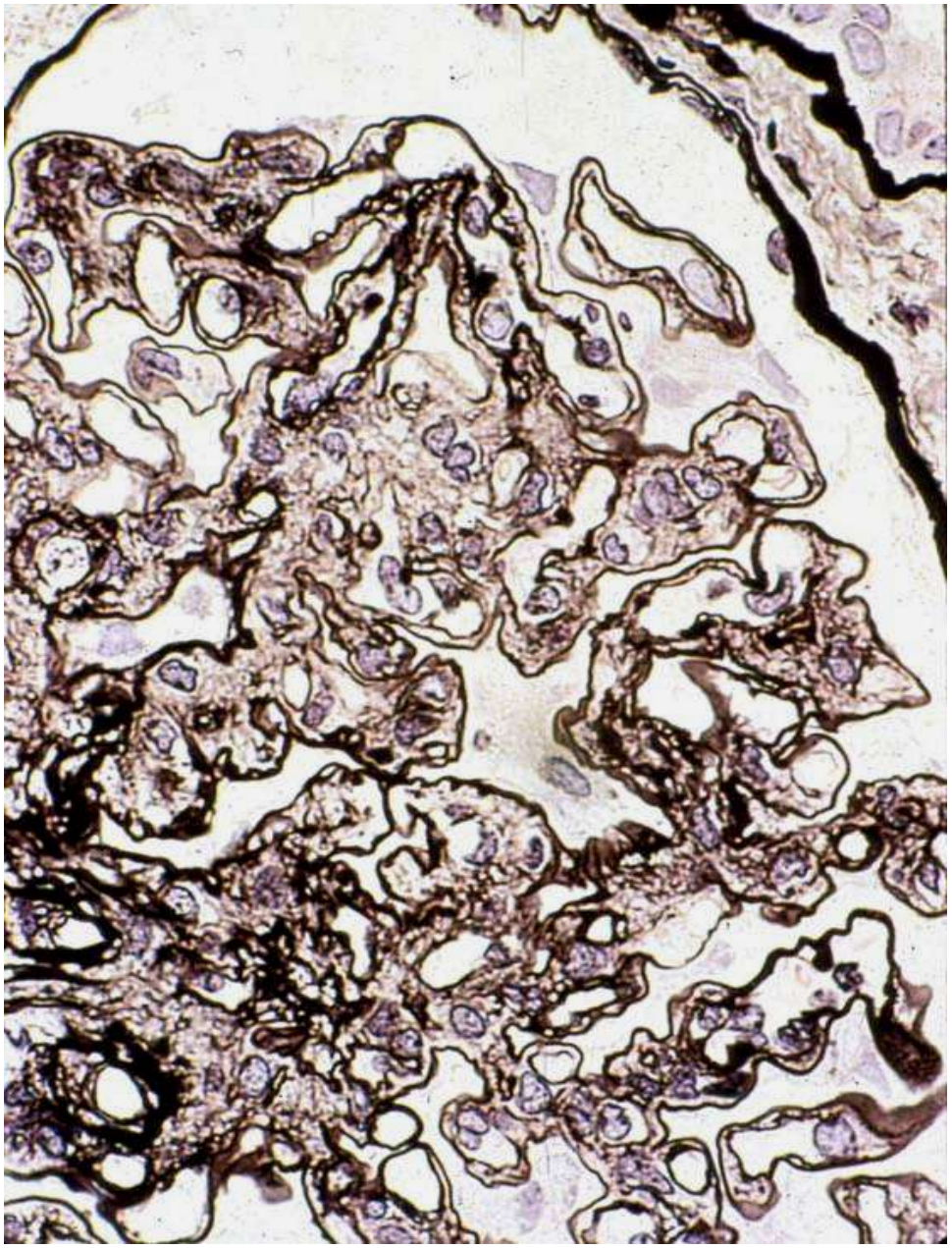


Concentric thickening of the arterial wall (“onion skinning”). Trichrome

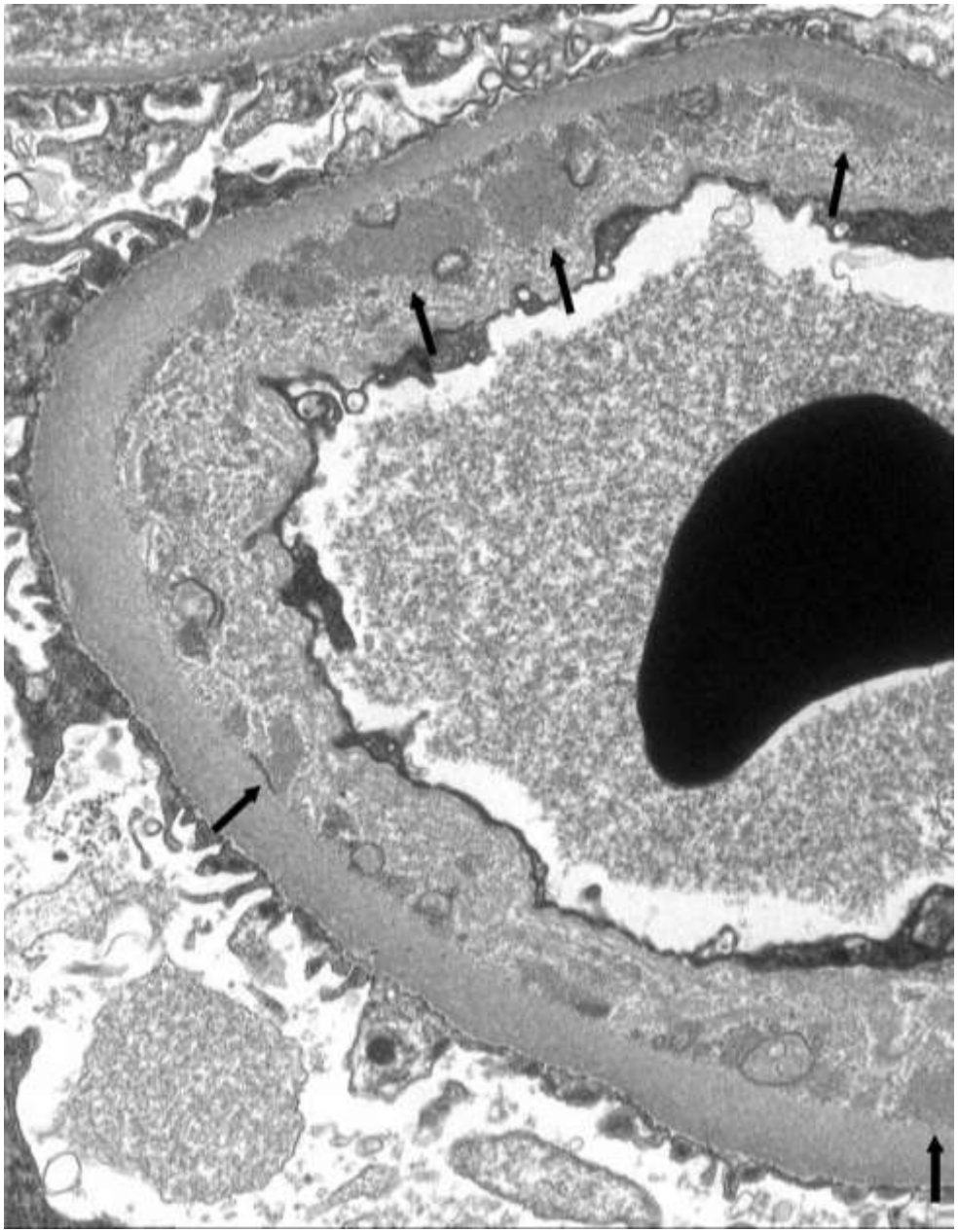


Amorphous material filling the glomerular capillaries (“bloodless glomerulus”). H&E

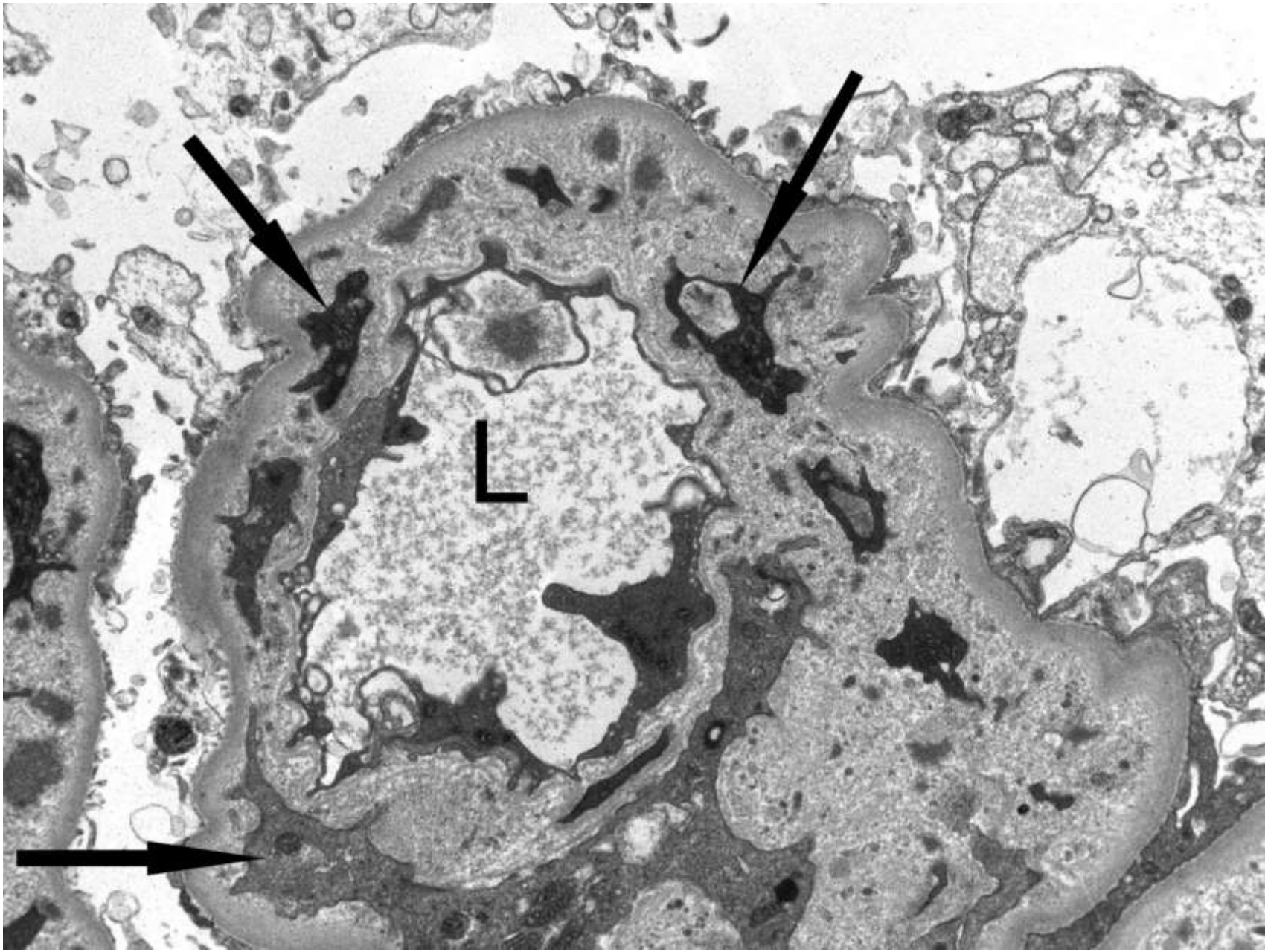
Glomerular changes in chronic stage TMA



Double contours along the glomerular capillary Loops, Jones methenamine silver



Subendothelial amorphous widening along a glomerular capillary wall on EM



Mesangial cell interposition (seen in chronic stage only)

The value of kidney biopsy in TMA

- Will provide the diagnosis of TMA
  - The diagnosis is frequently made by renal pathologists; the clinical presentation may not be typical (hence the name atypical HUS)
- Will tell wether the disease is acute active or chronic
- Will provide some therapeutic guidelines
  - Cases with advanced chronic injury may not respond to treatment
- Does not differentiate between different forms of TMA
  - There are some hints though, e.g. in malignant HTN there is usually less glomerular involvement

What to do if you have the biopsy diagnosis of TMA/aHUS?

Burn the renal biopsy report?

What to do if you have the diagnosis of TMA/aHUS?

- Careful review of history symptoms, lab values and medications
  - Make sure the case is not a d+HUS,
- Submit blood sample to test for complement regulatory gene abnormalities
- Test for ADAMTS13 levels
- Autoimmune panel, including anti-phospholipid antibody panel
- Coagulation workup
- If indicated, additional genetic testing
  - (e.g., thrombomodulin plasminogen, vWF etc.)

Diagnostic Tests

Shiga toxin

Stool culture for *E. coli* plus either toxicology for shiga toxin or PCR for *shiga toxin* gene

Urine culture for *E. coli*

Other bacterial testing as indicated

S. pneumoniae

T antigen expression on red cells

PCR of blood and/or secretions

Blood culture

Complement dysregulation

Plasma/serum protein levels

C3

Factor H, factor I, factor B

MCP (CD46) expression on PBMCs

Factor H autoantibodies

Mutations

Direct exon sequencing of *CFH, MCP, CFI, CFB, C3*

Copy number variation across *CFH-CFHR* locus

ADAMTS13 deficiency

ADAMTS13 activity

ADAMTS13 autoantibodies

ADAMTS13 mutations

Other associations

Pregnancy test

Liver and pancreas enzymes

Cobalamin (B12), homocysteine assay, methylmalonic acid (plasma and urine) ± mutation analysis of *MMACHC* gene

HIV and other viral serology as indicated

ANA, lupus anticoagulant, antiphospholipid antibodies

Pharyngeal swab and viral PCR for influenza A (H1N1)

Barbour et al NDT 27:2673, 2012

Why bother?

- Different types of TMA need different treatment
  - Not every TMA responds to plasmapheresis
- Effective treatment protocols are now available
  - E.g., eculizumab in complement regulatory problems-associated TMA
- Even if the patient develops ESRD, it is important to know the etiology of the TMA if the patient receives a kidney transplant
  - Recurrence rate of aHUS in renal allografts is high (60% +); different forms of TMA have different recurrent rates,
    - E.g., recurrence of factor H mutation-associated aHUS is >80%, recurrence of MCP mutation-associated aHUS is <10%, recurrence of scleroderma renal crisis: 60% to 80%, recurrence of drug-induced aHUS: should be 0%)
  - Recurrence of abnormal complement regulation-associated aHUS in renal allogfats can be prevented (eculizumab)
  - ESRD patients with genetically determined aHUS should not receive a living related renal allograft

Treatment of TMA

(i) ***Supportive measures only***

Paediatric STEC-HUS and invasive pneumococcal infection (p-HUS)

Cobalamin deficiency (children), HSCT- or malignancy-associated TMA, malignant HTN

(ii) ***Therapeutic plasma exchange*** (TPE)

First exclude paediatric STEC-HUS and p-HUS

Recommended in all other settings

Including TTP and aHUS (probably of no benefit in *MCP*-aHUS)

Controversial in adult STEC-HUS

Plasma infusion recommended in known congenital TTP

(iii) ***Eculizumab***

aHUS (related to alternate complement pathway abnormalities)

(iv) ***Steroids and/or rituximab***

Possibly in acquired TTP and aHUS with factor H autoantibodies

(v) ***Renal transplantation*** for ESKD

STEC-HUS

MCP-aHUS

Living-related donation contraindicated

(vi) Prophylactic strategies in high-risk transplantation (i.e. non-*MCP* aHUS)

Intensive perioperative TPE

Eculizumab

Rituximab (for factor H autoantibodies)

Combined kidney–liver transplantation

Barbour et al NDT 27:2673, 2012

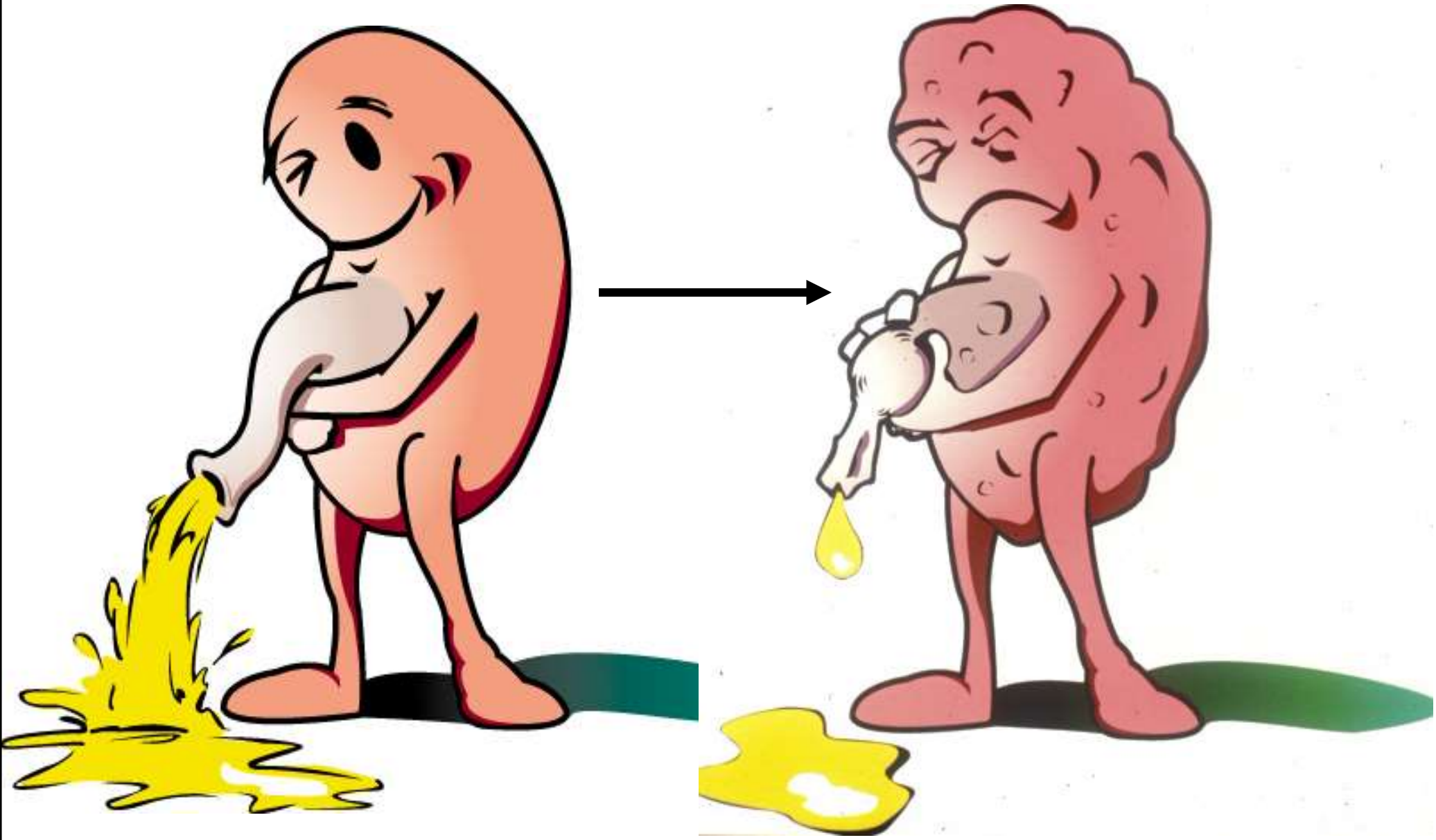
Data indicating primary TMA (aHUS) in the presented patient (Scenario #3)

- Low serum C3
- Elevated serum soluble MAC
- Elevated serum complement split product levels
- Genetic testing revealed a **mutation in the factor H gene** Allele 1 (NM_000186:c.1520-1G>A)

Follow-up information on our patient

- Patient was started on eculizumab
  - 900 mg IV weekly for 4 weeks then 1200 mg every two weeks indefinitely
- After 3 month of treatment, the serum creatinine stabilized around 3.8 to 4.2 mg/dl and the patient came off dialysis (and still is after 8 months)
- Blood pressure normalized, no evidence of MHA.

Usual disease course of aHUS



But not always; there are effective treatments available now

Suggested Reading

- Laszik ZG, Kambham N, Silva FG: Thrombotic microangiopathies. In: Jennette JC, Olson JL, Silva FG, D'Agati VD (eds.): Heptinstall's Pathology of the Kidney, Seventh Edition. Wolters Kluwer, Philadelphia, 2015, pp. 739-814.
- Nester CM, Barbour T, de Cordoba SR, et al. Atypical aHUS: State of the art. Mol Immunol. 2015; 67(1):31-42.
- Taylor CM, Machin S, Wigmore SJ, et al. Clinical practice guidelines for the management of atypical haemolytic uraemic syndrome in the United Kingdom. Br J Haematol. 2010; 148(1):37-47.
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- George JN, Nester CM. Syndromes of thrombotic microangiopathy. N Engl J Med. 2014; 371(7):654-66.