

A Close-Up on Sickle Cell Nephropathy through Electron Microscopy: A Case Report

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20 June 2024



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Outline

- Case History
- Sickle Cell Disease (SCD)
- Sickle Cell Nephropathy (SCN)
- Conclusion
- Acknowledgements
- References

Case History

Mr X

Case History – Mr X

- 45-year-old male
- Sub-Saharan Africa
- Moved to the UK a few years ago
- Sickle cell disease, no other known diseases
- Multiple complications
 - Chronic haemolytic anaemia
 - Retinopathy
 - Hepatopathy, gallstones
 - Cardiovascular complications (mild systolic impairment of the heart)
 - Cerebrovascular complications (left internal carotid artery aneurysm and silent cerebral infarcts)
 - **Nephropathy**

Case History – Mr X

- Different specialties
- Routine blood (FBC, Hb, LFTs, RFTs, iron, folate and B12..) and urine tests +/- imaging
- BMI 18.1
- Medications: hydroxycarbamide 1-1.5 g, lisinopril 20 mg OD, vitamin D supplements, folic acid 5 mg OD + emergency supply of Amoxicillin
- Vaccinations (vulnerable): various vaccinations and boosters including Hib/Men C, PCV13, PPV23, MenACWY, MenB, influenza, COVID and Hepatitis B.
- No admissions over the past year.

Sickle Cell Disease

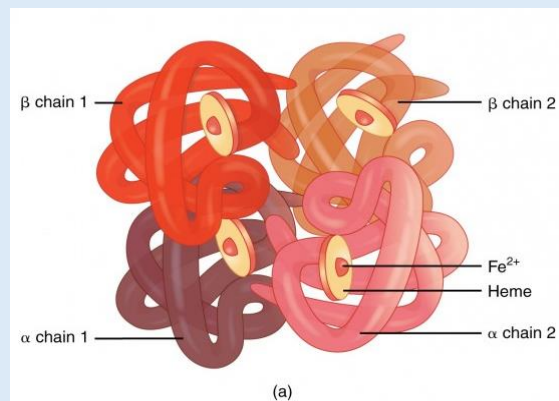
What is it?

Sickle Cell Disease

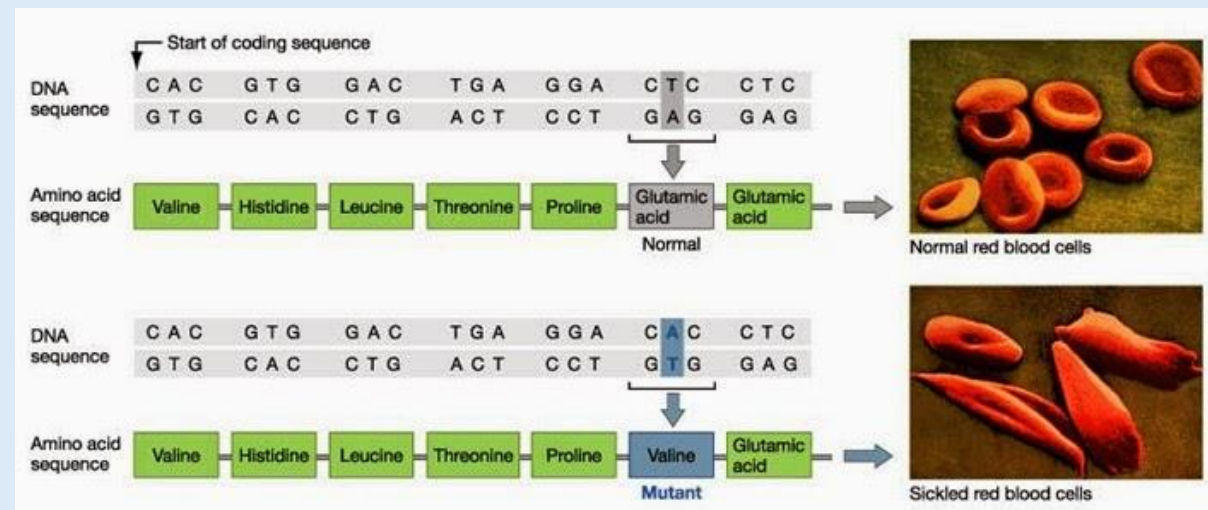
- A genetic disorder resulting from the presence of a mutated form of haemoglobin, haemoglobin S (HbS).
- Autosomal recessive
- Denotes all genotypes that contain at least one sickle gene
 - HbSS (sickle cell anaemia) being the most common and severe – Mr X
 - HbAS – sickle cell trait
 - Others include HbS/b-0 thalassemia, HbS/b+ thalassemia, HbSC disease and others..
- African ancestry, M=F
- Present after 6 months of age

Sickle Cell Disease - Etiology/Pathophysiology

- 5M RBC/uL, each can contain 300 million haemoglobin molecules and bind to 1.2 billion oxygen molecules
- A molecule of haemoglobin contains:
 - Four globin proteins (alpha 1 and 2, and beta 1 and 2), each of which is bound to one molecule of the iron-containing heme.
- Base substitution T>A in the gene coding beta globulin, resulting in a different amino acid and a mutant protein HbS.



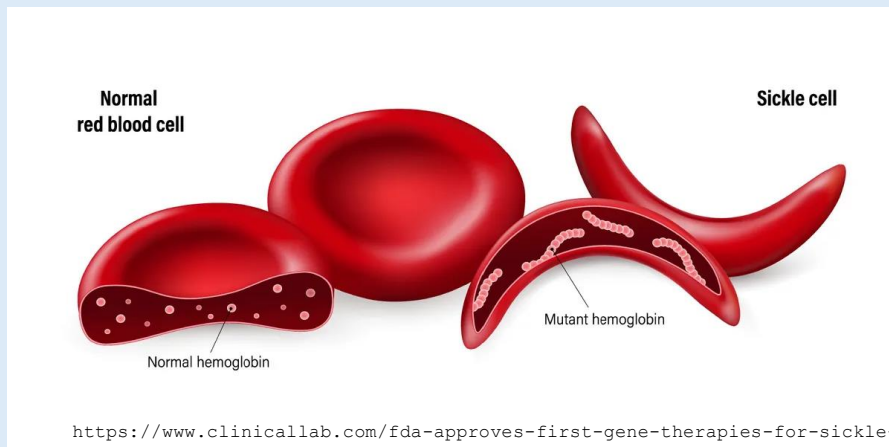
<https://studymind.co.uk/notes/transport-of-oxygen/>



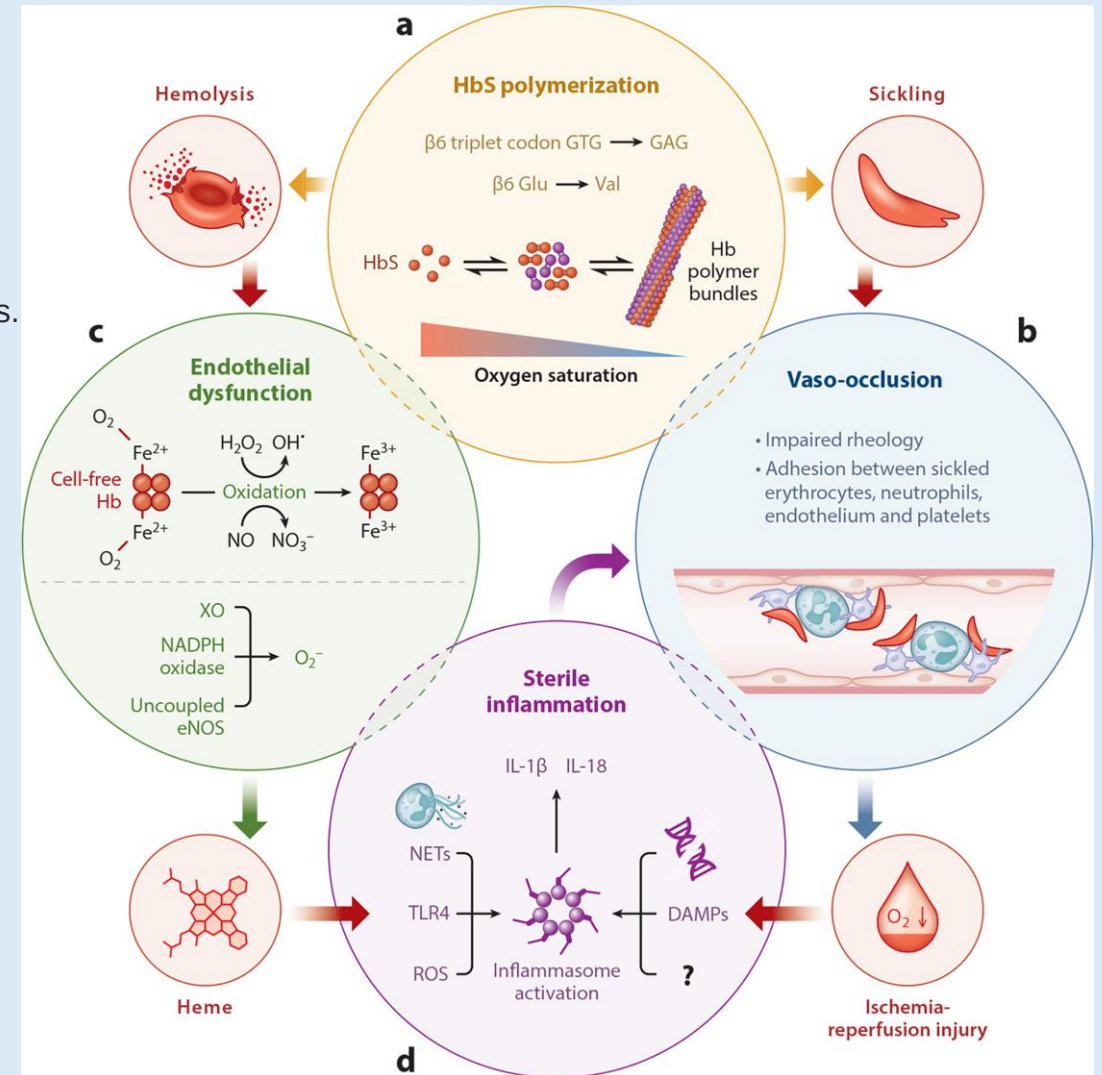
<https://biology4alevel.blogspot.com/2014/11/36-gene-mutation-sickle-cell-anemia.html>

Sickle Cell Disease – Pathophysiology

- HbS has different molecular/chemical properties to HbA.
 - Formation of polymers under conditions of stress
 - Low oxygen, acidic, cold temperatures, stress, dehydration, illness.
 - A range of clinical manifestations
 - Hemolytic anemia
 - Painful vaso-occlusive crisis
 - Multiple organ damage from microinfarcts



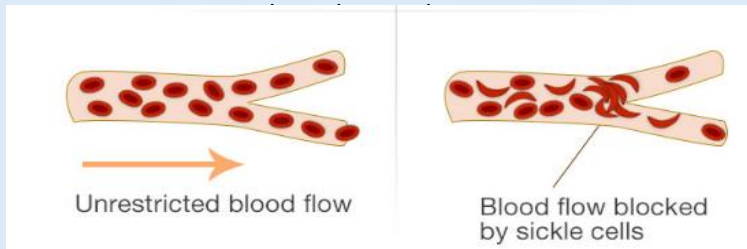
<https://www.clinicallab.com/fda-approves-first-gene-therapies-for-sickle-cell-disease-27641>



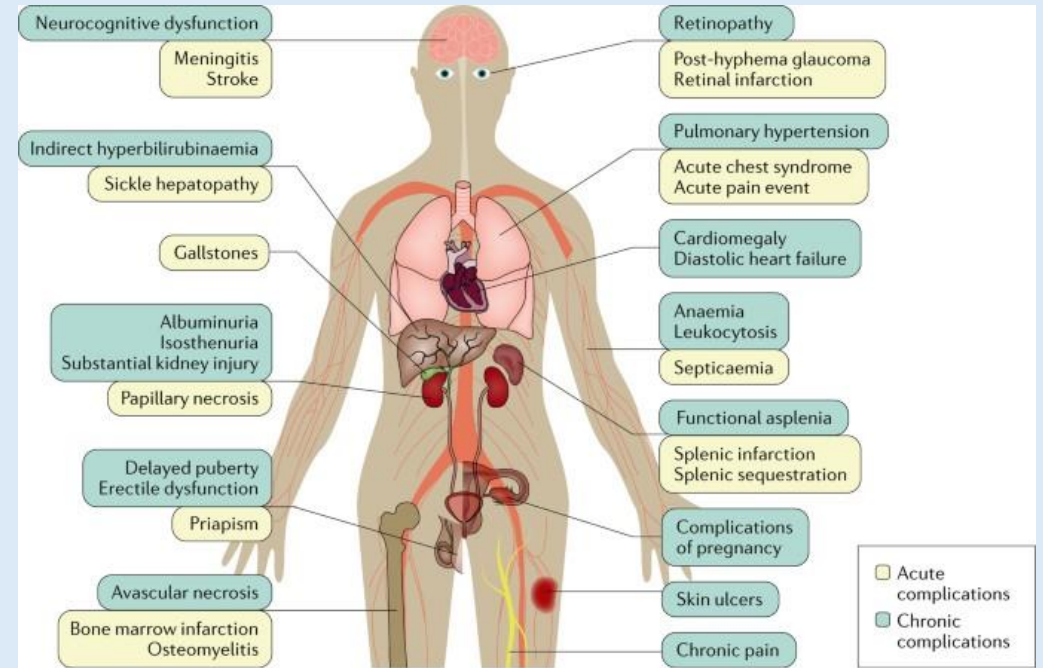
<https://europepmc.org/article/MED/30332562>

Sickle Cell Disease – Clinical Manifestations

- Diagnosis: Clinical, lab tests, genetic testing
- Mr X:
 - Mild pain – managed with paracetamol
 - Vaso-occlusive crisis, ischemia, infarction, infl
 - Chronic haemolytic anaemia (66-68 g/L)
 - Retinopathy
 - Hepatopathy, gallstones, ?haemochromatosis
 - ?Autosplenectomy – not seen on imaging
 - Cardiovascular complications (mild heart)



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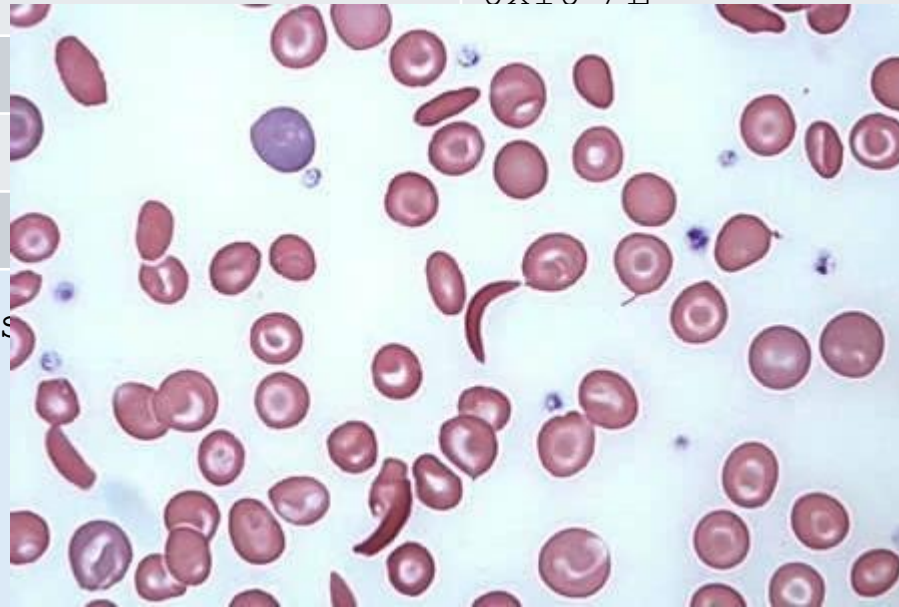
Nature Reviews | Disease Primers

[Sickle cell disease | Nature Reviews Disease Primers](#)

Case History – Investigations: CBC & hematinics

Test	Result	Reference
Hb	68	131-166 g/L (60-90 g/L)
WBCs	8.1	3.5-9.5x10 ⁹ /L
Platelets	197	150-400x10 ⁹ /L
nRBCs	1.51	0x10 ⁹ /L
Reticulocytes	405	
Ferritin	893	
B12	1021	

Blood film – numerous sickle cells
Urine dip – 3+ blood, 3+ protein



Case History - Investigations:

Biochemistry, RFTs, LFTs

Test	Result	Reference
Na	132	133-146 mmol/L
K	5.1	3.5-5.3 mmol/L
Urea	9.2	2.5-7.8 mmol/L
Cr	85	62-106 umol/L
eGFR	>90	>90 ml/min/1.73m2
Total protein	80	60-80 g/L
Albumin	45	35-50 g/L
Glucose	4.6	3.9 to 5.5 mmol/L
Total bilirubin	46	0-21 umol/L
Alkaline phosphatase	571	30-130 IU/L
AST	76	0-40 IU/L
VitD	43.3	50 to 125 nmol/L
PTH	7.6	1.6-6.9 pmol/L

UCR=29.6 (prerenal causes)

Latest K was high - lisinopril stopped

Lipid profile within normal limits

Nephrology Monitoring

- **Proteinuria** is a common feature of **sickle cell nephropathy** (kidney involvement secondary to **SCD**), reflecting kidney function.
- **uPCR**
 - indicates the the urine rel concentratic
 - Varies, <15
- **Nephrotic range** significantly eleva urine (>3.5 g/day)
- **Sudden rise**

Parameters	Diagnostic criteria
Proteinuria	>3-3.5 g/24 h or spot urine protein : creatinine ratio of >300-350 mg/mmol
Albumin	Serum albumin <25 g/l
Edema	Clinical evidence of peripheral edema
Hyperlipidemia	Severe hyperlipidemia (total cholesterol often >10 mmol/l) is often present

Urine Prot/Creat Ratio

Reference range: (0 - 15)	
Units: mg/mmol Creat	
Collected ▲	Result
12 Sep 2022 17:00	287
27 Jan 2023 15:55	438
24 Jul 2023 14:40	981
04 Aug 2023 00:00	*665
14 Sep 2023 11:30	*321
03 Oct 2023 18:30	*290
26 Oct 2023 11:20	*539
10 May 2024 11:35	*636

https://www.researchgate.net/figure/Diagnostic-criteria-for-nephrotic-syndrome_tb11_3402627

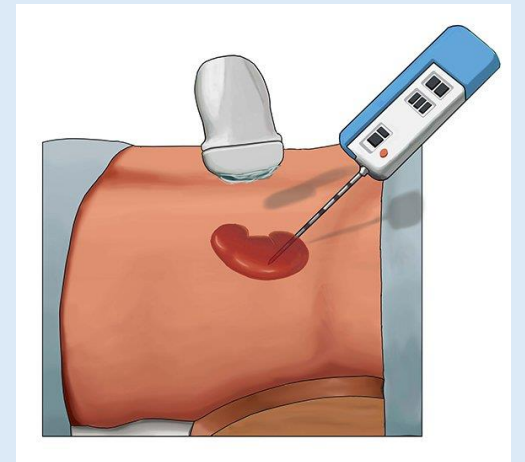
Lower Range Upper Range Value

Case History – Investigations: Immunology

- **RF** raised; RA, other autoimmune diseases, **non-specific**: chronic inflammation, infection..
- **ANA** weakly positive, **negative on repeat**; SLE, other autoimmune diseases, **non-specific**: chronic inflammation, infection..
- **Anti-dsDNA** **negative**
- **Complement** **normal**
- **ANCA** **negative on repeat**; autoimmune and inflammatory diseases
- **IgA** raised; **non-specific, could be elevated in liver disease, infections, chronic inflammation**, autoimmune diseases.. **IgA nephropathy was considered**
- **Complement level** **normal**; if increased or decreased could indicate autoimmune diseases, chronic inflammation, infection..
- **Polyclonal increase in immunoglobulins** – (IgG elevated too) generalized activation of the immune system not unusual in **SCD** which is an inflammatory condition
- No specific pattern

Biopsy

- Elective kidney biopsy to investigate the cause of nephrotic-range proteinuria and its sudden rise
- Rule out secondary diseases – IgA nephropathy
- Admitted overnight for monitoring – crisis
- Improved uPCR after bx, no changes to management though



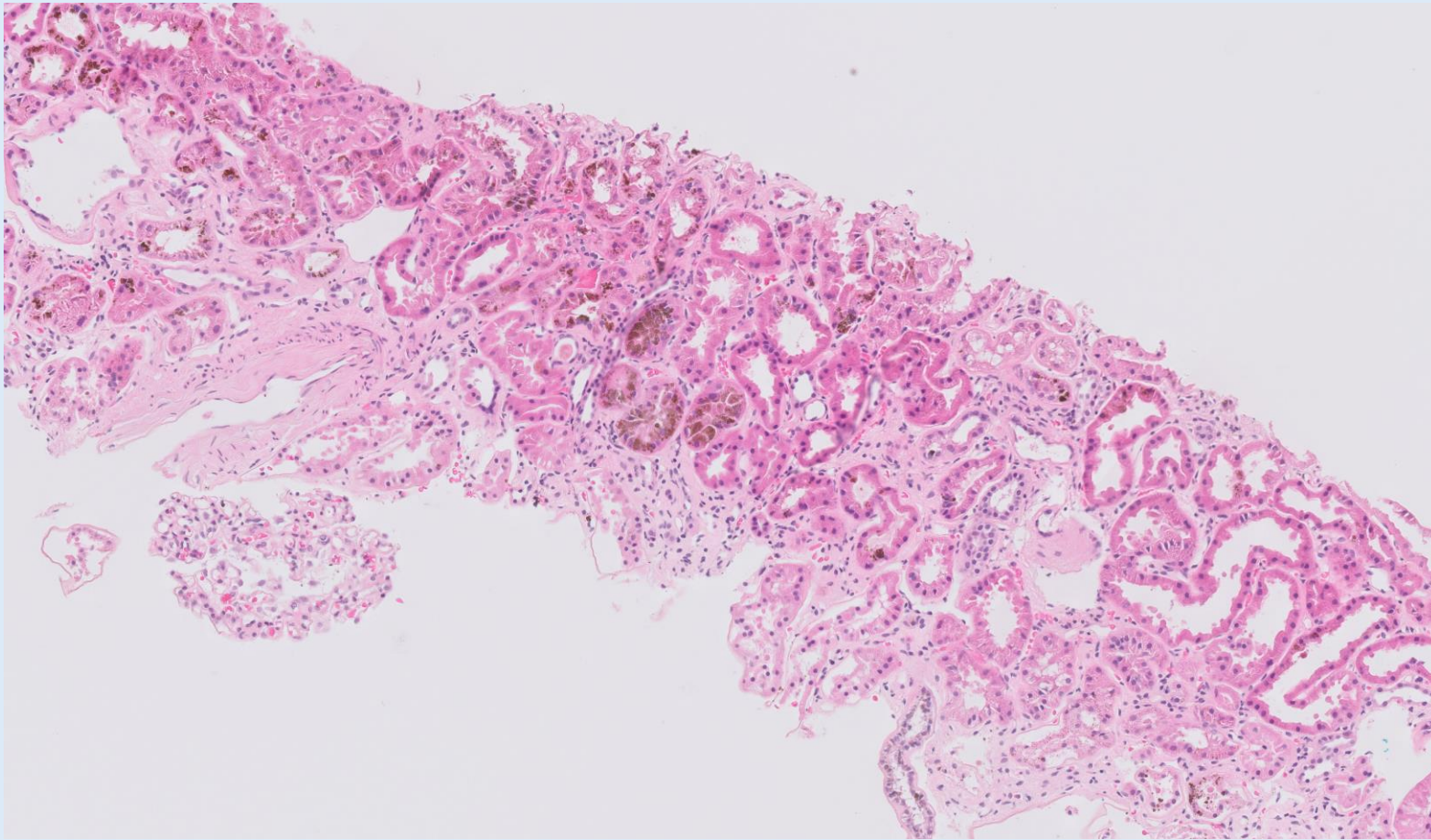
<https://www.healthtravellersworldwide.com/treatments/kidney-biopsy/>

Light Microscopy

Light Microscopy

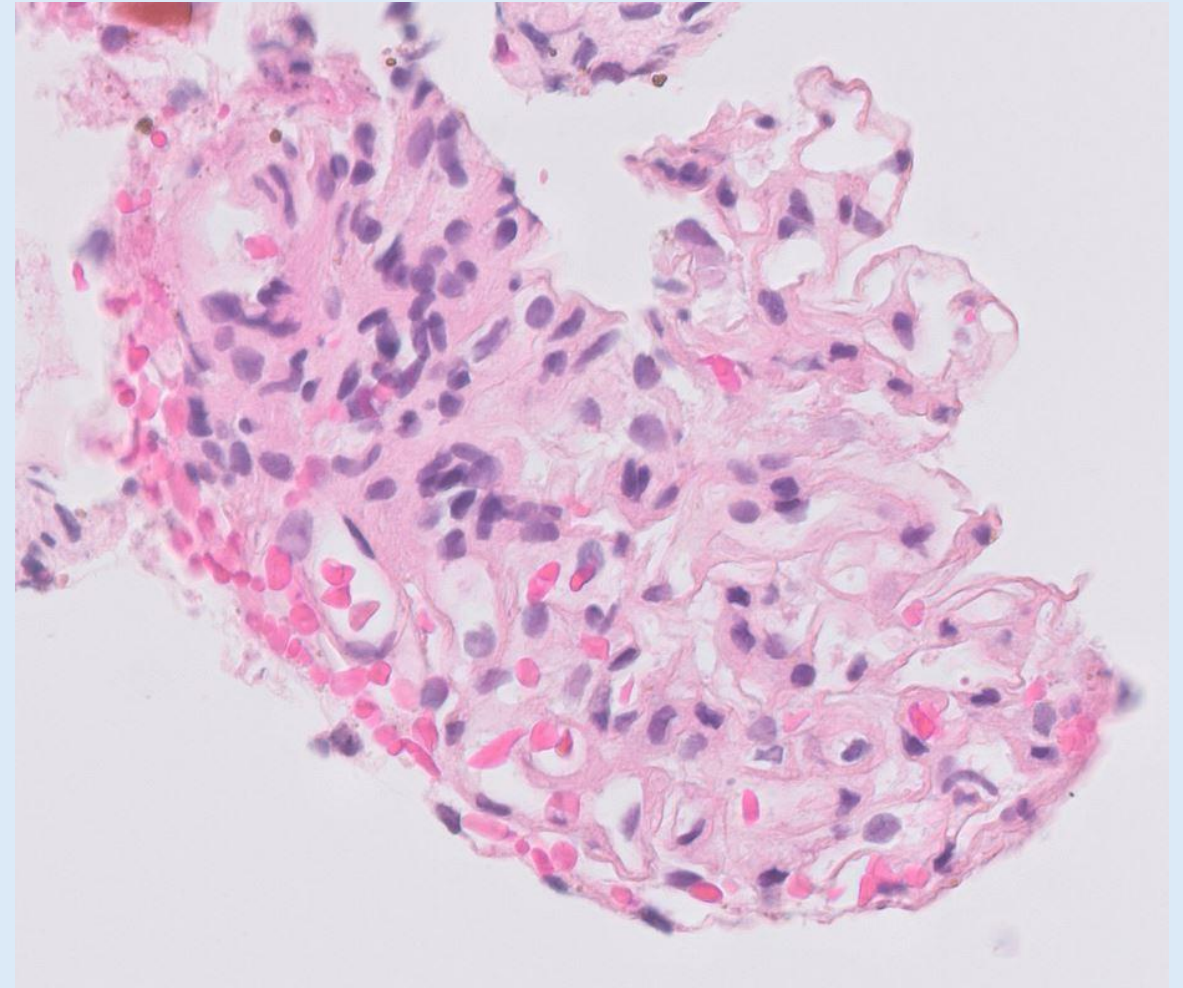
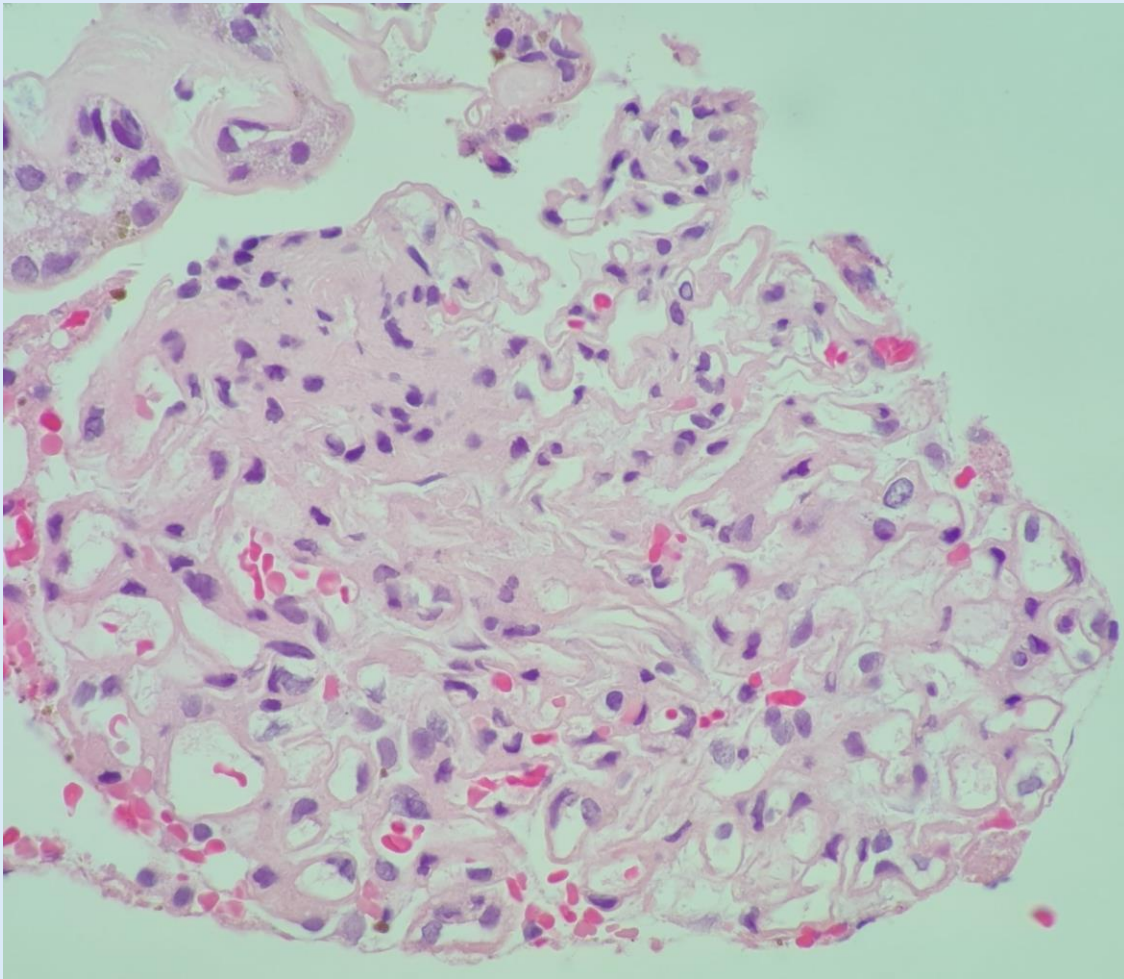


Light Microscopy



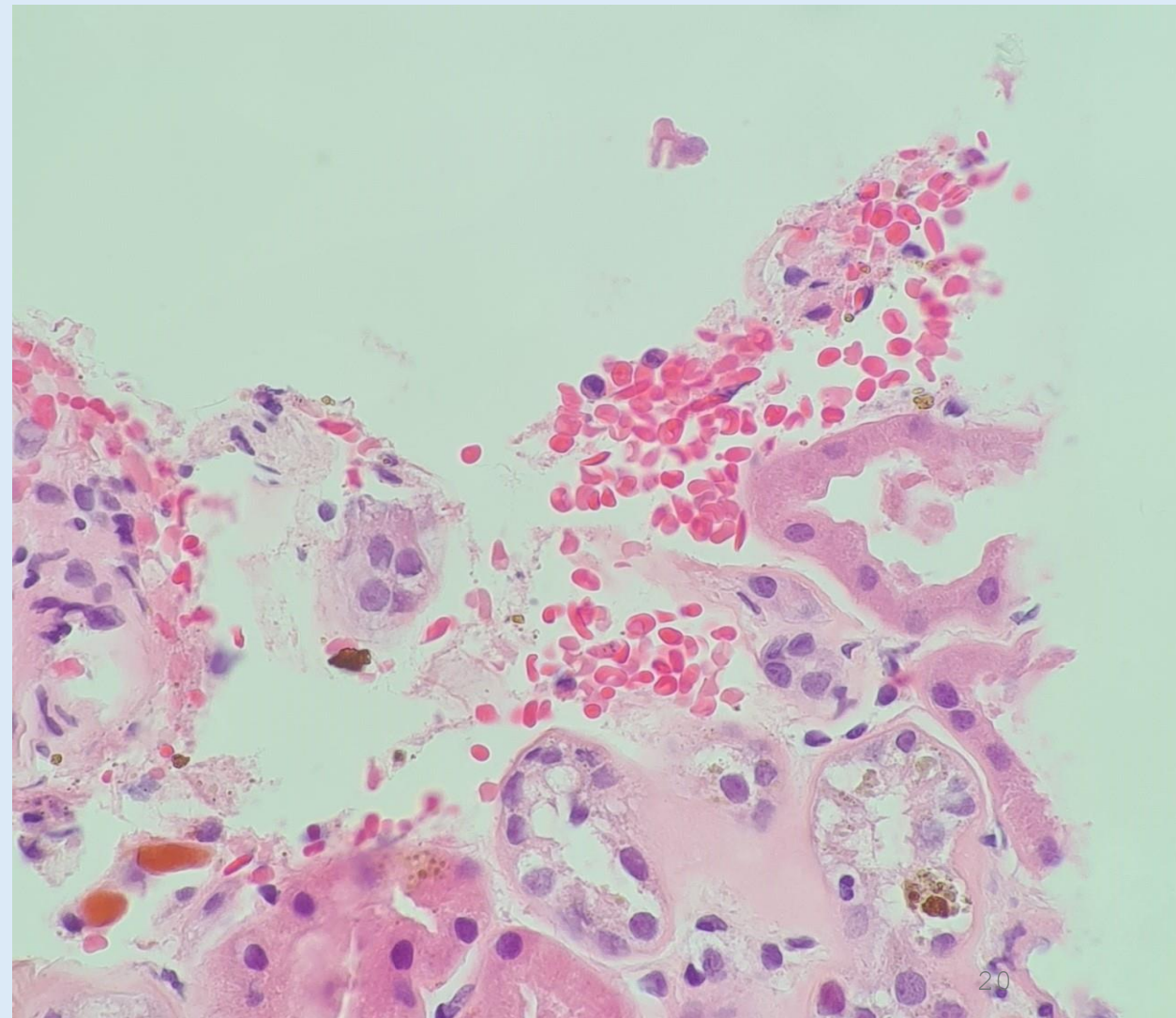
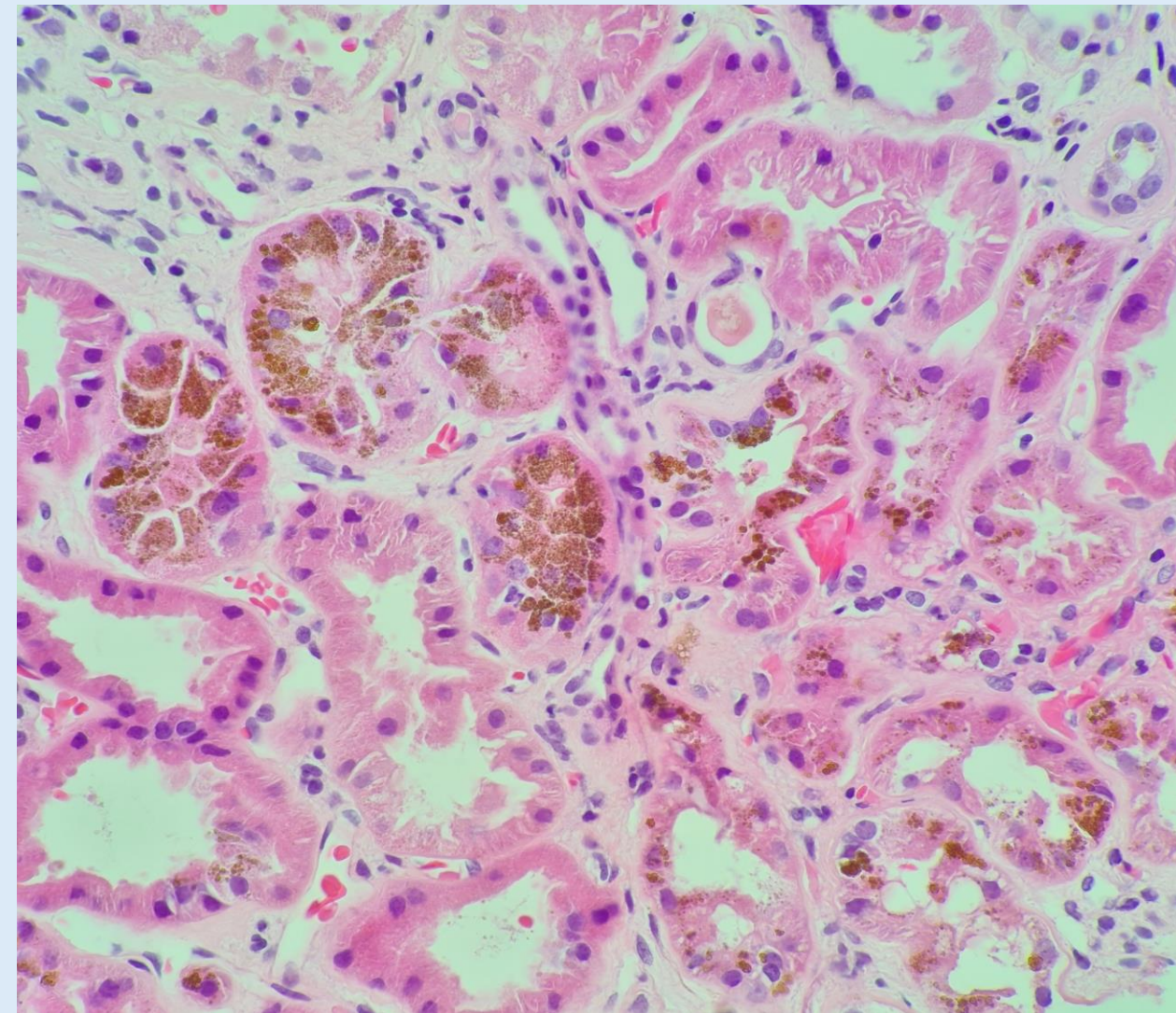
Light Microscopy 400x

A glomerulus showing focal segmental glomerulosclerosis.



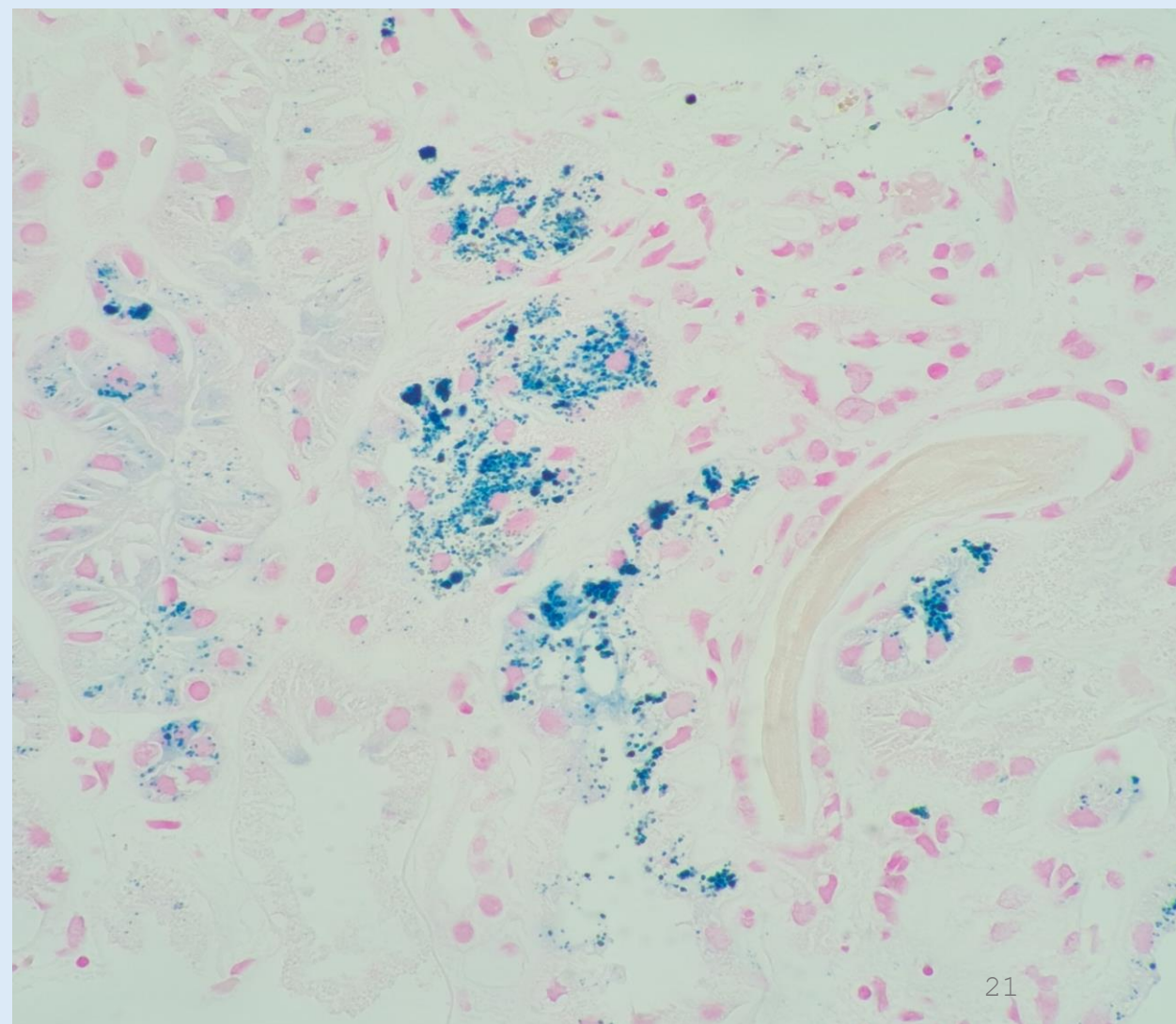
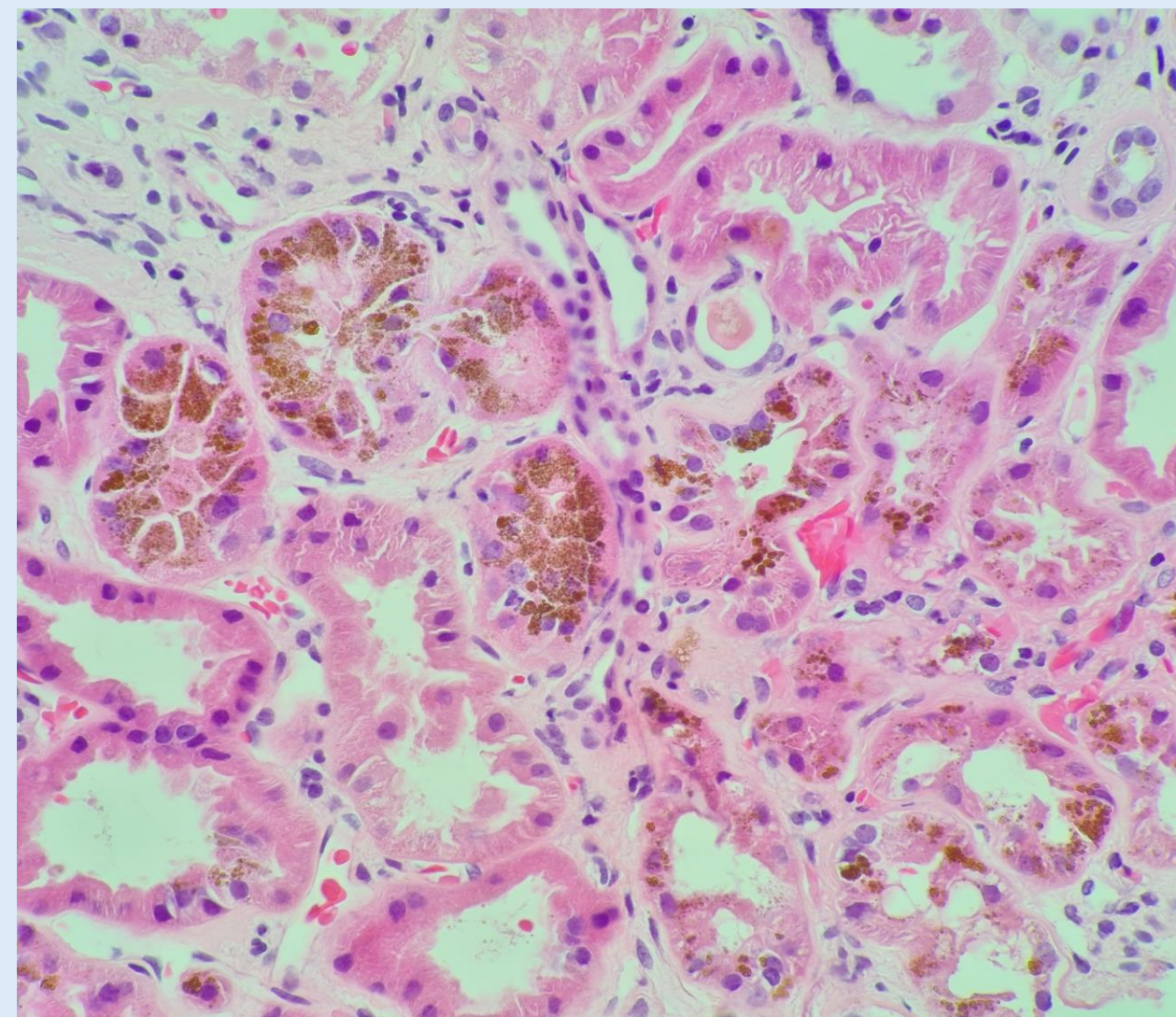
Light Microscopy 400x

- Hemosiderin deposition in tub
- Sickled erythrocytes
- Aggregates



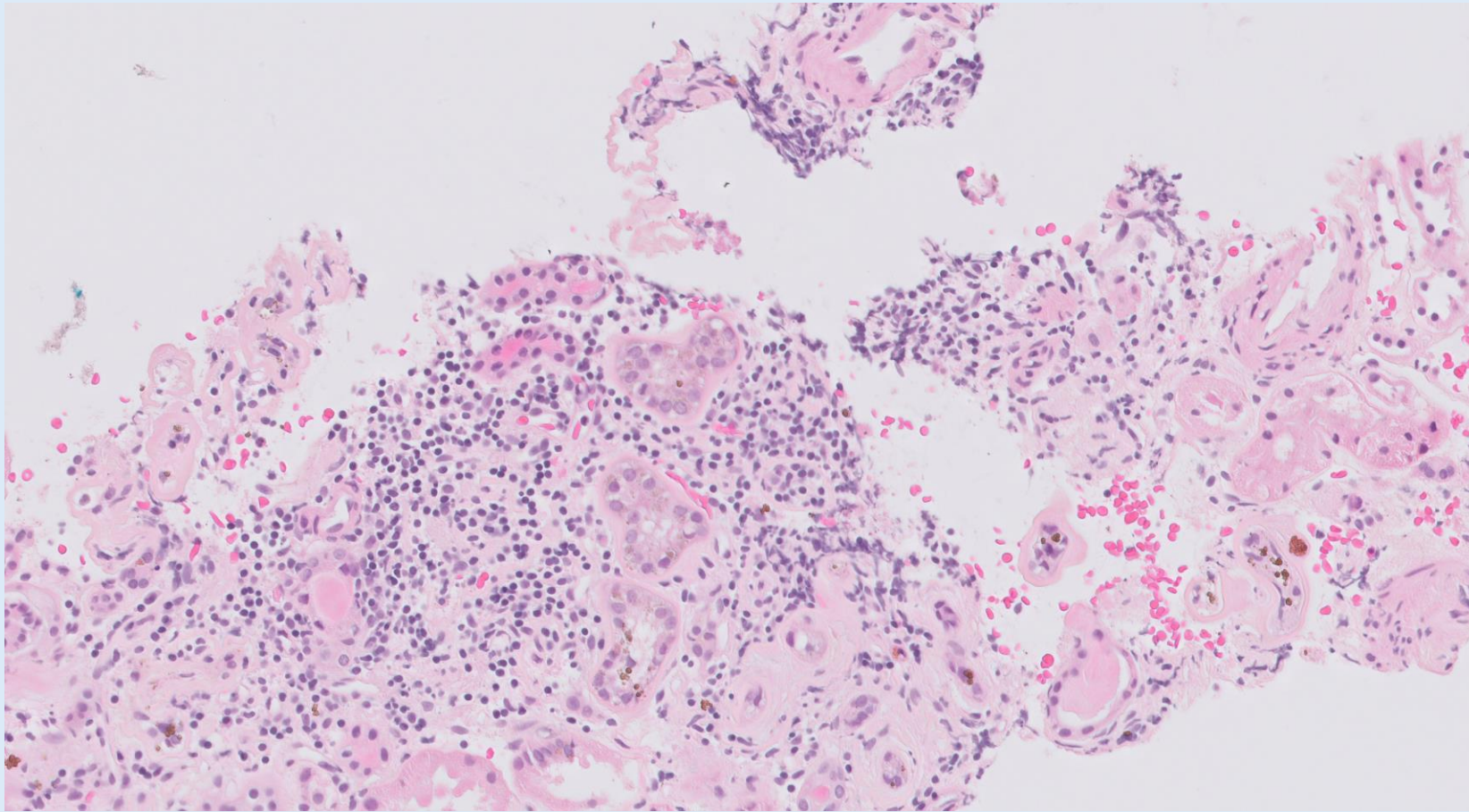
Light Microscopy 400x

- Perls' Prussian blue stain highlighting ferric iron associated with hemosiderin predominantly in renal tubular cells.



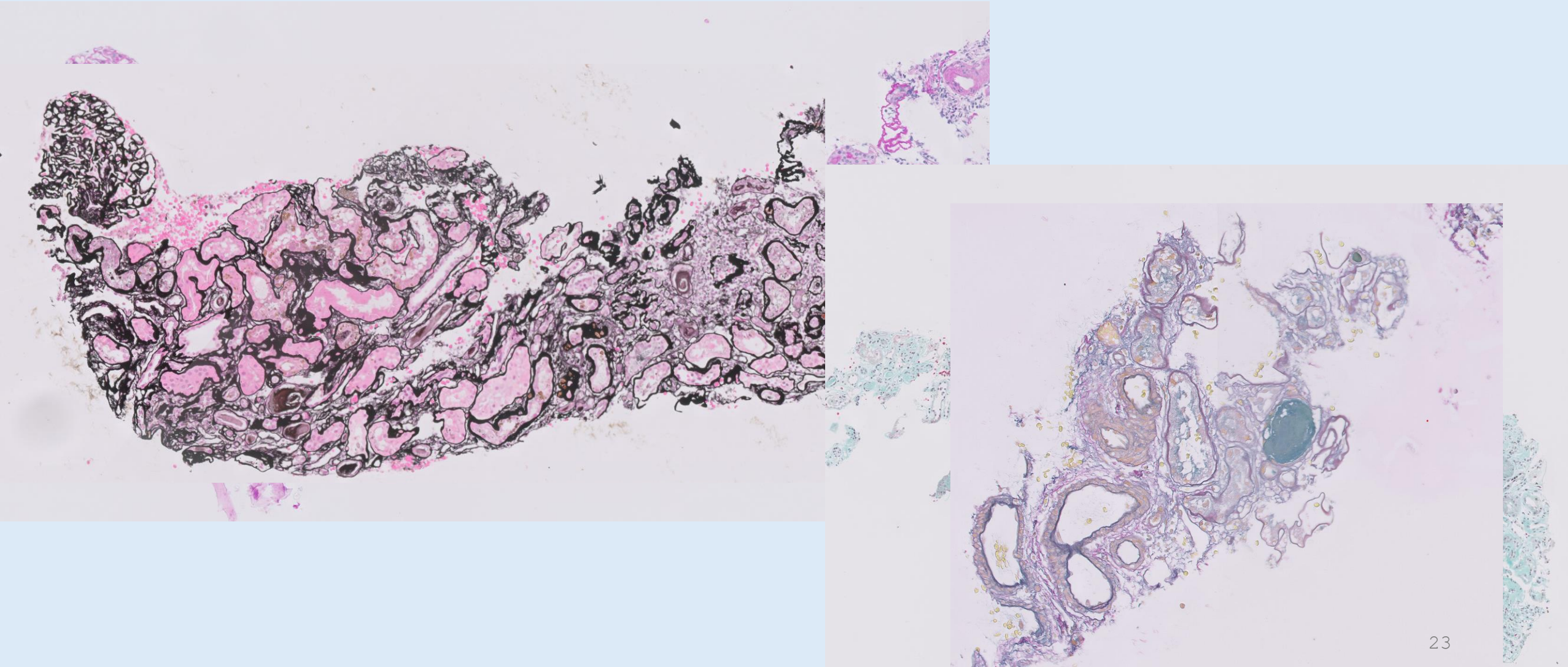
Light Microscopy

- Moderate tubular injury



Light Microscopy

- 20% IFTA
- Vessels unremarkable

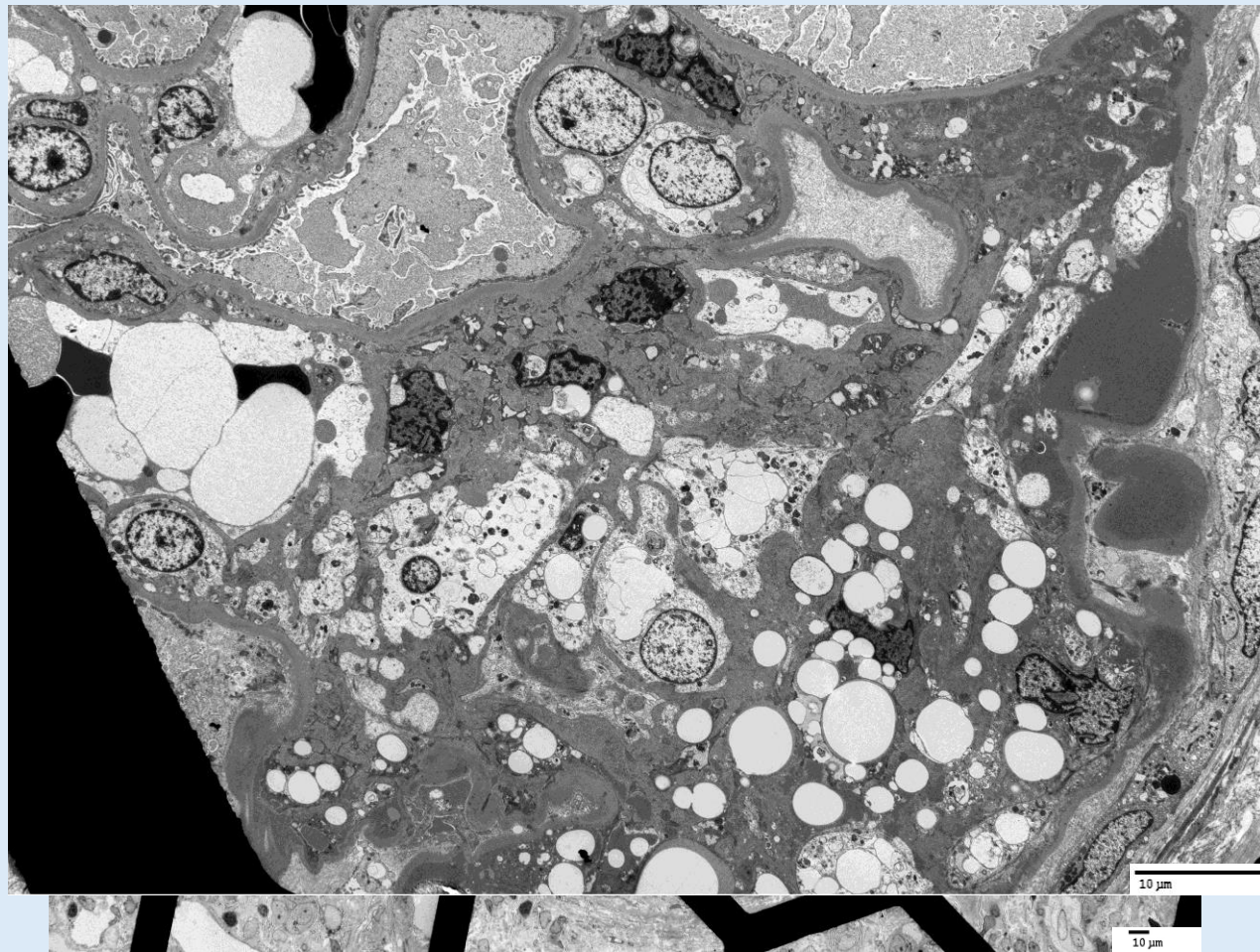


Immunofluorescent Microscopy

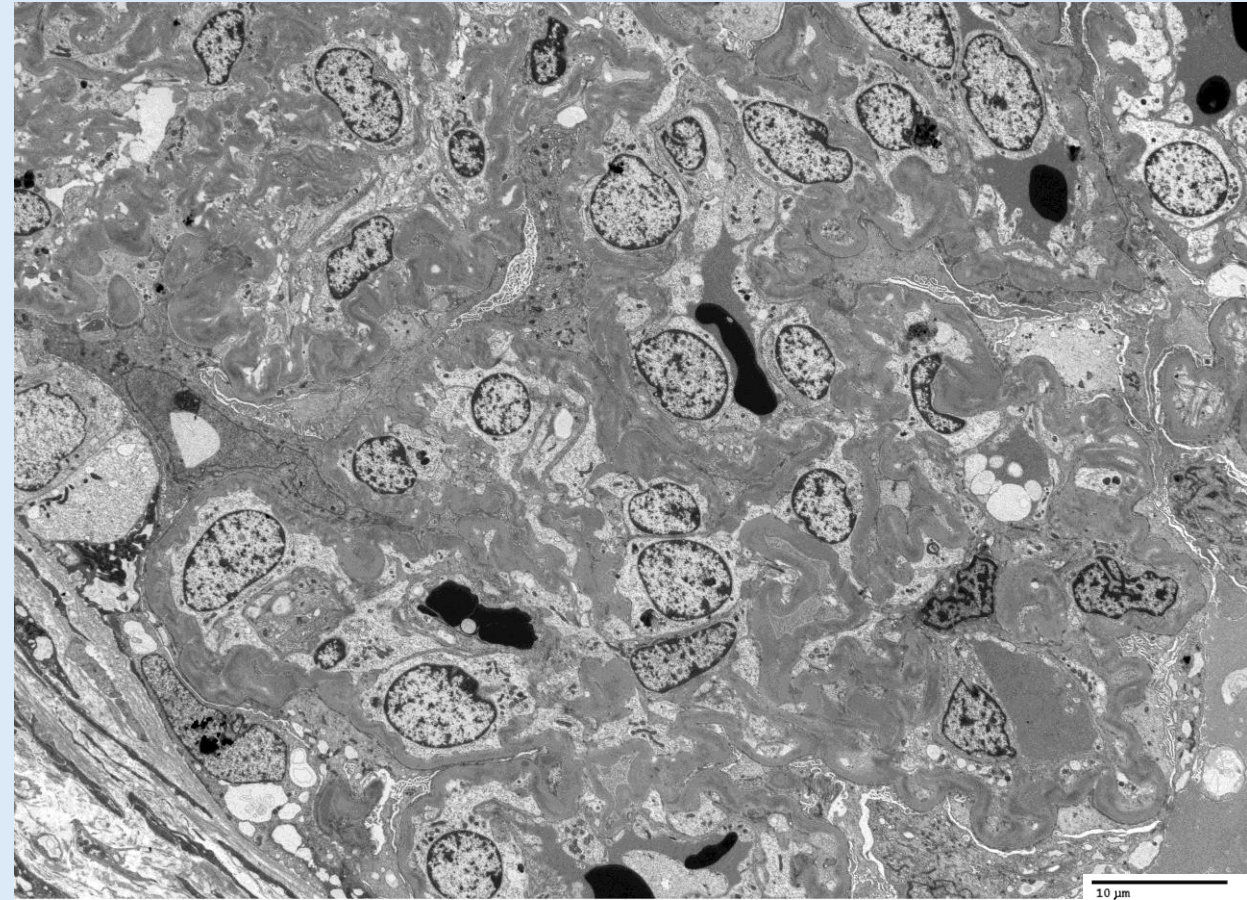
No immune reagents

Electron Microscopy

Electron Microscopy



- Glomerulomegaly/ hypertrophy
- Focal segmental glomerulosclerosis



- Almost obsolete with severe GBM wrinkling

- Aggregated of sickled (elongated misshapen)

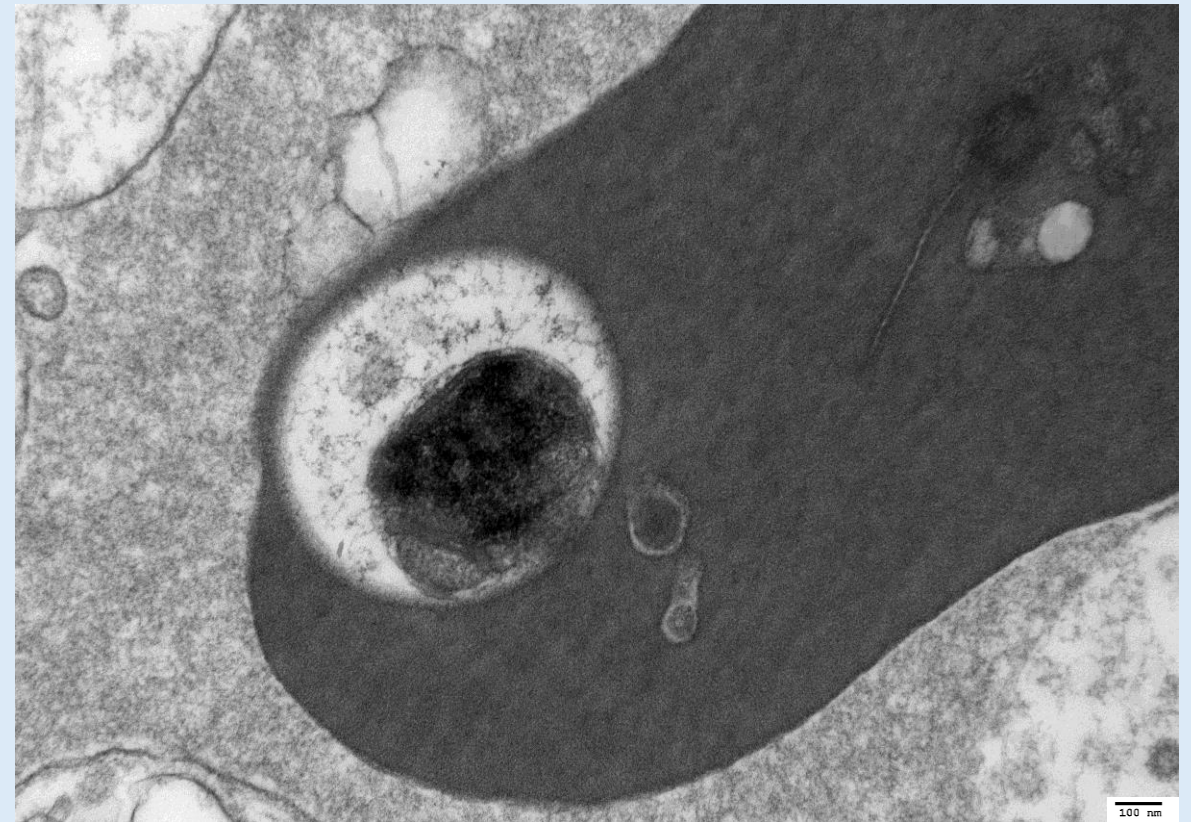
Ele

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ted mesangial cell

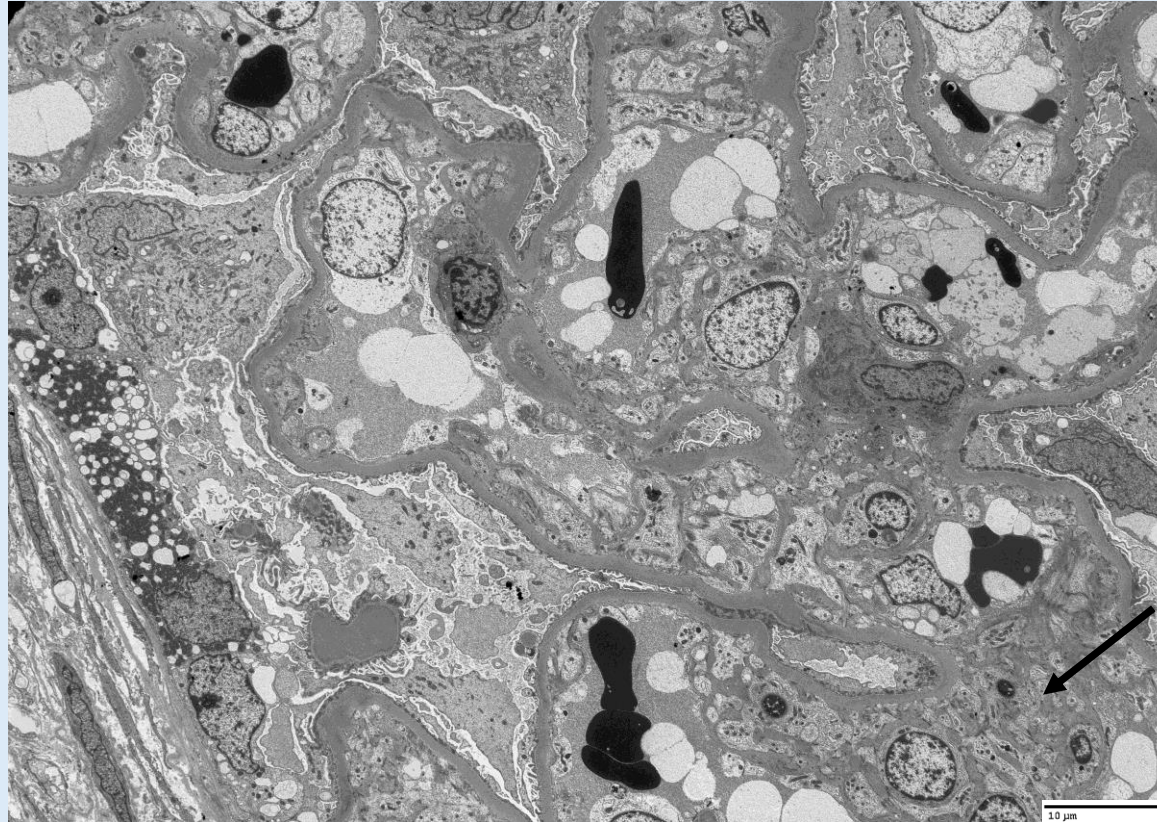


Electron Microscopy

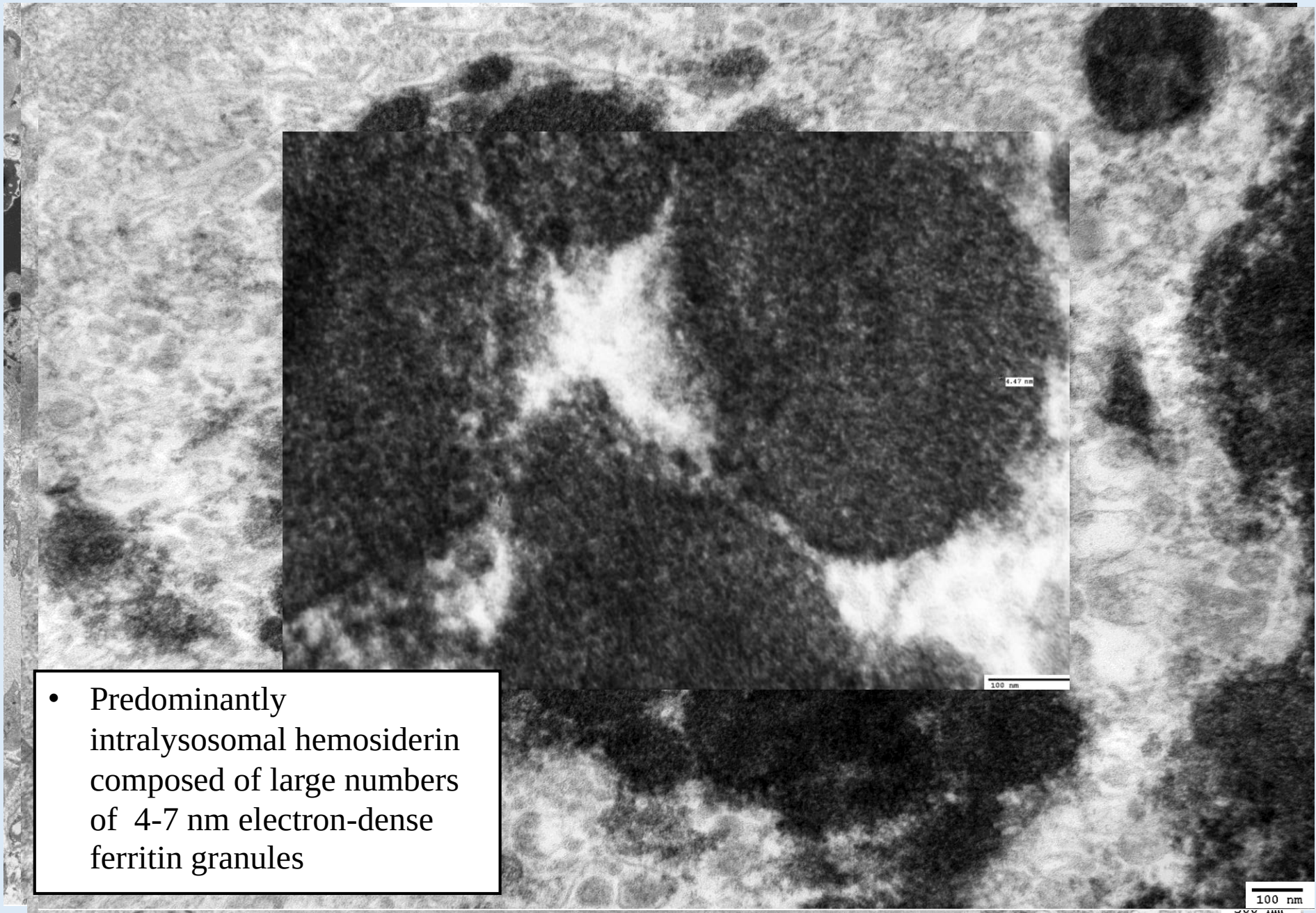
- Howell-Jolly bodies are present within erythrocytes.
 - Nuclear remnants usually removed in the spleen - a/w (auto)splenectomy



Electron Microscopy



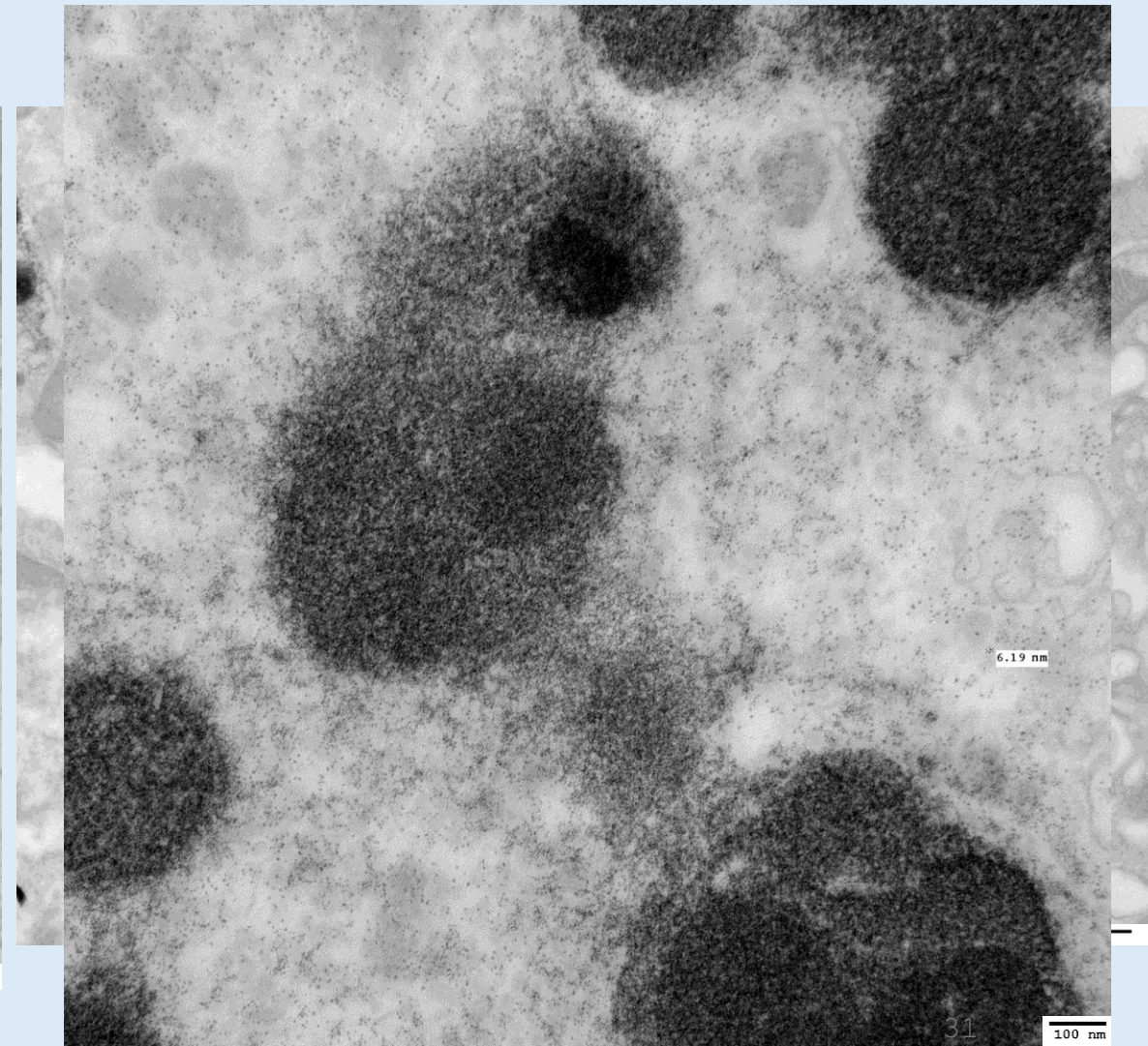
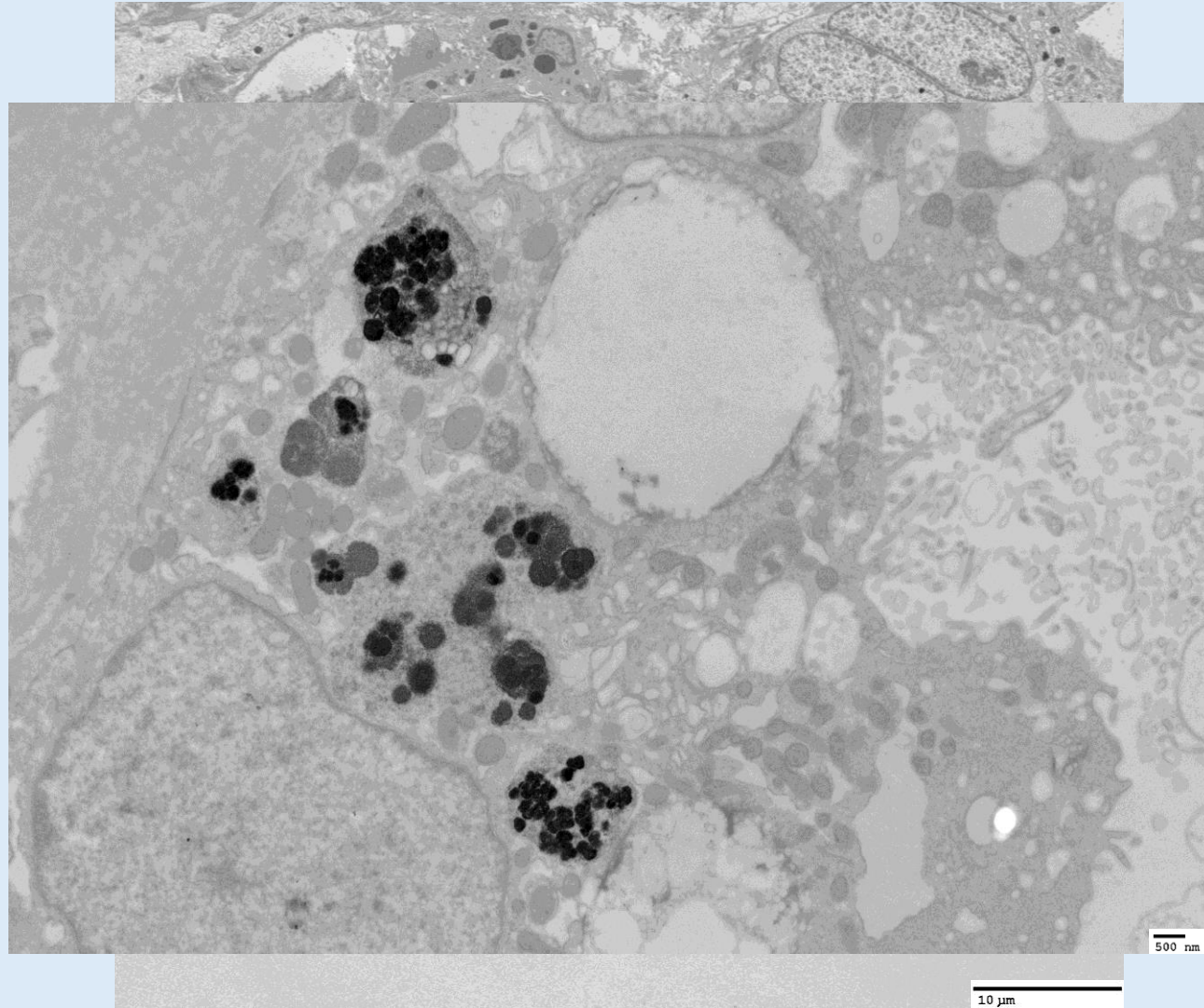
- Focal mesangial proliferation
- No mesangial, subendothelial or subepithelial electron-dense deposits.



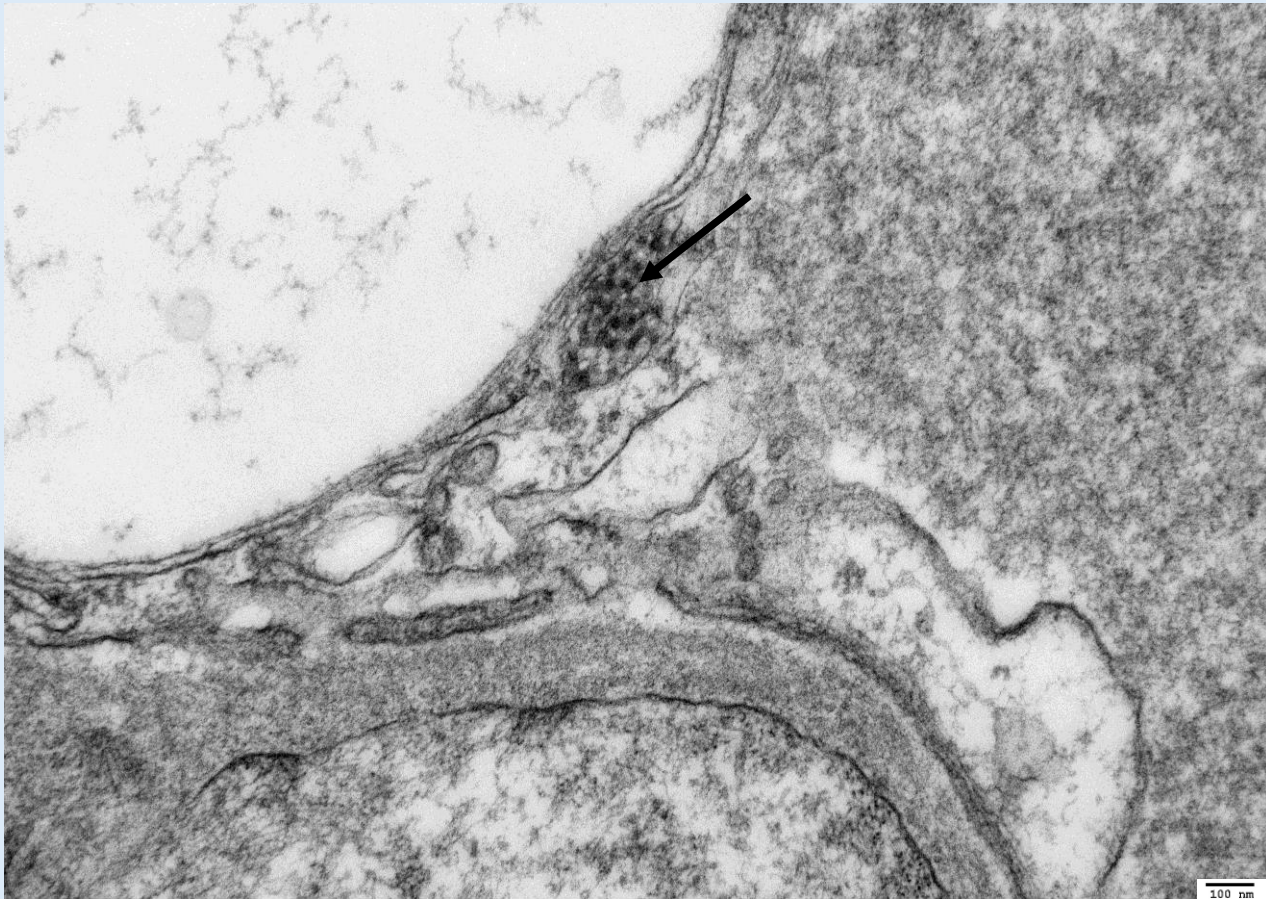
- Predominantly intralysosomal hemosiderin composed of large numbers of 4-7 nm electron-dense ferritin granules

Electron Microscopy

- Predominantly intralysosomal hemosiderin composed of large numbers of 4-7 nm electron-dense ferritin granules



Electron Microscopy



- Moderate numbers of endothelial tubuloreticular inclusions (TRI's) up to 368 nm.
- Tightly packed electron-dense microtubules forming a reticular (network-like) pattern.
- Their presence is indicative of systemic immune activation.
- In literature associated with SLE (autoimmune diseases), HIV, HCV (viruses) & interferon therapy. But in practice we see them often – Non-specific
- Mr X - HIV, Hep C and Hep B screen negative.

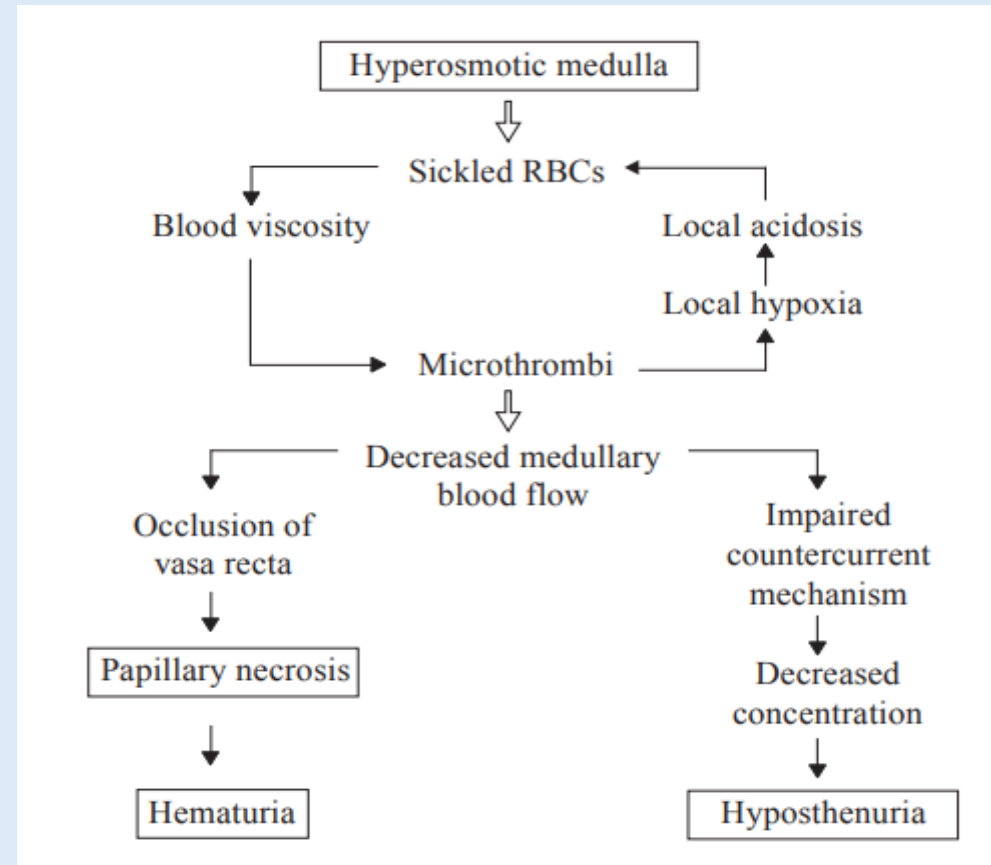
Overall Impression

- In keeping with the clinical impression of sickle cell nephropathy
 - Glomerulomegaly
 - Aggregates of sickled erythrocytes, many containing Howell-Jolly bodies
 - Hemosiderin deposition
 - GBM wrinkling
 - IFTA
- The possibility of FSGS (primary vs secondary)
 - Nephrotic-range proteinuria
 - FSGS morphology
 - Severe podocyte foot effacement
 - No electron-dense deposits or immune-reactants
- Thickening of the GBM
 - Uncertain significance?

Sickle Cell Nephropathy (SCN)

SCN – Clinical Manifestations

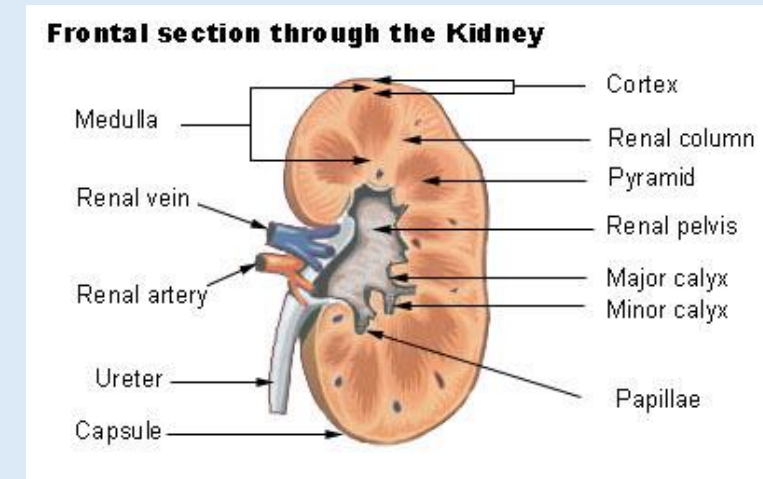
- Renal damage/dysfunction due to SCD.
- Varies from patient to patient in its clinical and histological manifestations.
- Usually does not need a biopsy for diagnosis (history, blood tests, urine tests +/- imaging suffice)
- Multifactorial process:
 - Vaso-occlusion and ischemia (chronic hypoxia)
 - Chronic hemolysis with the release of toxins and injury
 - Compensatory hyperfiltration and hypertrophy



<https://www.sciencedirect.com/science/article/pii/S1561541309601204>

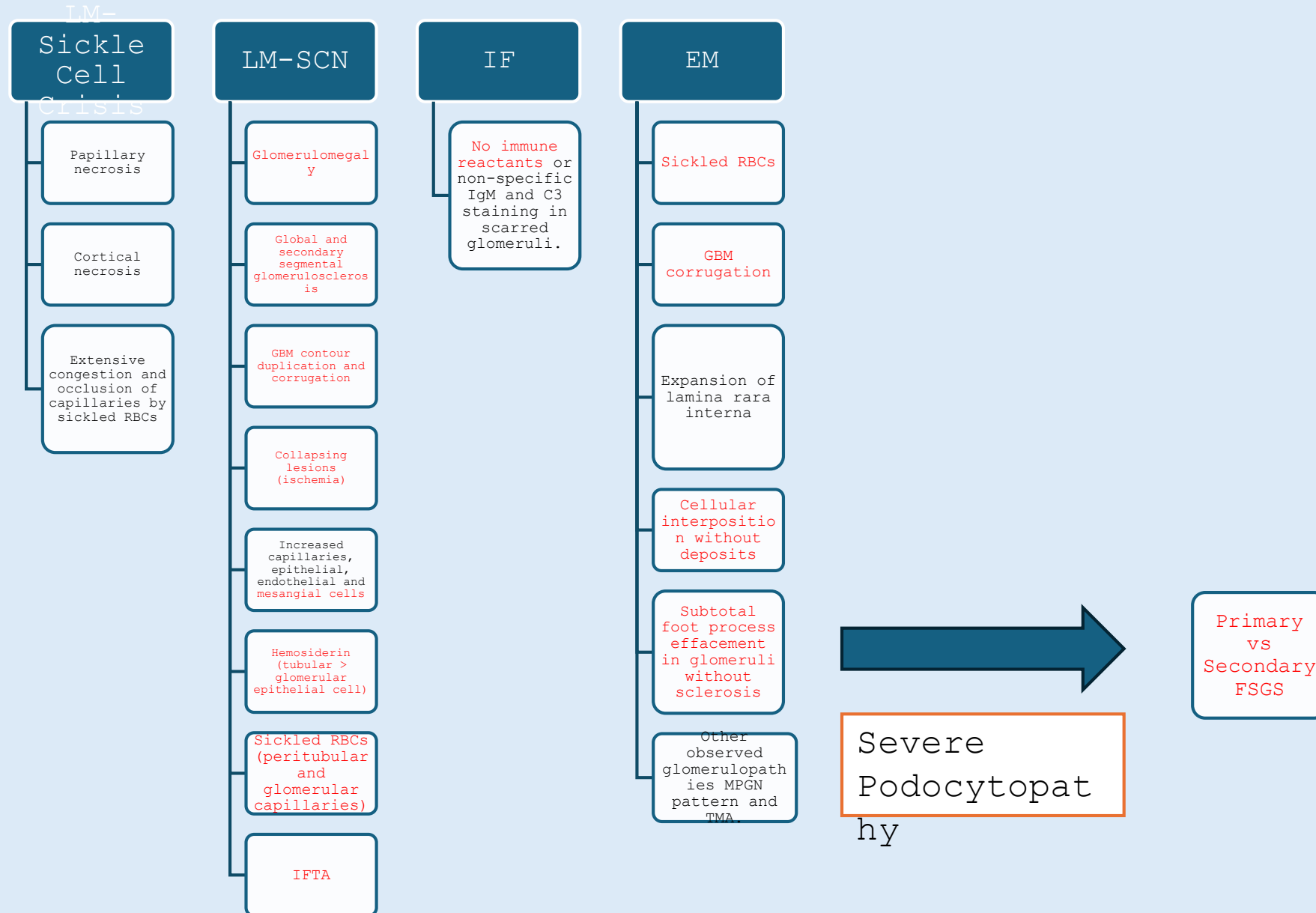
SCN – Clinical Manifestations

- Asymptomatic hematuria (RPN)
- Tubular abnormalities:
 - Hyposthenuria (reduced concentration of solutes in the urine)
 - RTA (do not properly remove acids from the blood into the urine)
 - Concentrating problems (PO, Na, K)
 - Hyperfunctioning proximal tubules (Cr & uric acid secretion preserved – GFR maintains normal for longer (hyperfiltration as compensation to intravascular hemolysis))
- Sickle cell glomerulopathy:
 - Microalbuminuria in childhood (ACR: 3-30 mg/mmol)
 - Overt proteinuria in 20-25% (PCR Mild: 30-150, Moderate 150-500, Severe >500 mg/mmol)
 - Nephrotic syndrome in 5% (PCR >300 mg/mmol + criteria)
 - Progressive GFR loss (CKD) after the age of 20 years in 5-8%
- Sickle cell crises: Renal papillary necrosis and renal infarction/AKI/ARF
- CKD



<https://training.seer.cancer.gov/anatomy/urinary/components>

SCN – Pathologic Features



FSGS – Which subtype did Mr X have?

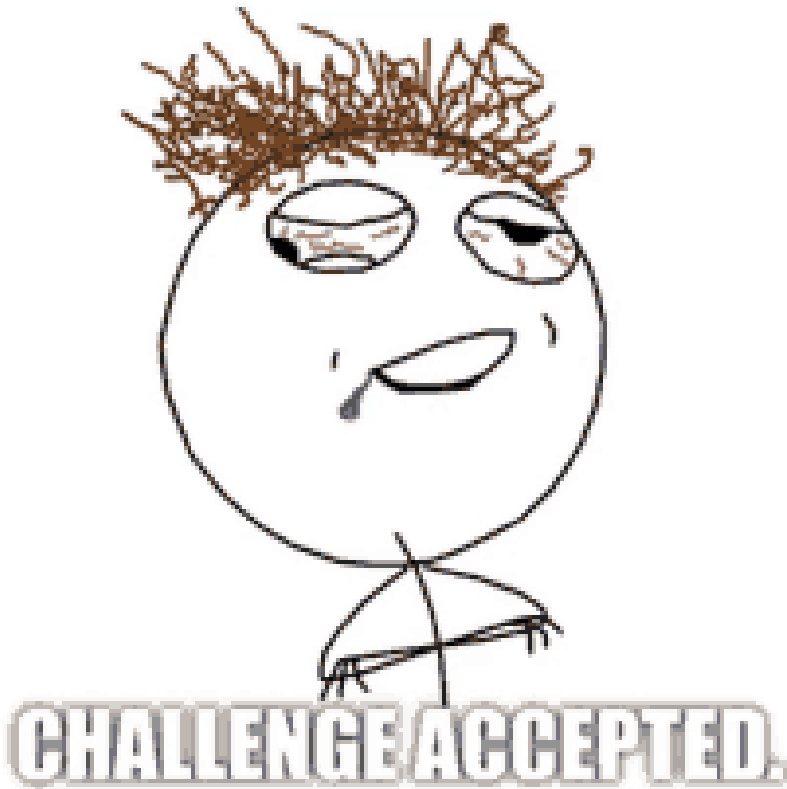
- Scarring/sclerosis of a segment of some glomeruli.
- Subclasses of FSGS include:
 - Primary (?circulating factors causing generalized injury to podocytes, corticosteroids responsive)
 - Genetic (mutations encoding vital podocyte proteins, NPHS1/2 coding Nephrin and Podocin, steroid resistant, calcineurin inhibitors may be effective)
 - Secondary (manage the cause)
 - Maladaptive (mismatch between glomerular load and capacity due to conditions like hyperfiltration and glomerulomegaly)
 - Viral
 - Drug
- Features overlap
- No light microscopic features are pathognomonic for a particular FSGS subcategory.
- Clinicopathological approach

Differentiating Primary, Genetic, and Secondary FSGS in Adults: A Clinicopathologic Approach

An S. De Vriese,¹ Sanjeev Sethi,² Karl A. Nath ,³ Richard J. Glassock,⁴ and Fernando C. Fervenza ³

¹Division of Nephrology, AZ Sint-Jan Brugge-Oostende, Brugge, Belgium; ²Department of Anatomic Pathology and
³Division of Nephrology and Hypertension, Mayo Clinic, Rochester, Minnesota; and ⁴Geffen School of Medicine at the University of California, Los Angeles, California

Primary FSGS	
Clinical	NS
LM	FSGS Often no other damage (unless late in disease course) Glomerulomegaly uncommon
EM	Diffuse FPE (>80%)
NS, nephrotic syndrome. ^a Characterized by thickening and wrinkling of	

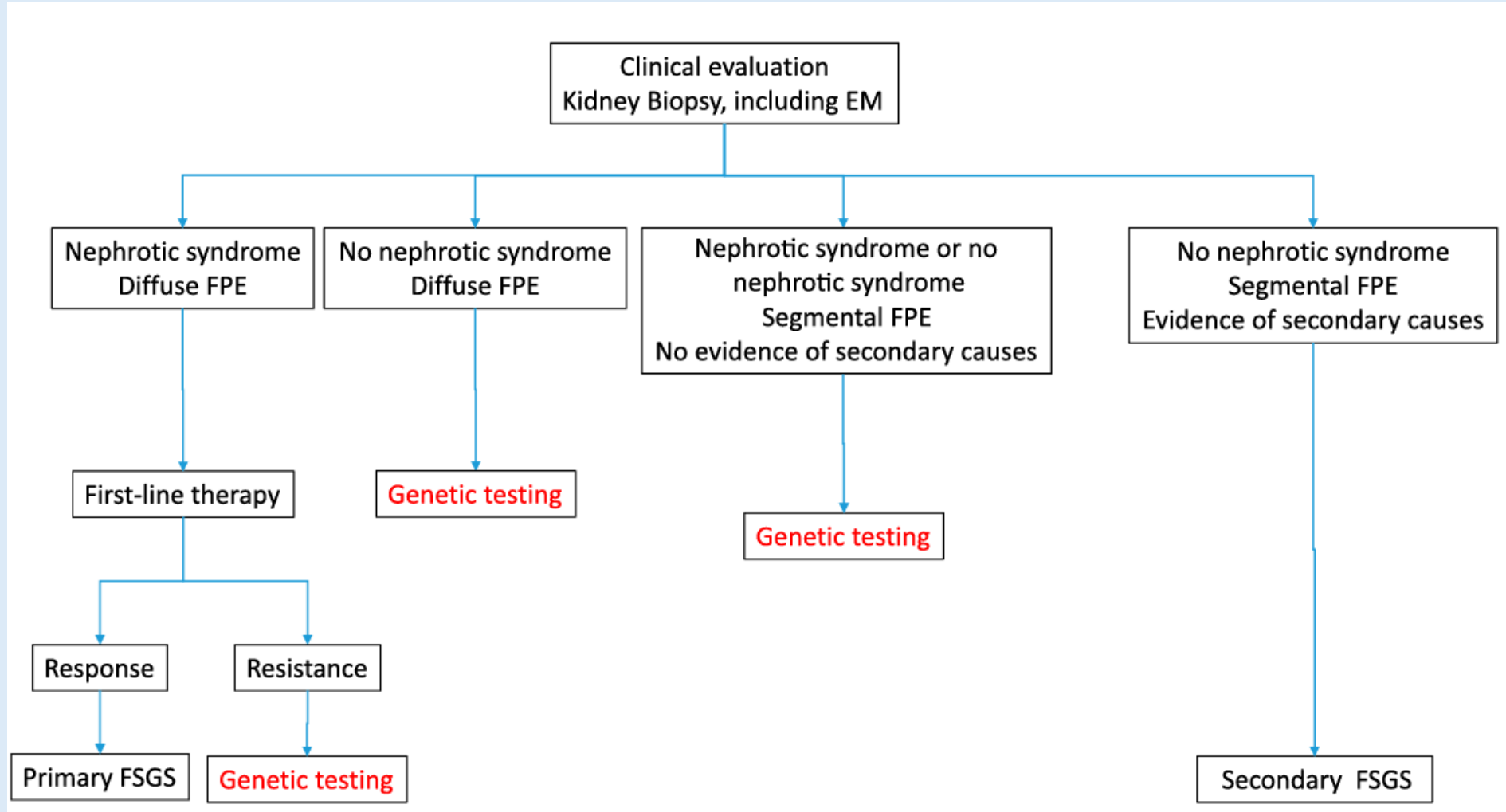


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IF: All show nonspecific IgM and C3 staining.

Differentiating Primary, Genetic, and Secondary FSGS in Adults: A Clinicopathologic Approach

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Management

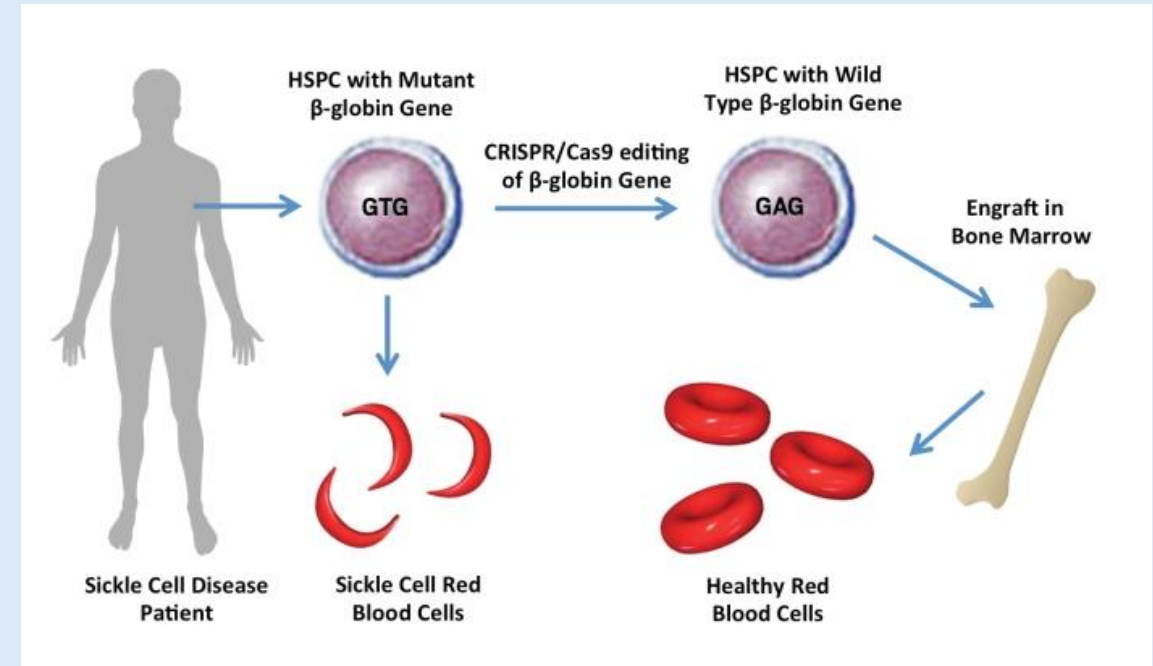
- Multidisciplinary team effort
 - Patient education (detect and prevent crisis, lifestyle, importance of follow up, psychosocial support, family planning)
 - Supplements – vitamin D, folate, B12
 - Prevention and early control of infections – prophylactic antibiotics and vaccines
 - Manage multisystem complications under relevant teams. In terms of SCN:
 - Hematuria and RPN – self-resolving, hydration with hypotonic solutions +/- transfusion
 - Proteinuria – ACE-I and ARBs for proteinuria (controls bp, manage hyperfiltration injury, cardio-renal protective, dilate bv reduce pressure)
 - AKI - manage cause (infection, dehydration..)
 - CKD and ESRD – erythropoiesis-stimulating agents, dialysis, transplant (immunosuppression risky)
 - APOL1 high risk variants testing – higher risk of FSGS, SCN and albuminuria and incurs a worse prognosis, African descent – clinical criteria

Management

- Managing pain during vaso-occlusive crises - PCM, avoid NSAIDs if possible (renal vessels constriction)
- Hydroxycarbamide - HbF, severe genotypes, disease complications, guidelines
- Blood transfusion - acute vs chronic, guidelines
- BMT or hematopoietic stem cell transplantation (HSCT)
 - Potential for cure
 - HSCT commissioned
 - Severe disease not controlled by medication/ requiring regular RBC exchange
 - Transplant-related complications like GvHD, infections, reduced immunity toxicity, long-term monitoring
 - Mr X was stable so was not offered this as risks outweigh benefits

Management

- Genome editing
 - CRISPR-Cas9, (gene editing technology) which allows precise modification of DNA sequences (single nucleotide substitution).
 - The goal is to correct the mutation in the HBB gene in HSCs to produce functional hemoglobin and eliminate the production of sickle hemoglobin.
 - Ex-vivo and in-vivo approaches
 - Off-target effects, efficiency, immunogenicity, ethical considerations related to genoma editing
 - Labs to clinical trials to clinical practice
 - FDA approved, used in the US
 - Currently under consideration by NICE, provisional MHRA approval, cost might be an issue, potential for cure



<https://cen.acs.org/articles/94/i41/CRISPR-edits-sickle-cell-mutati>



Conclusion

- Case presentation – Mr X
- Histological light and electron microscopy findings
- SCD and SCN
 - Pathophysiology
 - Clinical manifestations
 - Management

Sickle cell nephropathy is a **complex disease** with **varying manifestations** which necessitates an **effective collaborative** approach between haematologists, nephrologists, pathologists and all teams involved to best **optimise patient outcomes.**

Acknowledgments

- Mr Bart E Wagner
- Dr Ali Al-Omari
- Dr Clare Samuelson
- Dr Ragada El-Damanawi
- Mr Graeme Wild

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