Role of Novel Biomarkers in Glomerular Disease

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Introduction

Effective management of glomerular kidney disease requires:

- Diagnosis
- Risk Prediction
- Therapy Guidance
- Ideally, prediction of drug response

For glomerular diseases, kidney biopsy remains the gold standard that for decades has provided diagnostic and prognostic information that forms the basis of current therapies.

Introduction

limitations to kidney biopsies:

- Biopsies are processed for light (LM), immunofluorescence (IF), and electron microscopy (EM) and provide a "snapshot" in time of the disease.
- Do not necessarily reflect on the dynamic nature of disease activity
- Do not always differentiate between primary or secondary disease
- Do not provide an association between appearance and prognosis or responsiveness to treatment.

- Moreover, biopsies are invasive.

Objectives

- Novel glomerular biomarkers have provided clinicians with insight into glomerular disease pathogenesis and have advanced care by enabling tailored therapy.

- The aim of this talk is to present some available information on the diagnostic, prognostic, and predictive tissue biomarkers currently available for the management of glomerular diseases.

Novel Biomarkers in Glomerular Disease

1- primary Membranous Nephropathy :

After discovery of the autoantigen phospholipase A2 receptor in 2009, the serologic evaluation of glomerular diseases has become more detailed for nephrologists. And then

- Thrombospondin type 1 domain-containing 7A (THSd7A)

- Neural epidermal growth factor-like 1 protein (NELL-1)

Novel Biomarkers in Glomerular Disease

Additionally, discoveries of specific biomarkers in:

2- C3 Glomerulopathy
3- Fibrillary Glomerulonephritis

with the major focus on their clinical applicability.

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Review

Non-Invasive Biomarkers for Diagnosis, Risk Prediction, and Therapy Guidance of Glomerular Kidney Diseases: A Comprehensive Review

Lorenzo Catanese [1,2,3], Harald Rupprecht [1,2,3], Tobias B. Huber [4], Maja T. Lindenmeyer [4], Felicitas E. Hengel [4], Kerstin Amann [5], Ralph Wendt [6], Justyna Siwy [7], Harald Mischak [7] and Joachim Beige [6,8,9,*]

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Narrative Review

The Evolving Role of Novel Biomarkers in Glomerular Disease: A Review

Corey Cavanaugh and Mark D. Okusa

Recent advances in glomerular biology have expanded our understanding of glomerular diseases, leading to more precise therapeutic options. Since the discovery of the autoantigen phospholipase A_2 receptor in primary membranous nephropathy 10 years ago, the serologic evaluation of glomerular diseases has become more detailed and nuanced for nephrologists. In addition to phospholipase A_2 receptor antibodies, circulating autoantibodies now include thrombospondin type 1 domain–containing 7A and most recently, neural epidermal growth factor–like 1 protein for membranous nephropathy. Additionally, discoveries in C3 glomerulopathy and fibrillary glomerulonephritis are poised to improve the diagnostic approach to these disorders by using novel biomarkers to complement traditional histologic patterns on kidney biopsy. Although kidney biopsies are considered the gold standard in profiling glomerular diseases, validated novel glomerular biomarkers contribute substantially to the diagnostic and therapeutic approaches through their ability to improve sensitivity, permit dynamic longitudinal monitoring of disease activity, and capture genetic heterogeneity. We describe the value of specific biomarkers in selected glomerular diseases, with the major focus on their clinical applicability.

Complete author and article information provided before references.

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doi: 10.1053/j.ajkd.2020.06.016

Biomarkers of Membranous Nephropathy in Adults

Table 1. Biomarkers of Membranous Nephropathy in Adults

Biomarker	Disease	Method of Detection	Malignancy Screening and Rate	Incidence	Comments
Phospholipase A_2 receptor 1 (PLA_2R)	Primary MN	Serum: ELISA,[a] IIF,[a] WB Tissue: IHC, IF	Age-appropriate screening; rate of malignancy: ~9%[32]	~70%-80% of idiopathic MN	• Most common antigen in primary MN • Biopsy not necessary if eGFR > 60 without evidence of secondary/superimposed cause • IgG4 dominant
Neural epidermal growth factor-like 1 protein (NELL-1)	Primary MN	Serum: WB Tissue: IF, IHC	Search for malignancy; rate of malignancy: 11.7-33%[5,92]	~3.8%-16% of PLA_2R, THD7A-negative idiopathic MN	• 2nd most common antigen in MN • IgG1 dominant
Thrombospondin type 1 domain containing 7A (THSd7A)	Primary MN	Serum: ELISA, IIF,[a] WB Tissue: IHC, IF	Aggressive screening including urogenital and gastrointestinal/colorectal: rate of malignancy: 6%-20%[56,59,60]	1%-5% of idiopathic MN (~10% of PLA_2R negative)	• 3rd most common antigen in MN • ELISA not commercially available • IgG4 dominant
Exostosin 1/exostosin 2 (EXT1/EXT2)	Secondary MN	Tissue: IHC, IF	Limited data to recommend screening; rate of malignancy: 7.6%[6]	11.6% of PLA_2R-negative MN	• Tissue marker of class V lupus ~1/3 of cases & autoimmune disease, typically young, female • IgG1 dominant

Abbreviations: eGFR, estimated glomerular filtration rate (in mL/min/1.73 m^2); ELISA, enzyme-linked immunosorbent assay; IF, immunofluorescence; IgG4, immunoglobulin G4; IHC, immunohistochemical; IIF, indirect immunofluorescence; MN, membranous nephropathy; PLA_2R, phospholipase A_2 receptor; WB, Western blot.
[a]Commercially available.

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Membranous Nephropathy

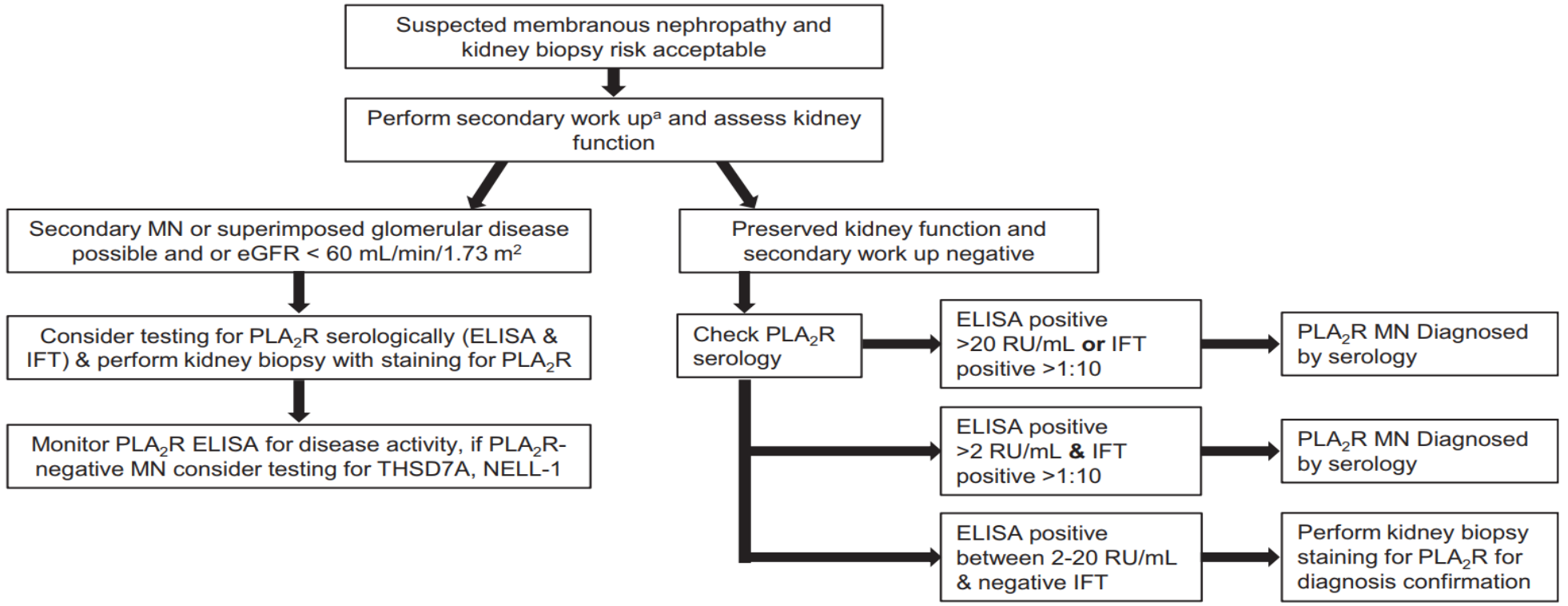

Figure 2. Proposed approach to serologic diagnosis of phospholipase A_2 receptor (PLA_2R) membranous nephropathy (MN). Sensitivity of the serologic assays is not uniform and depends on ethnicity. This algorithm is based on the study by Bobart et al[33] (predominantly White North American cohort). [a]In general it should include a search for autoimmune disease (lupus), medications (nonsteroidal anti-inflammatory drugs), malignancy, and infections (viral hepatitis). Abbreviations: eGFR, estimated glomerular filtration rate; ELISA, enzyme-linked immunosorbent assay; IFT, immunofluorescence testing.

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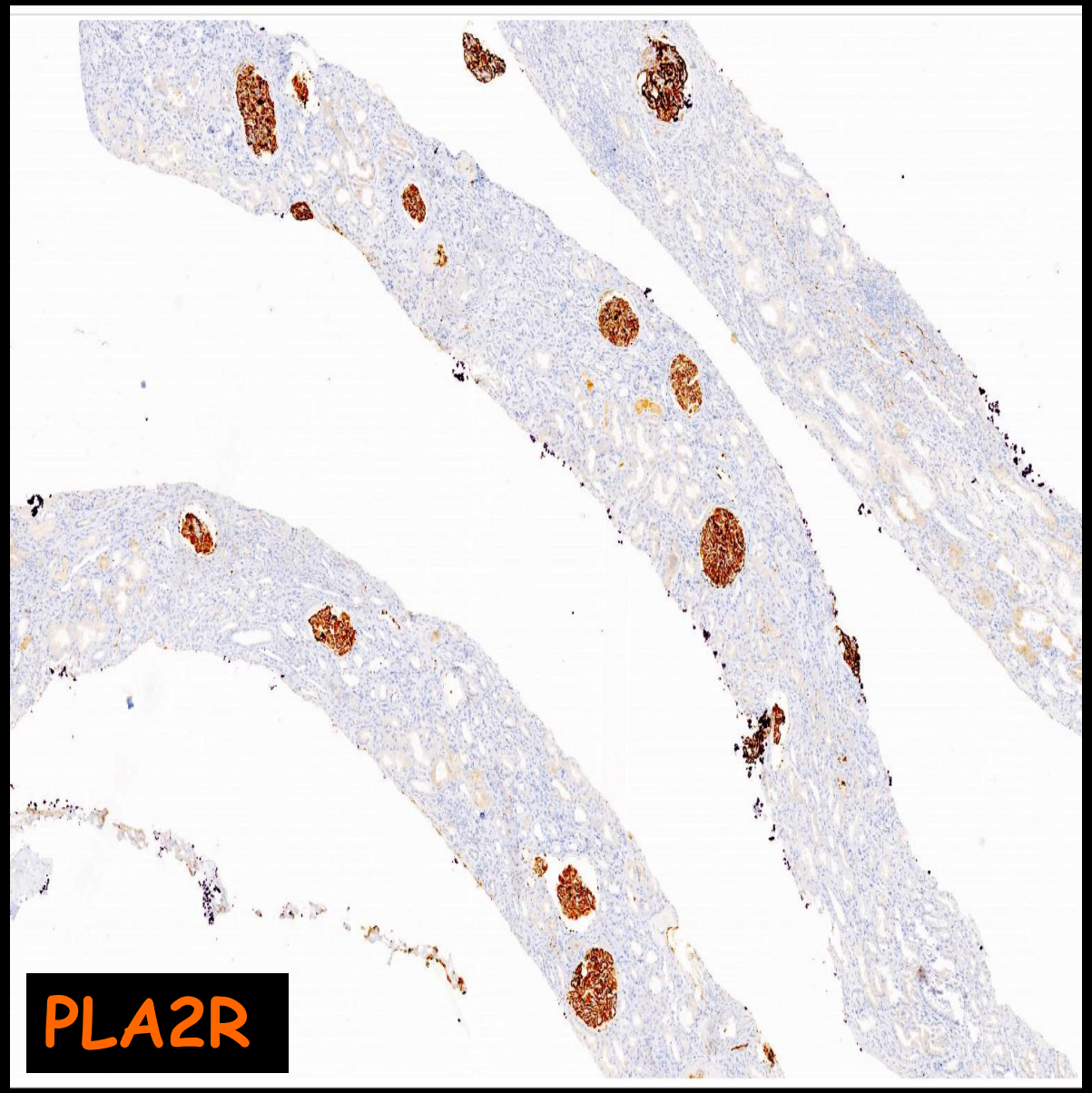
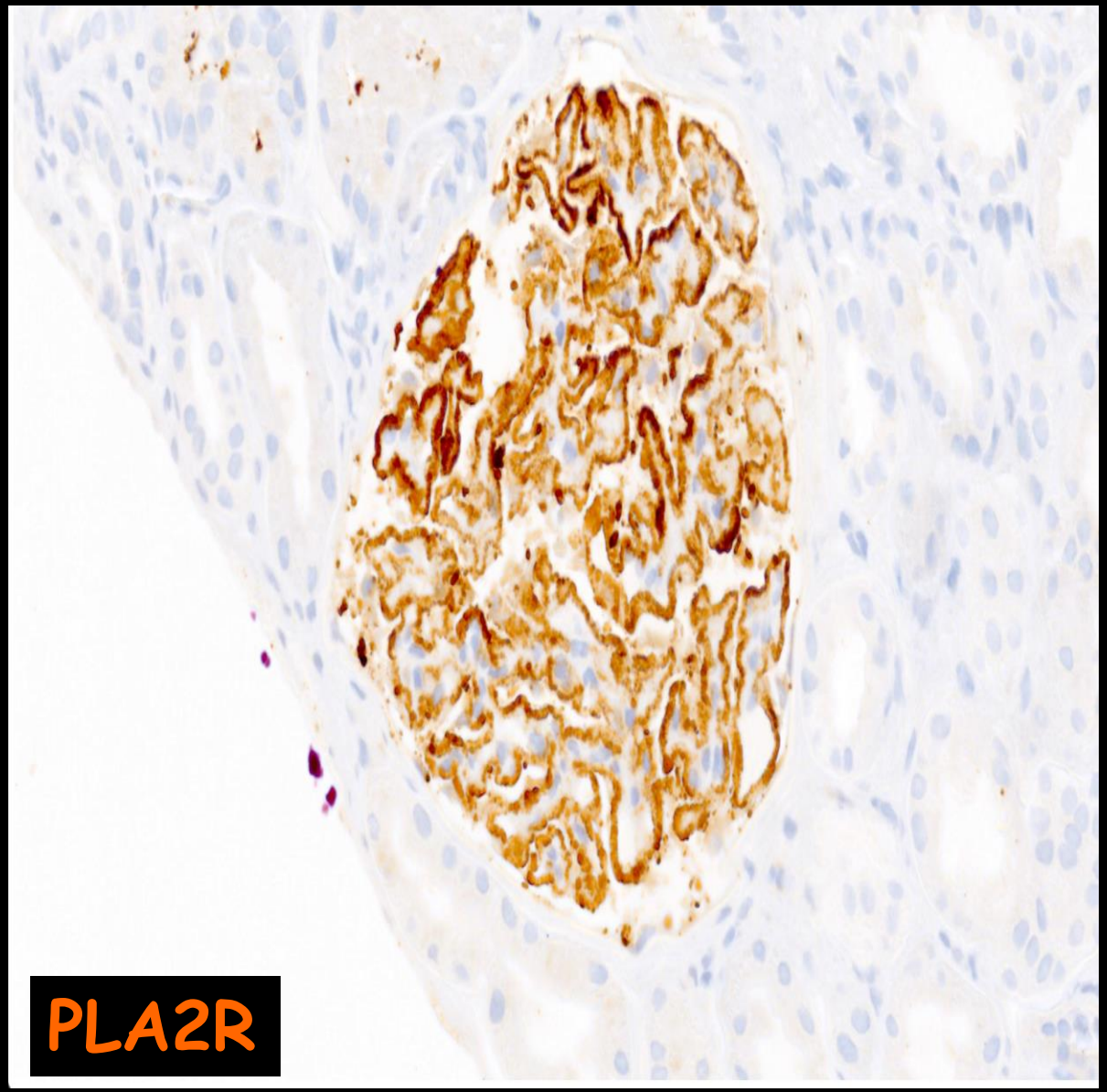
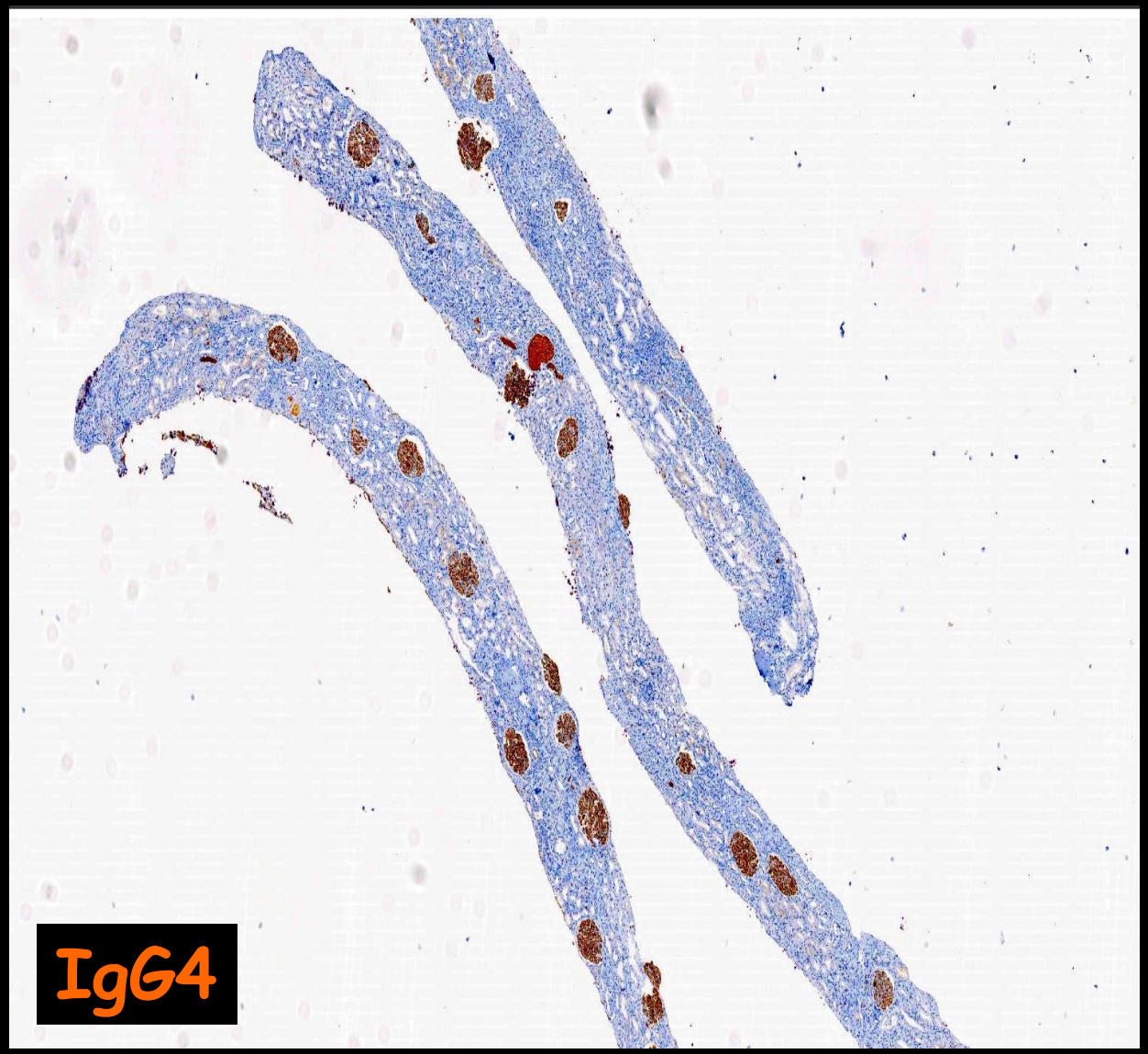
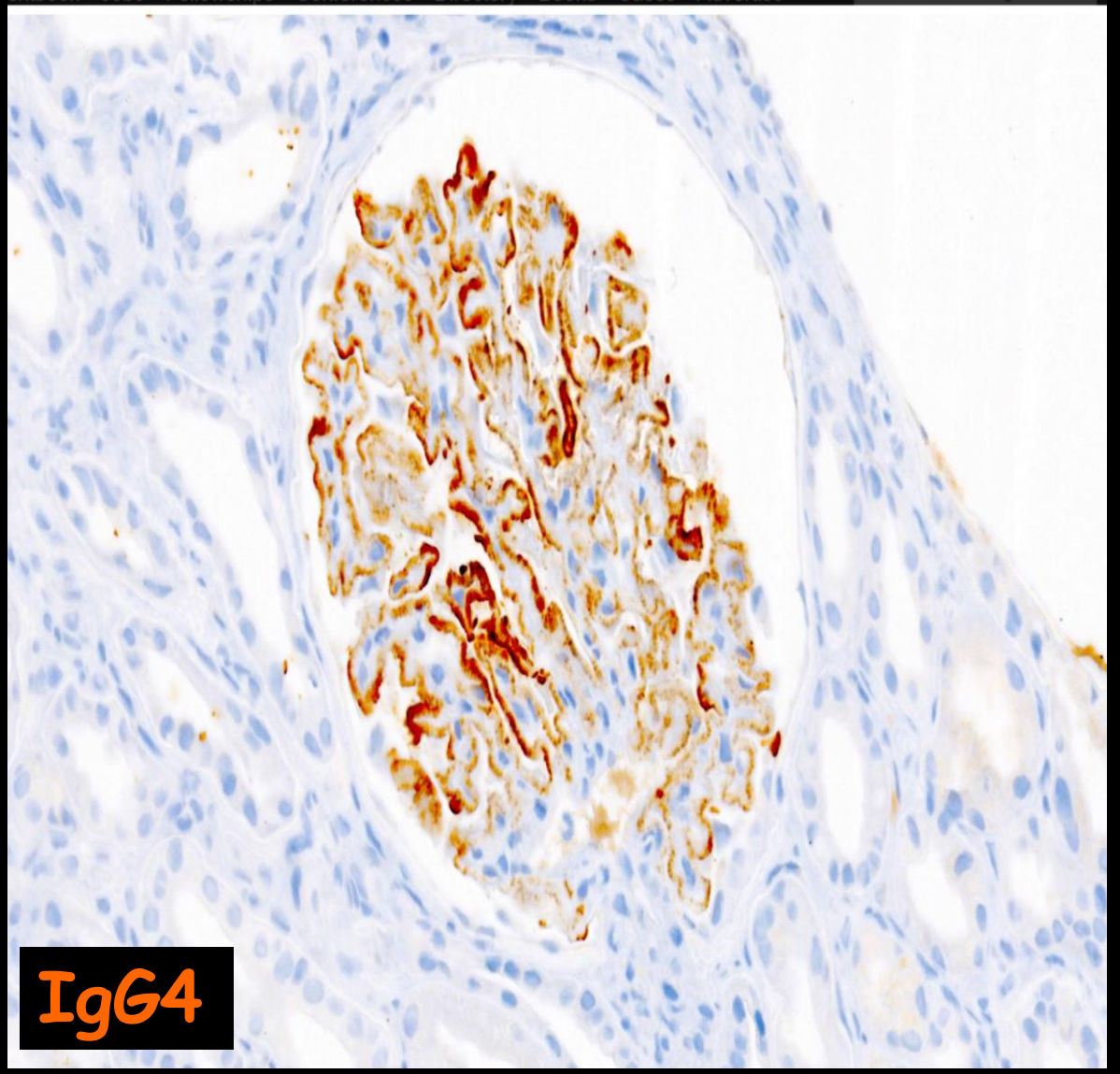

PLA2R


PLA2R


IgG4


IgG4

Key message

- Appropriate utilization of PLA2R testing can aid in management of MN patients.
- Serial anti-PLA2R levels provide valuable information regarding response to therapy and likelihood of remission.
- Because auto-antibody levels are often negative during quiescent disease, tissue testing may be required to correctly categorize MN as PLA2R+.

Glomerular Diseases

Review Article

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C3 Glomerulopathy: A Review with Emphasis on Ultrastructural Features

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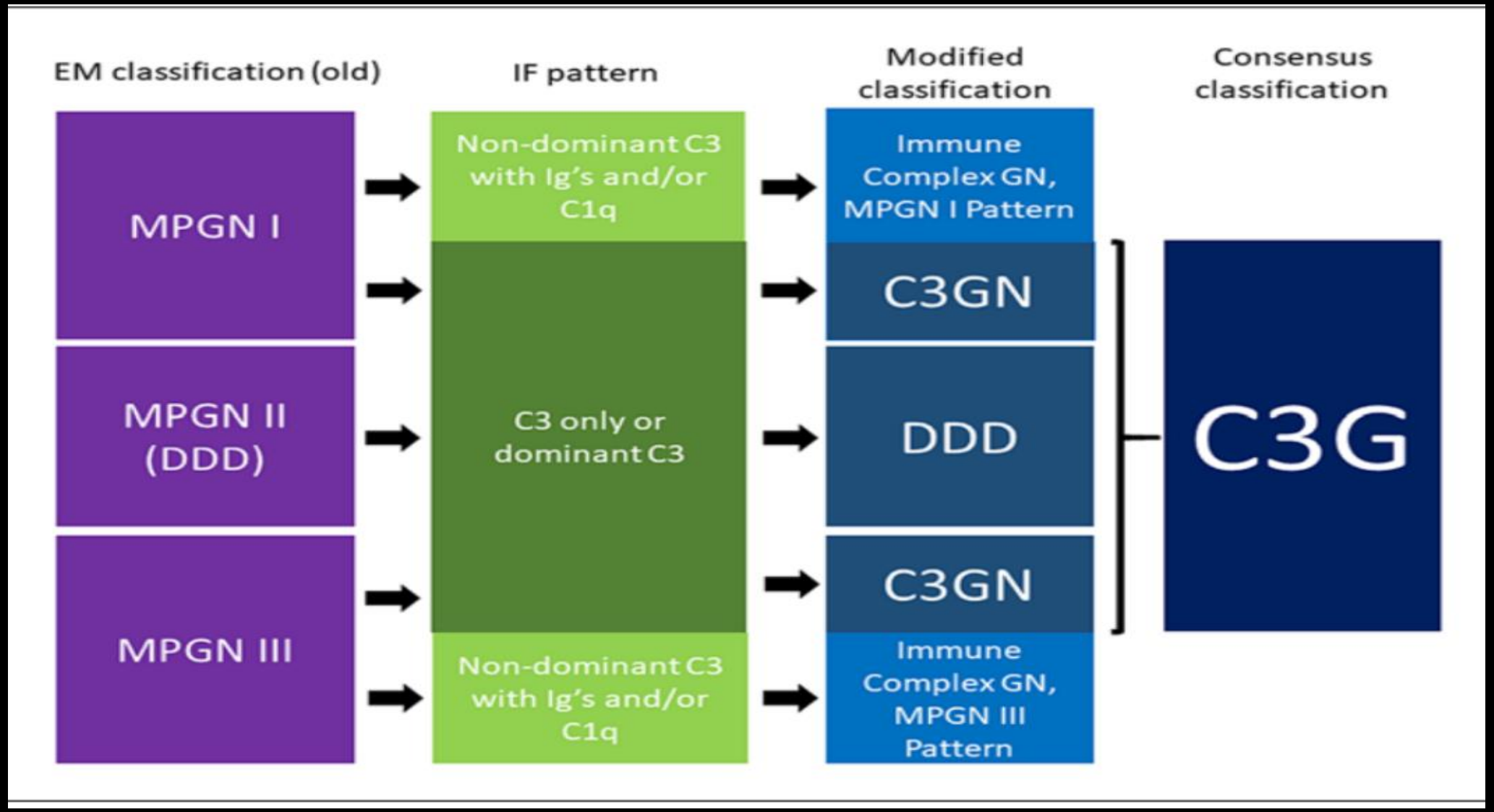
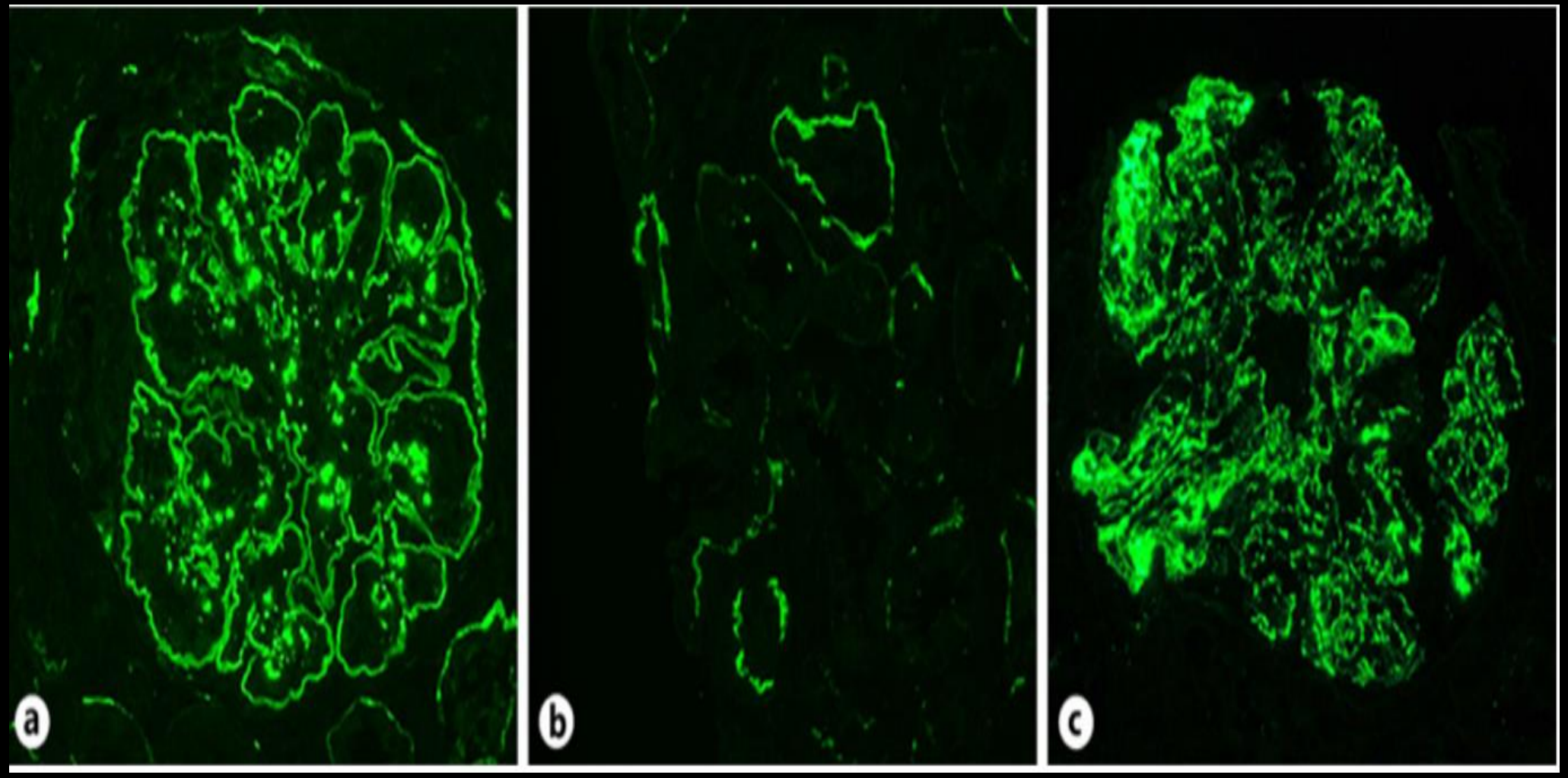
[b]Department of Pathology and Molecular Medicine, Queen's University, Kingston, ON, Canada

C3 Glomerulopathy



Glomerular Dis 2022;2:107-120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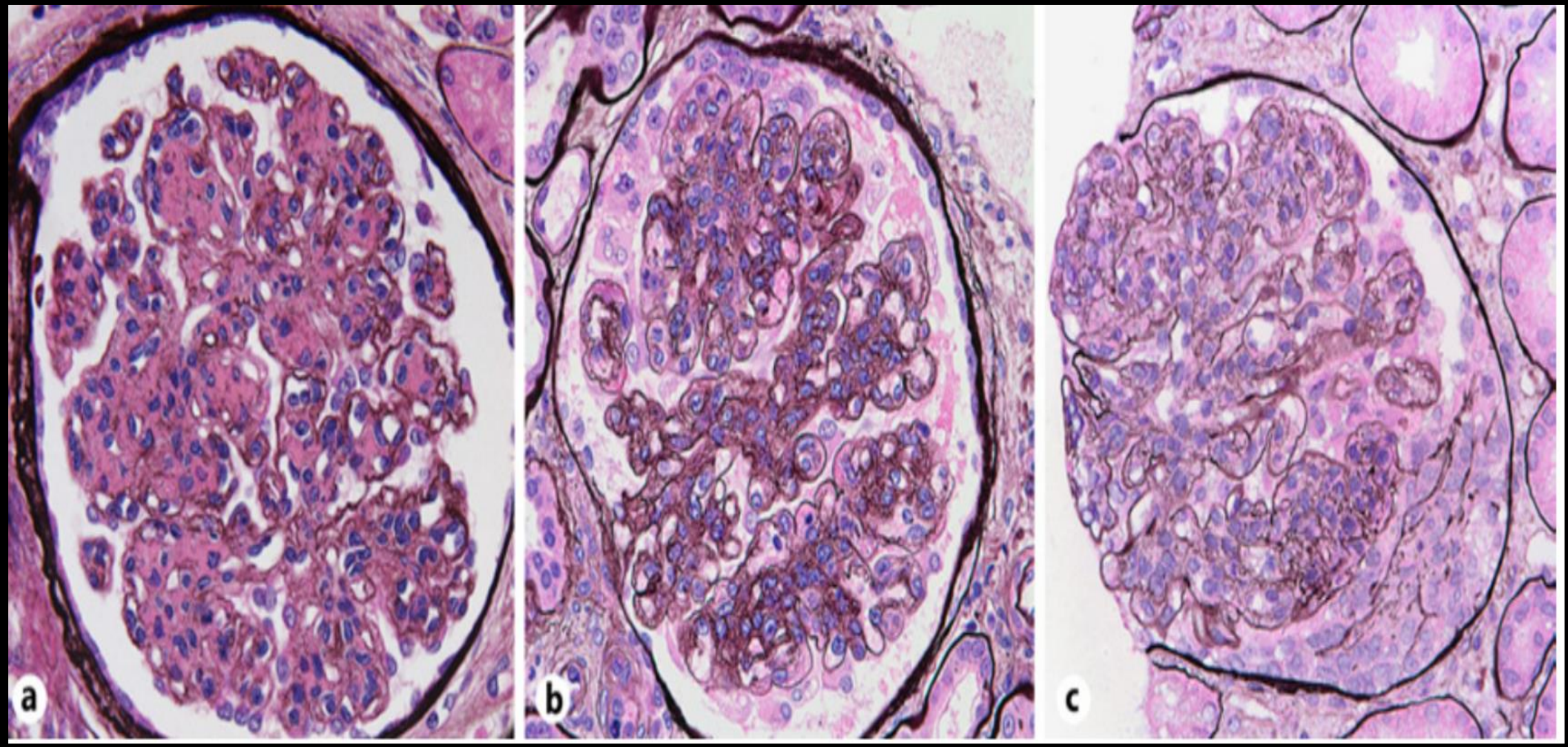
c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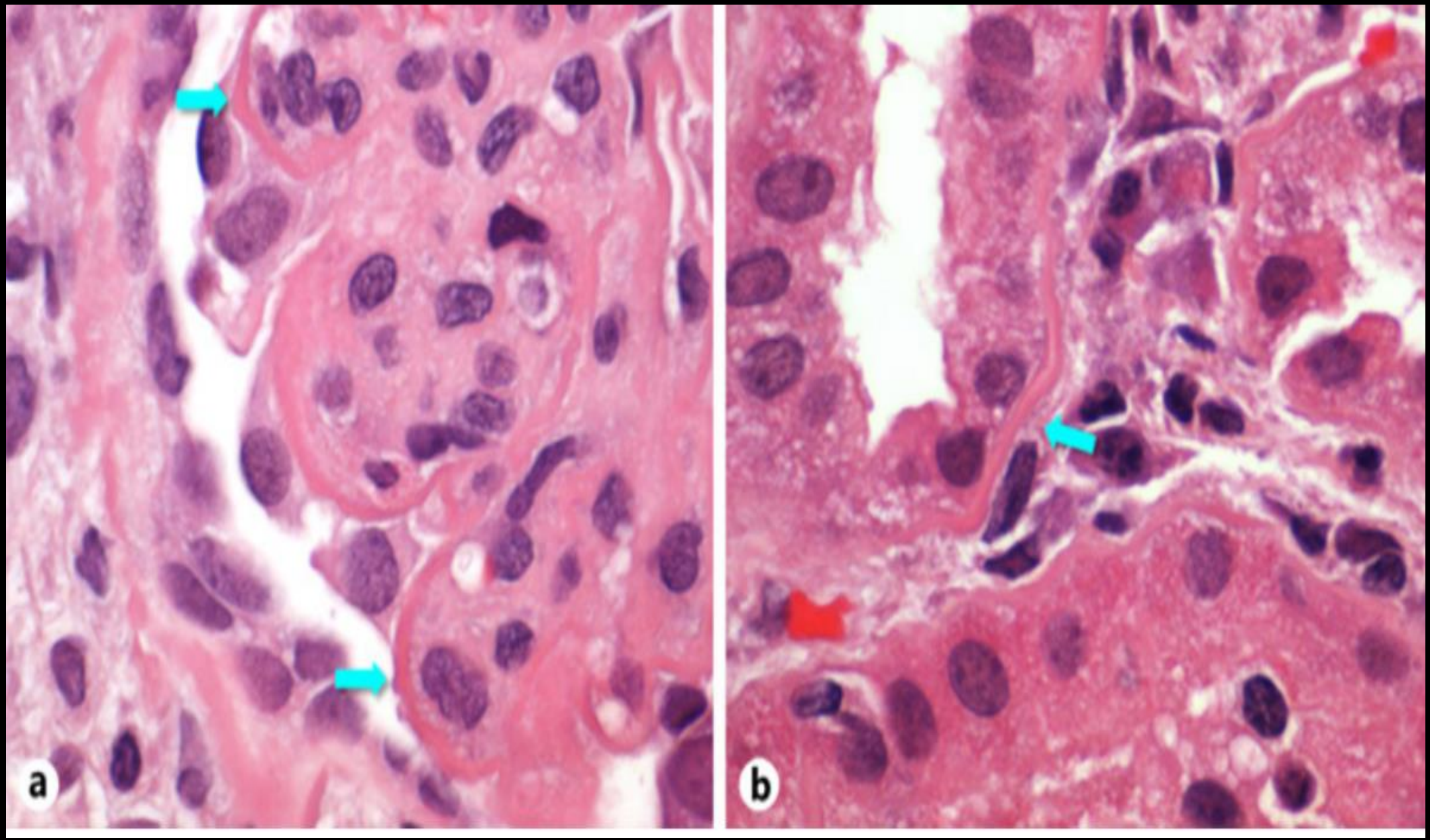
a
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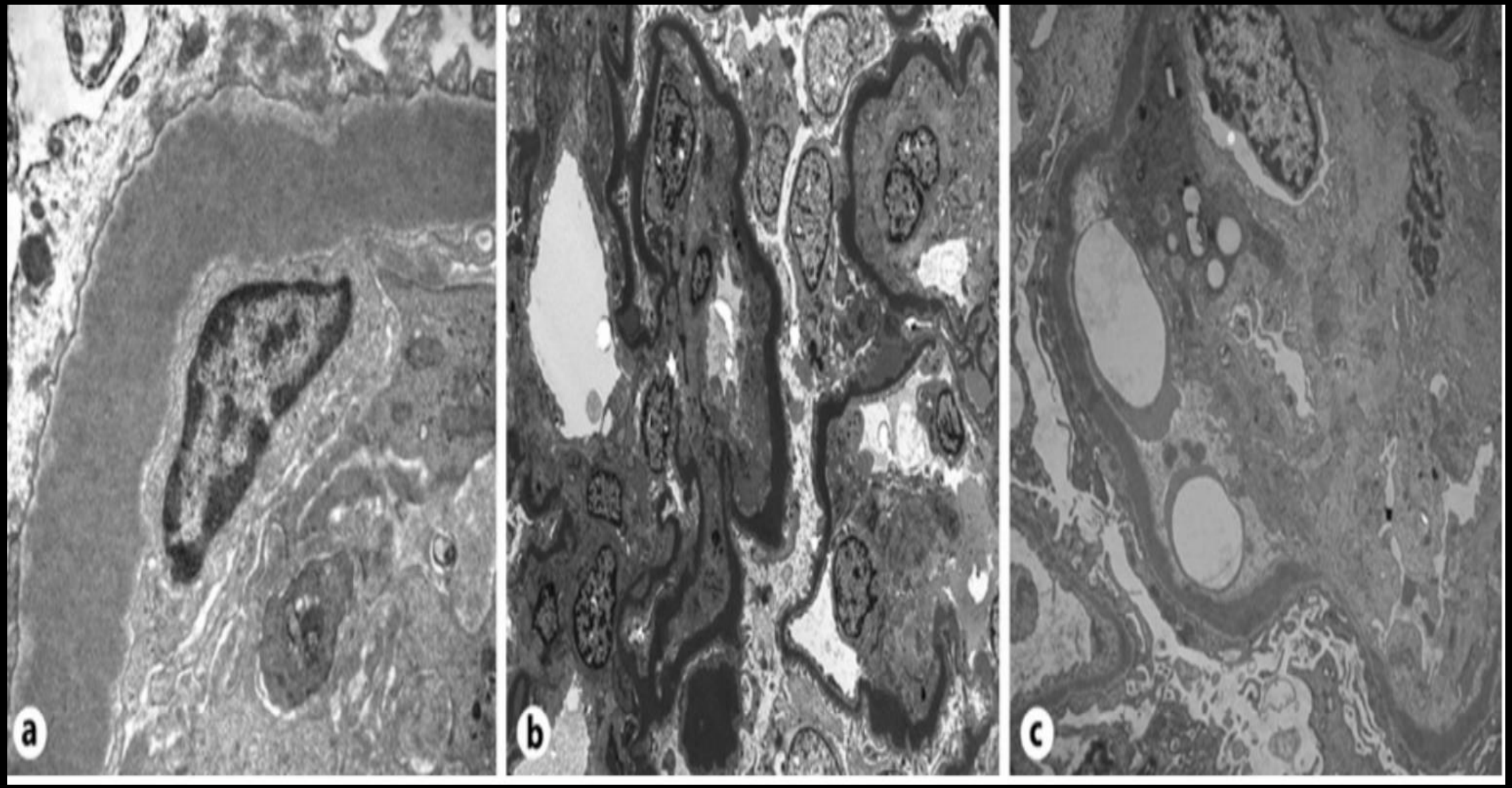
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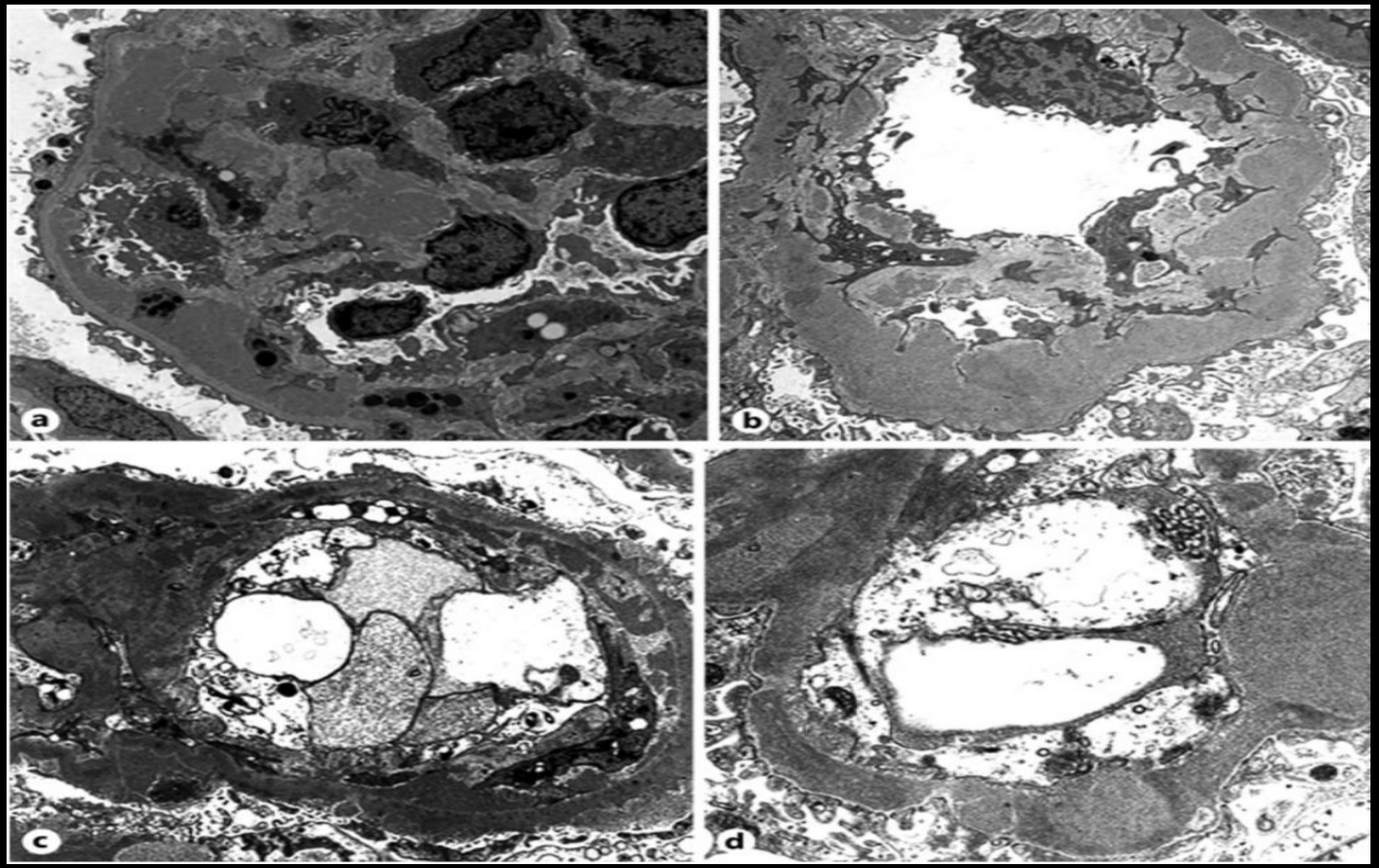
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c
d

C3 Glomerulopathy

- The panel of antibodies used for immunofluorescence studies in most renal pathology laboratories includes antisera to immunoglobulin heavy chains

(IgG, IgM and IgA), immunoglobulin light chains (κ and λ) and the complement components C1q and C3.

- The antibody to C3 is specific for C3c, a stable C3 cleavage product.

- Laser microdissection and mass spectrometry studies identify large amounts of C3, most commonly C3dg

(a cleavage product of C3) with limited amounts of C5, C6, C7, C8 and C9, as well as of

the five complement factor H-related proteins (FHR1–FHR5).

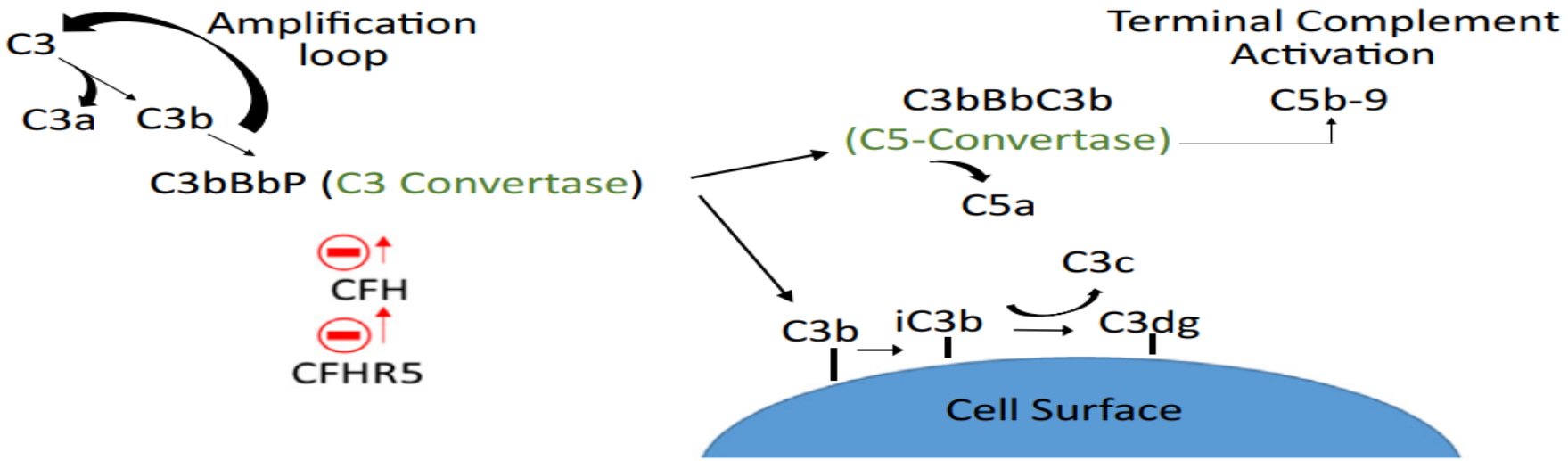


Figure 3. Alternative complement cascade and hypothesized role of complement factor H (CFH)-related protein 5 (CFHR5). Formation of C3 convertases leads to cleavage of C3 and formation of C5 convertase, creating potent anaphylatoxins (C3a and C5a) that mediate the inflammatory response. C3b is degraded into iC3b and C3dg, which mediate phagocytosis and an adaptive immune response. CFH is a strong inhibitor of C3 convertase, whereas CFHR5 preserves C3 convertase activity by inhibiting CFH. C5b causes terminal complement activation membrane attack complexes.

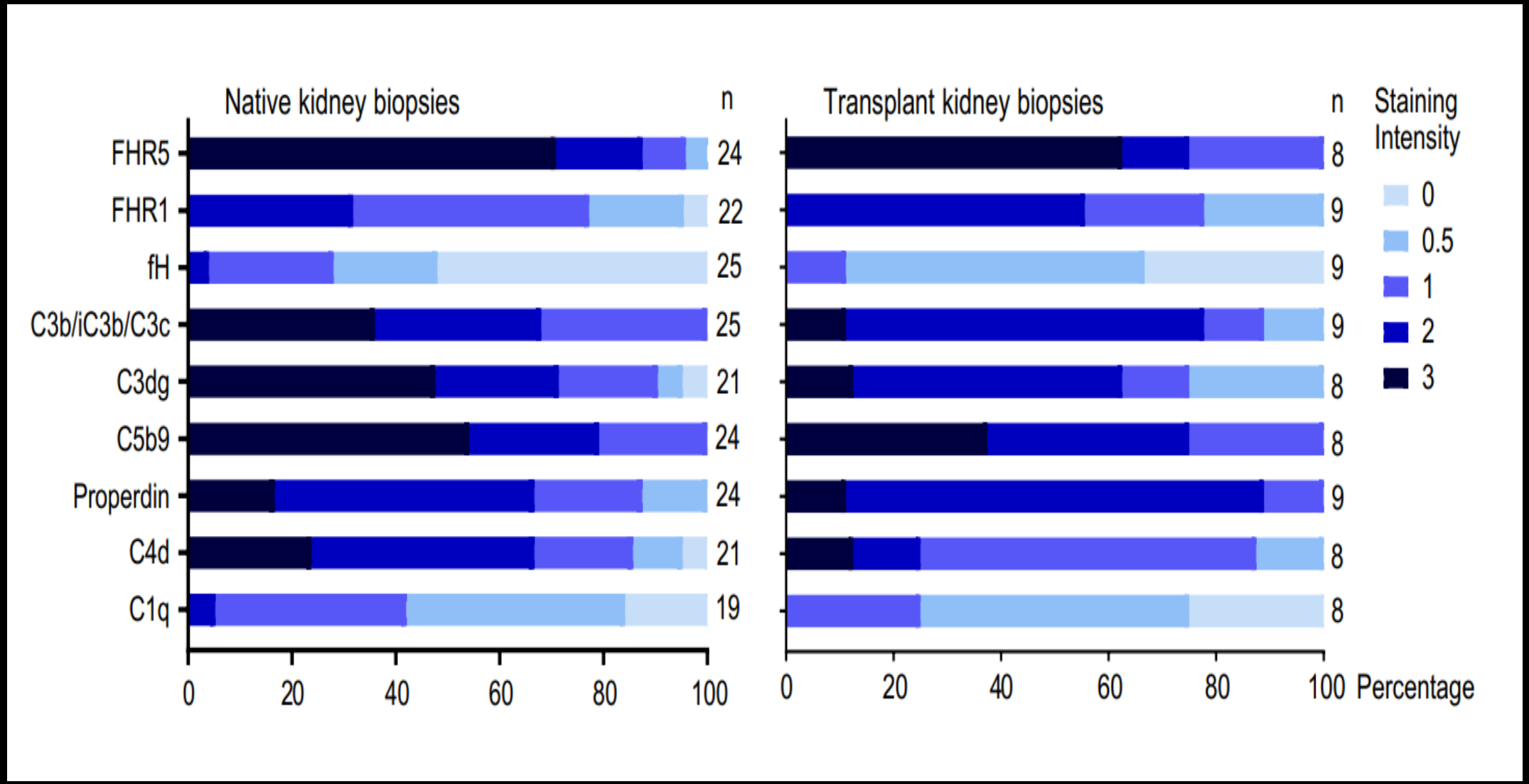
KI REPORTS
KIReports.org
CLINICAL RESEARCH

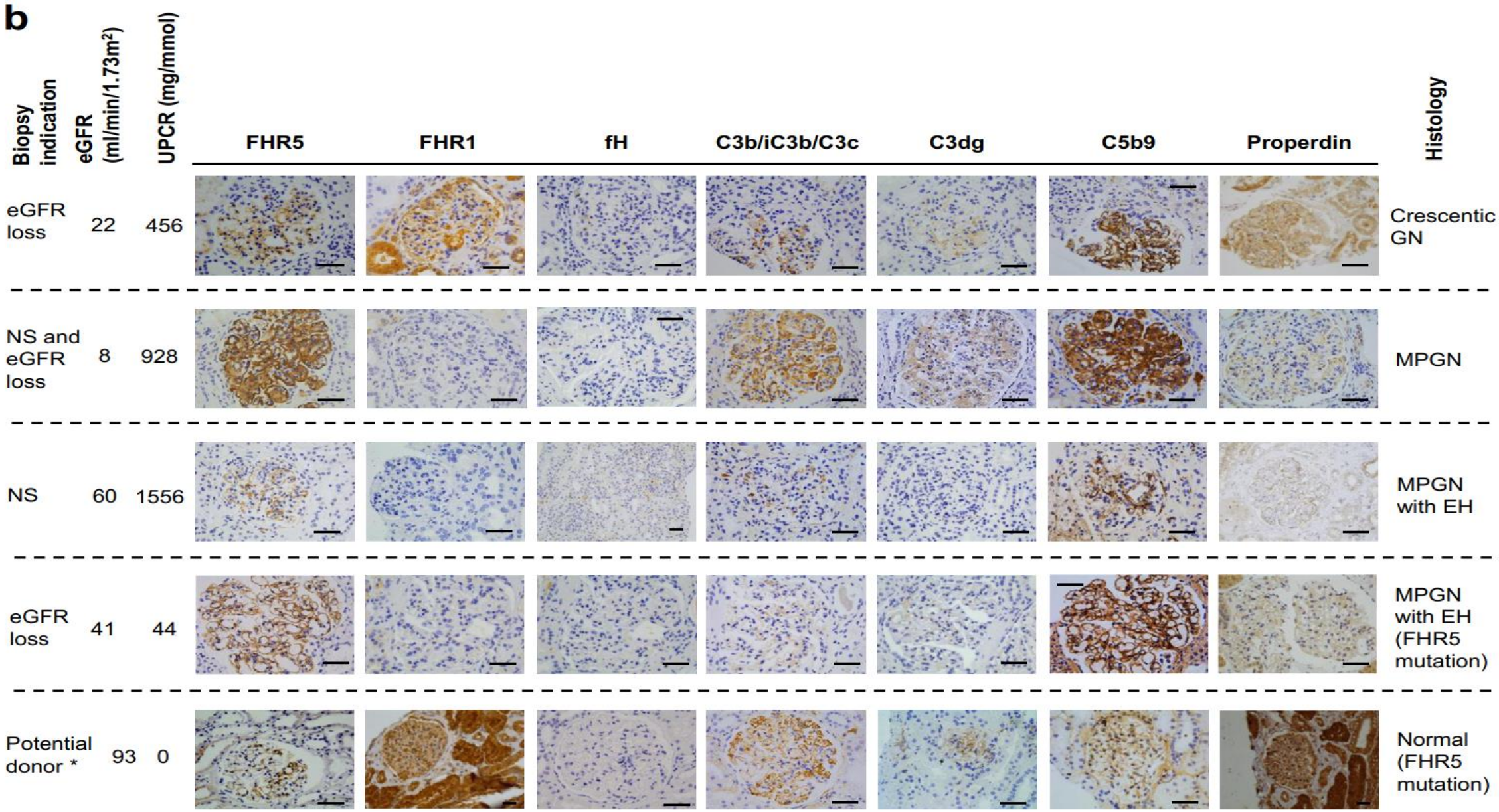
Glomerular Complement Factor H–Related Protein 5 (FHR5) Is Highly Prevalent in C3 Glomerulopathy and Associated With Renal Impairment

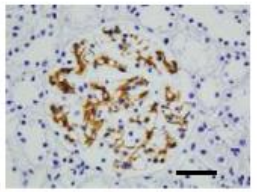
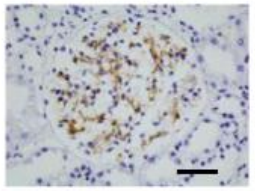
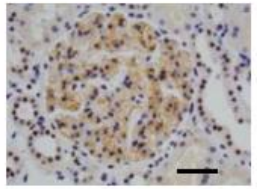
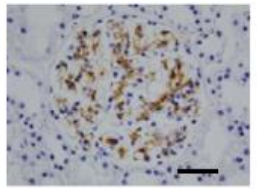
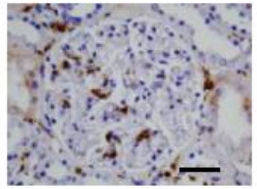
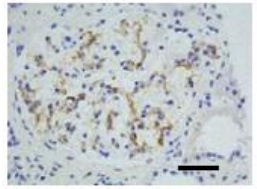
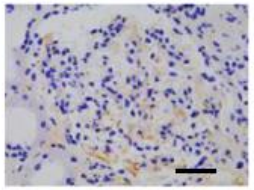
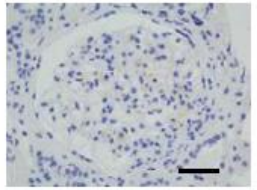
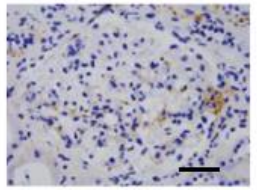
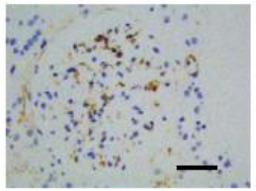
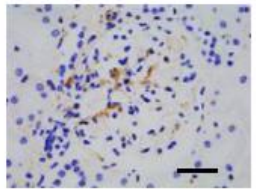
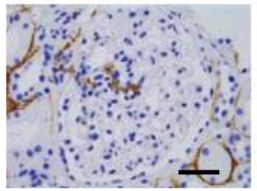
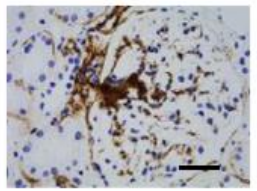
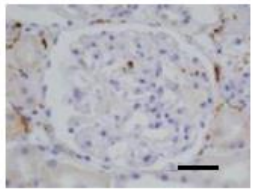
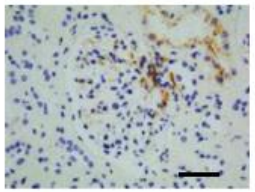
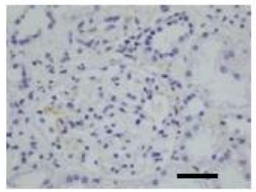
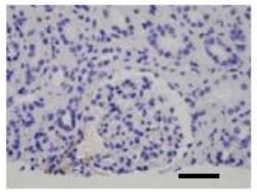
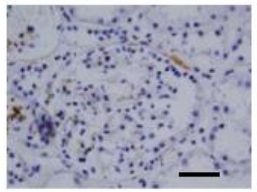
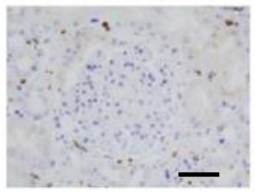
Nicholas R. Medjeral-Thomas[1], Hilary Moffitt[1], Hannah J. Lomax-Browne[1], Nicholas Constantinou[1], Tom Cairns[2], H. Terence Cook[1] and Matthew C. Pickering[1]

[1]Centre for Inflammatory Disease, Division of Immunology and Inflammation, Department of Medicine, Imperial College London, UK; and [2]Renal and Transplant Centre, Imperial College Healthcare NHS Trust, UK

Kidney International Reports (2019) 4, 1387–1400


Native kidney biopsies
n
FHR5
FHR1
fH
C3b/iC3b/C3c
C3dg
C5b9
Properdin
C4d
C1q
24
22
25
25
21
24
24
21
19
0
20
40
60
80
100
Transplant kidney biopsies
n
8
9
9
9
8
8
9
8
8
0
20
40
60
80
100
Percentage
Staining Intensity
0
0.5
1
2
3


b
Biopsy indication
eGFR (ml/min/1.73m²)
UPCR (mg/mmol)
FHR5
FHR1
fH
C3b/iC3b/C3c
C3dg
C5b9
Properdin
Histology
eGFR loss
22
456
Crescentic GN
NS and eGFR loss
8
928
MPGN
NS
60
1556
MPGN with EH
eGFR loss
41
44
MPGN with EH (FHR5 mutation)
Potential donor *
93
0
Normal (FHR5 mutation)

Biopsy / months post-transplant	eGFR (ml/min/1.73m²)	UPCR (mg/mmol)	FHR5	C3b/iC3b/C3c	C3dg	C5b9	CD68	Histology	Transplant rejection?	Histology on subsequent biopsies
Protocol 3	56	0						C3GN with EH	No	Crescentic C3GN
Indication 16	52	35						C3GN with focal and segmental proliferation	No	C3GN with widespread proliferation
Indication 1	63	26						C3GN no proliferation	No	T-cell–mediated rejection
Protocol 6	54	0						Normal	No	Recurrent C3G

Key message

- The diagnosis of C3G remains challenging and often relies on both a structural and functional assessment, which poses significant challenges in interpretation for many nephrologists given that they do not routinely use these assays.
- While the diagnosis of C3G is dependent on IF studies and dominant staining for C3, EM analysis is necessary for diagnostic confirmation.
- In addition, clinical correlation with serum complement C3 levels as well as targeted testing for abnormalities in the alternative complement pathway is necessary for proper personalized patient management.

Key message, Cont

- Glomerular FHR5 is highly prevalent in C3G, interacts with glomerular C3, and is associated with markers of disease severity.
- Glomerular FHR5 interaction with glomerular complement might be exploited to target complement therapeutic agents.

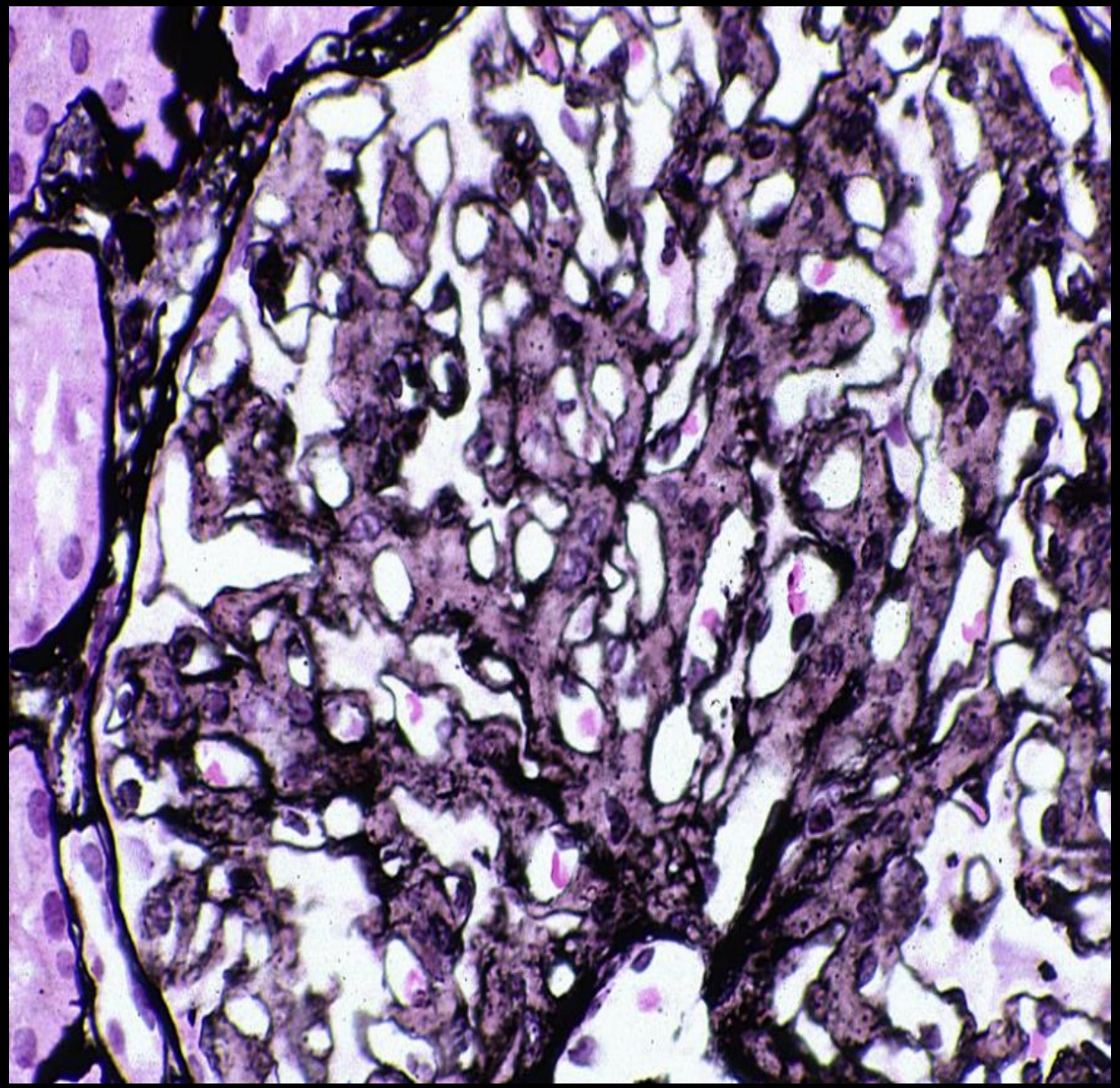
Fibrillary Glomerulonephritis

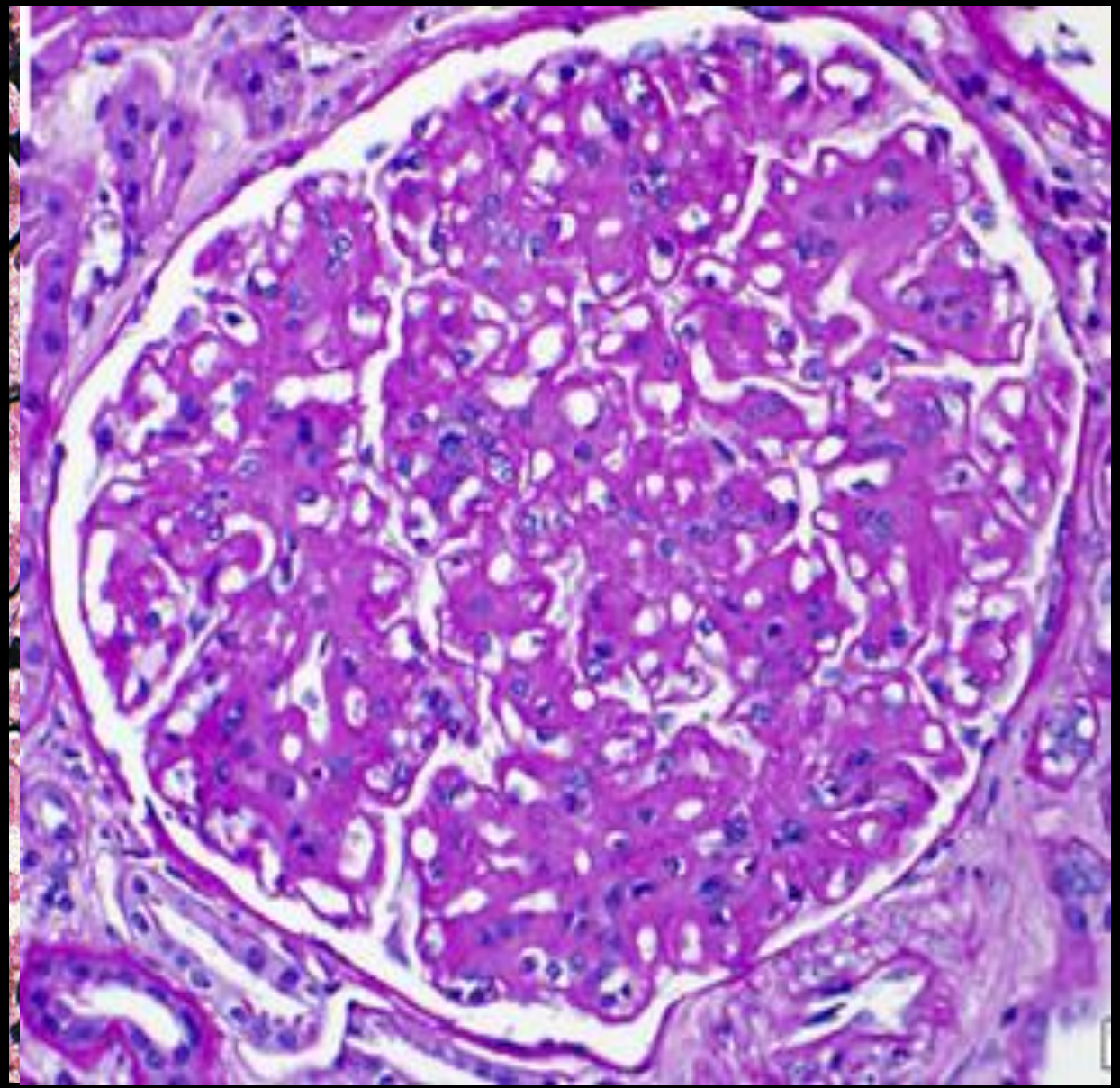
- Mainly in adults with an average age of around 50 years.
- It presents with nephrotic syndrome, hematuria, and reduced GFR in about two-thirds of patients.
- Half of patients progress to ESRD within 2 to 4 years.
- Recurrence in allograft kidneys developed in 36% of patients in one small series.

Fibrillary Glomerulonephritis

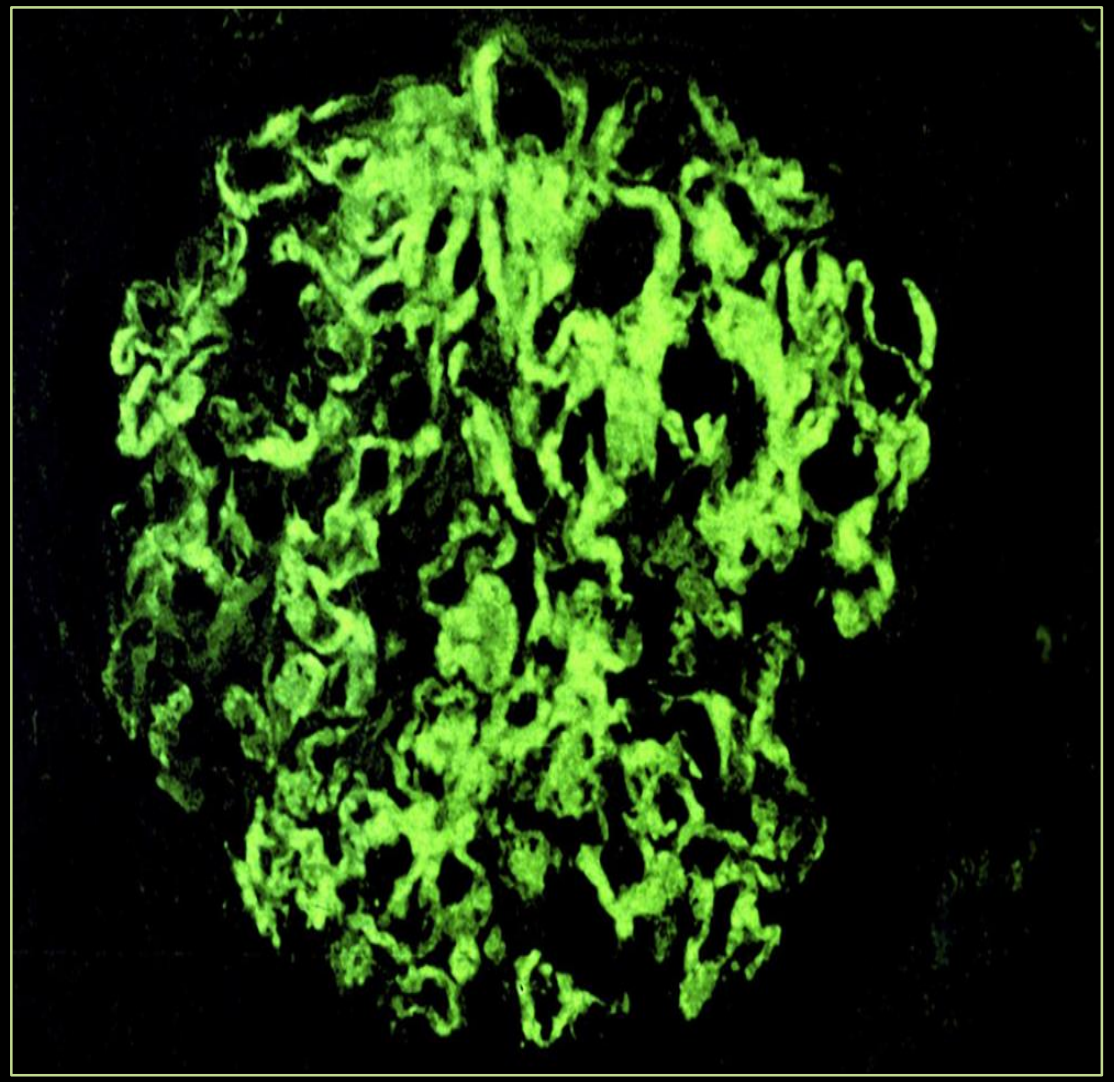
Key Diagnostic Features :

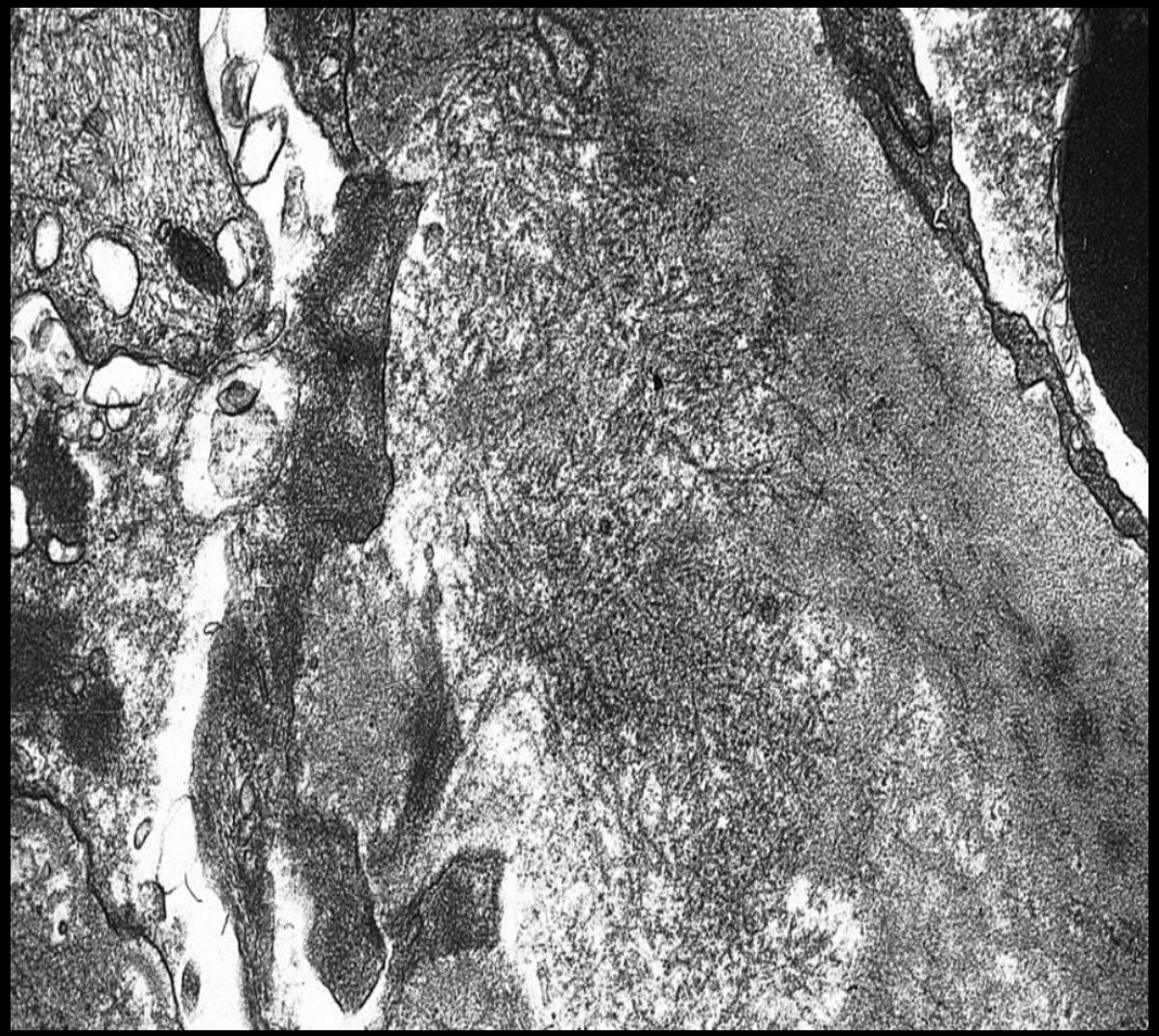
- Mesangial proliferation and variable endocapillary proliferation
- Polyclonal immunoglobulin G and C3
- Randomly arranged fibrils in the mesangium, and variably in the GBM, 12-22 nm in diameter
- Negative Congo red stain





Am J Kidney Dis. 2015;66(4):e27-e28





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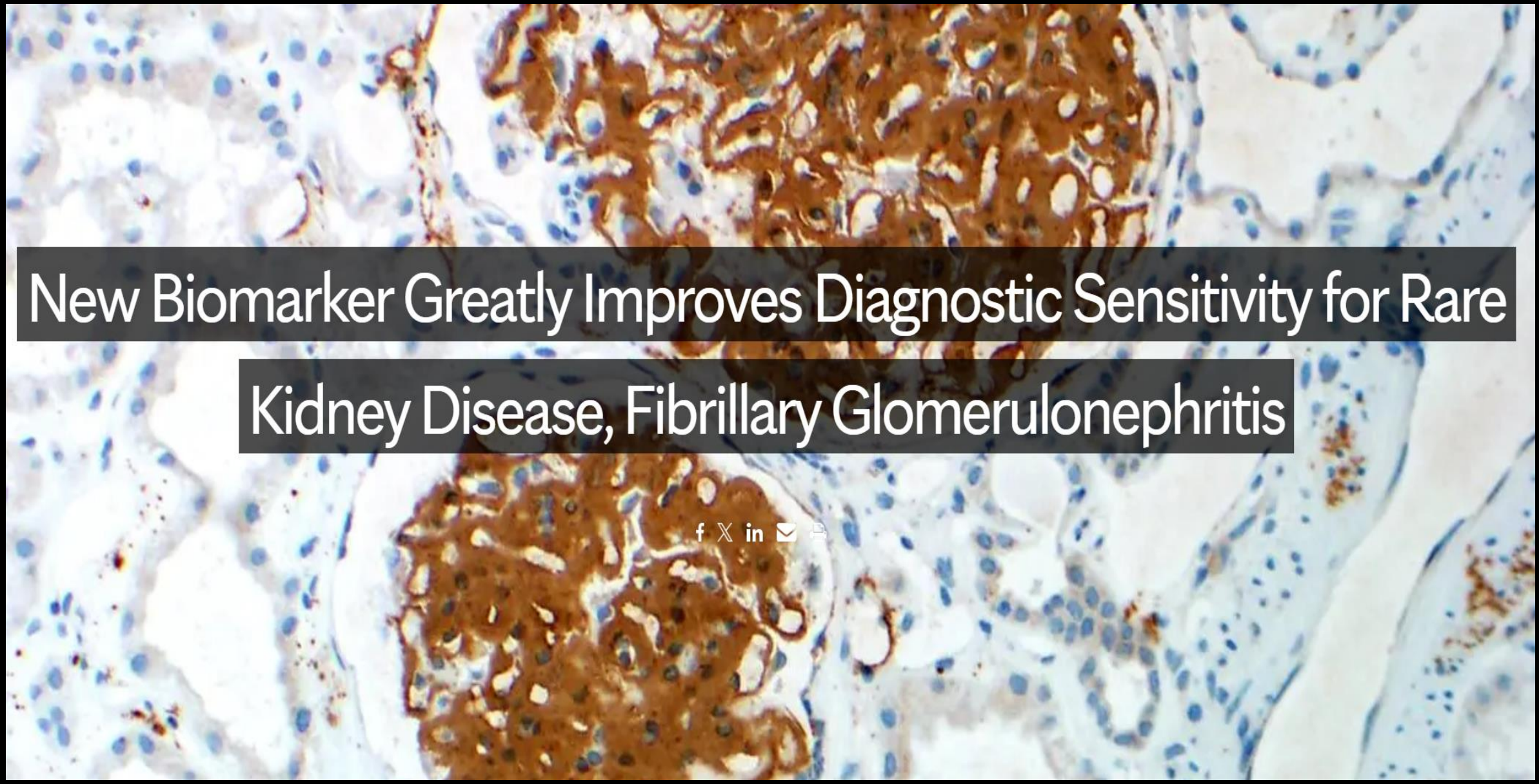

New Biomarker Greatly Improves Diagnostic Sensitivity for Rare Kidney Disease, Fibrillary Glomerulonephritis

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CLINICAL RESEARCH

DNAJB9 Is a Specific Immunohistochemical Marker for Fibrillary Glomerulonephritis

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Kidney International Reports (2018) 3, 56–64

www.kidney-international.org review

New developments in the diagnosis of fibrillary glomerulonephritis

Samih H. Nasr[1] and Agnes B. Fogo[2]

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Fibrillary glomerulonephritis is a glomerular disease historically defined by glomerular deposition of Congo red–negative, randomly oriented straight fibrils that lack a hollow center and stain with antisera to immunoglobulins. It was initially considered to be an idiopathic disease, but recent studies highlighted association in some cases with autoimmune disease, malignant neoplasm, or hepatitis C viral infection. Prognosis is poor with nearly half of patients progressing to end-stage renal disease within 4 years.

Fibrillary glomerulonephritis (FGN) is a rare glomerular disease that was first described by Rosenmann and Eliakim in an Arabic patient in 1977[1] and was subsequently recognized as a distinct entity by Duffy *et al.* in 1983.[2] The term "fibrillary glomerulonephritis" was coined by Alpers *et al.* in 1987 when describing a series of 7 patients with non-congophilic fibrillar glomerular deposits that measured 10 to 20 nm in thickness.[3] Several other names were used in the 1980s to describe this lesion, including Congo red–negative

Kidney International (2019) 96, 581–592

Recently, a novel tissue biomarker of FGN, DNAJ homolog subfamily B member 9 (DNAJB9), has been identified .

- DNAJB9 belongs to a family of proteins that function as "co-chaperones" to heat-shock protein 70 (hsp-70).

- It is expressed in all healthy tissues and is localized to endoplasmic reticulum (ER), and is upregulated by inflammatory mediators.

- DNAJB9 immunohistochemistry has a 98% sensitivity and >99% specificity for FGN and has become the gold standard in the diagnosis of FGN.

Int. J. Mol. Sci. 2024, 25, 3519

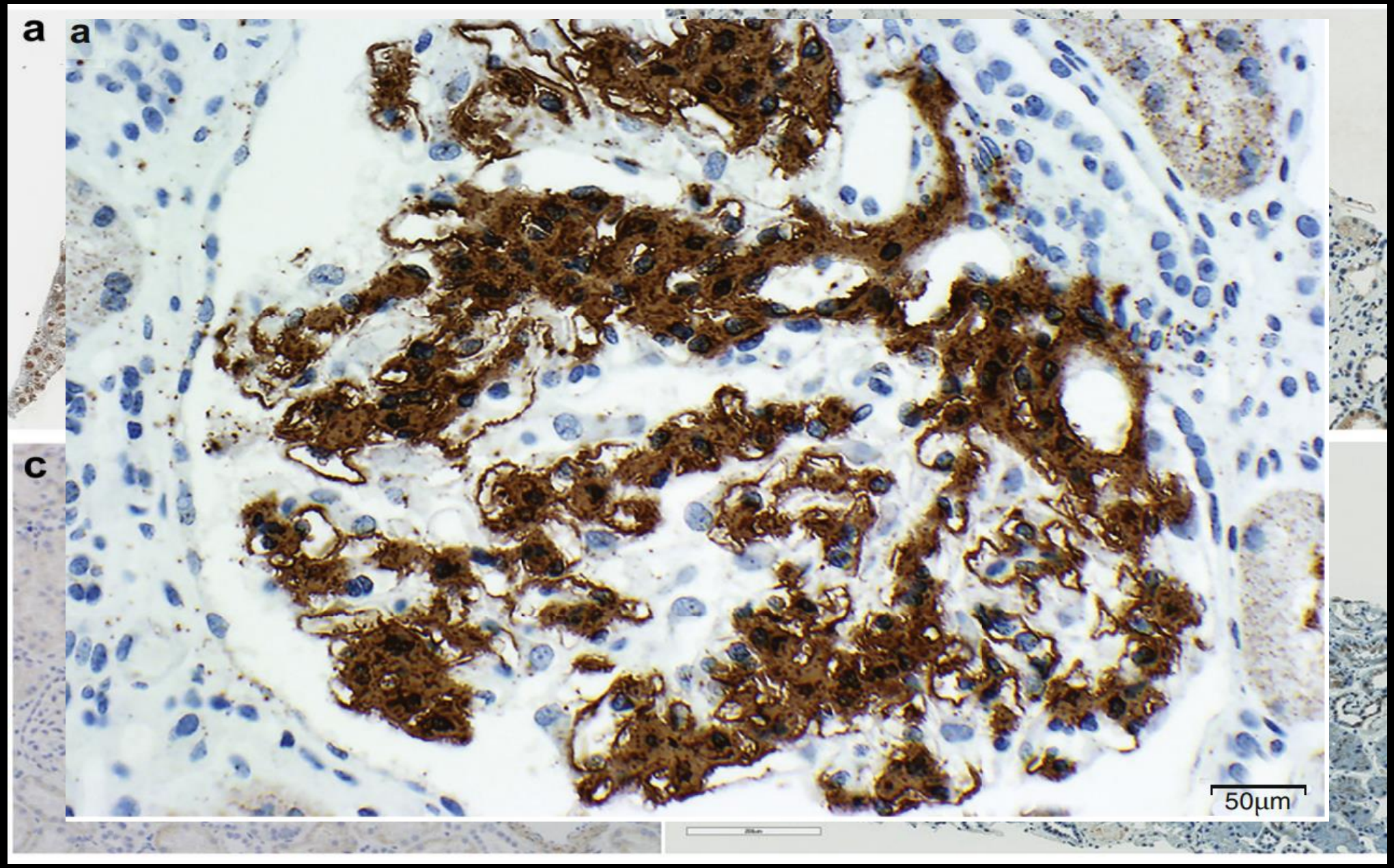
• Blood Biomarkers:

• The group of researchers that discovered the role of DNAJB9 immunohistochemistry in the diagnosis of FGN also detected a 4-fold higher abundance of serum DNAJB9 in FGN patients when compared to controls.

• Serum DNAJB9 levels accurately predicted FGN with moderate sensitivity (67%), high specificity (98%), and a positive and negative predictive value of 89% and 95%, respectively.

• Urine Biomarkers

Urine DNAJB9 has not been investigated so far and its role as a potentially useful non-invasive biomarker in the future remains unclear.



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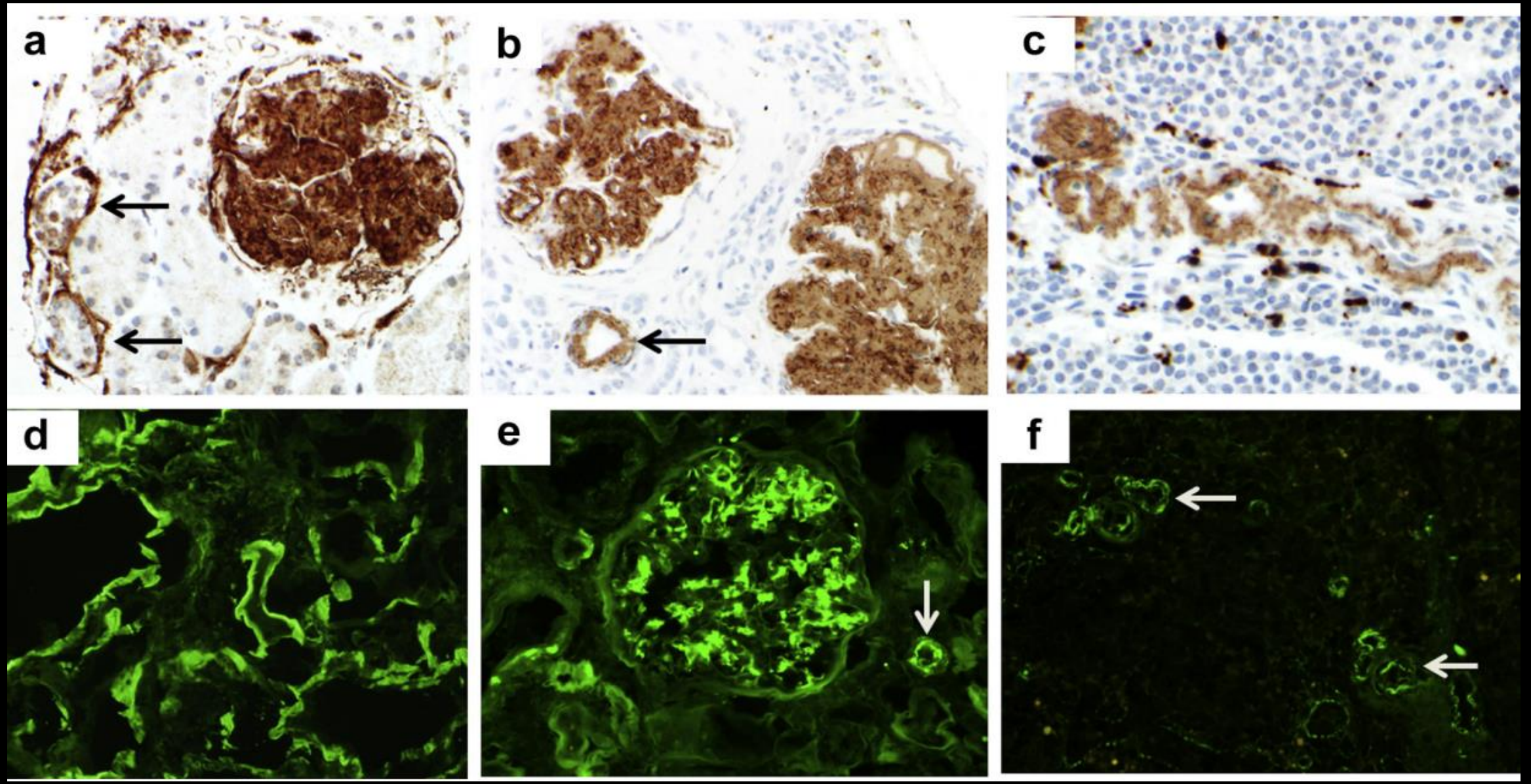

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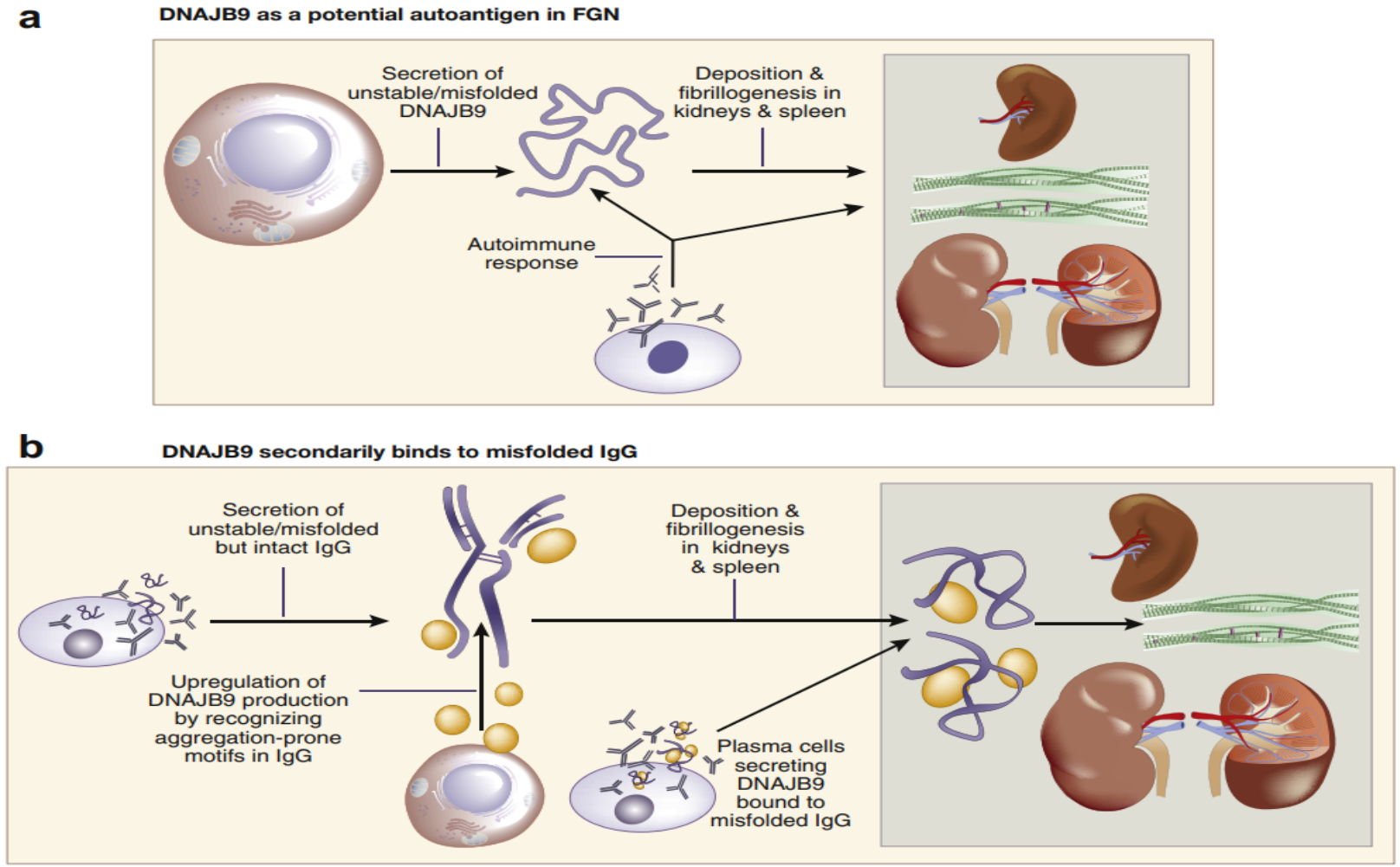

Figure 3 | Potential pathogenetic role of DNAJB9 in fibrillary glomerulonephritis (FGN). (**a**) DNAJB9 could be an autoantigen in FGN. Potentially, a misfolded DNAJB9 molecule is formed (possibly facilitated by protein posttranslational modification) and deposited in glomeruli (through entrapment and/or interaction with glomerular constituents) and in the spleen, which then triggers an autoimmune response. (**b**) Alternatively, DNAJB9 may not be an autoantigen in FGN, but rather a protein that secondarily binds to misfolded (but intact) IgG molecules by recognizing aggregation-prone motifs that are constitutively present or are induced by somatic mutations. This binding could be critical for the IgG aggregates to undergo non-amyloidogenic fibrillogenesis in the kidneys and spleen. Plasma cells could be the source of secreted DNAJB9 bound to the misfolded IgG.

Utility DNAJB9 Immunohistochemistry In The Diagnosis of FGN

1. Alleviates the need for electron microscopy for diagnosing FGN
   - In centers in which electron microscopy is not performed (particularly in developing countries in which the disease is likely underdiagnosed)
   - In limited biopsies without glomerular sampling for electron microscopy
2. Provides prompt diagnosis of FGN in laboratories with a long turnaround time for electron microscopy
3. Distinguishes FGN from other lesions in the differential diagnosis
   - By light microscopy: IgA nephropathy, diabetic nephropathy, immunotactoid GN, fibronectin glomerulopathy, and collagenofibrotic glomerulopathy
   - By immunofluorescence: Amyloidosis (AHL amyloidosis, AH amyloidosis, and AA amyloidosis with entrapped immunoglobulins), lupus nephritis, immunotactoid GN, and anti–glomerular basement membrane nephritis
   - By electron microscopy: Amyloidosis, immunotactoid GN associated with chronic lymphocytic leukemia (which tends to show small microtubules), diabetic fibrillosis, and fibronectin glomerulopathy
4. Discriminates the rare cases of congophilic FGN (which account for ≈4% of FGN cases[28,62]) from amyloidosis
5. Confirms the diagnosis of FGN when concurrent with other diseases (e.g., IgA nephropathy, membranous nephropathy, and diabetic nephropathy), which is encountered in ≈17% of cases[15]

Key message

DR. DASARI:

Collaboration between the clinician, renal pathologist and biochemical or genetic laboratory is required to elucidate both the underlying pathogenesis and the optimal therapeutic approach.

Team of Mayo Clinic pathologists

Pictured from left to right: Mariam (Priya) Alexander, M.D.; Surendra Dasari, Ph.D.; Paul Kurtin, M.D.; and Samih Nasr, M.D.