

Gammapathie monoclonale avec atteinte rénale: un nouveau kaléidoscope?

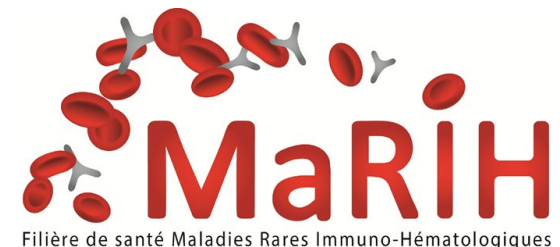
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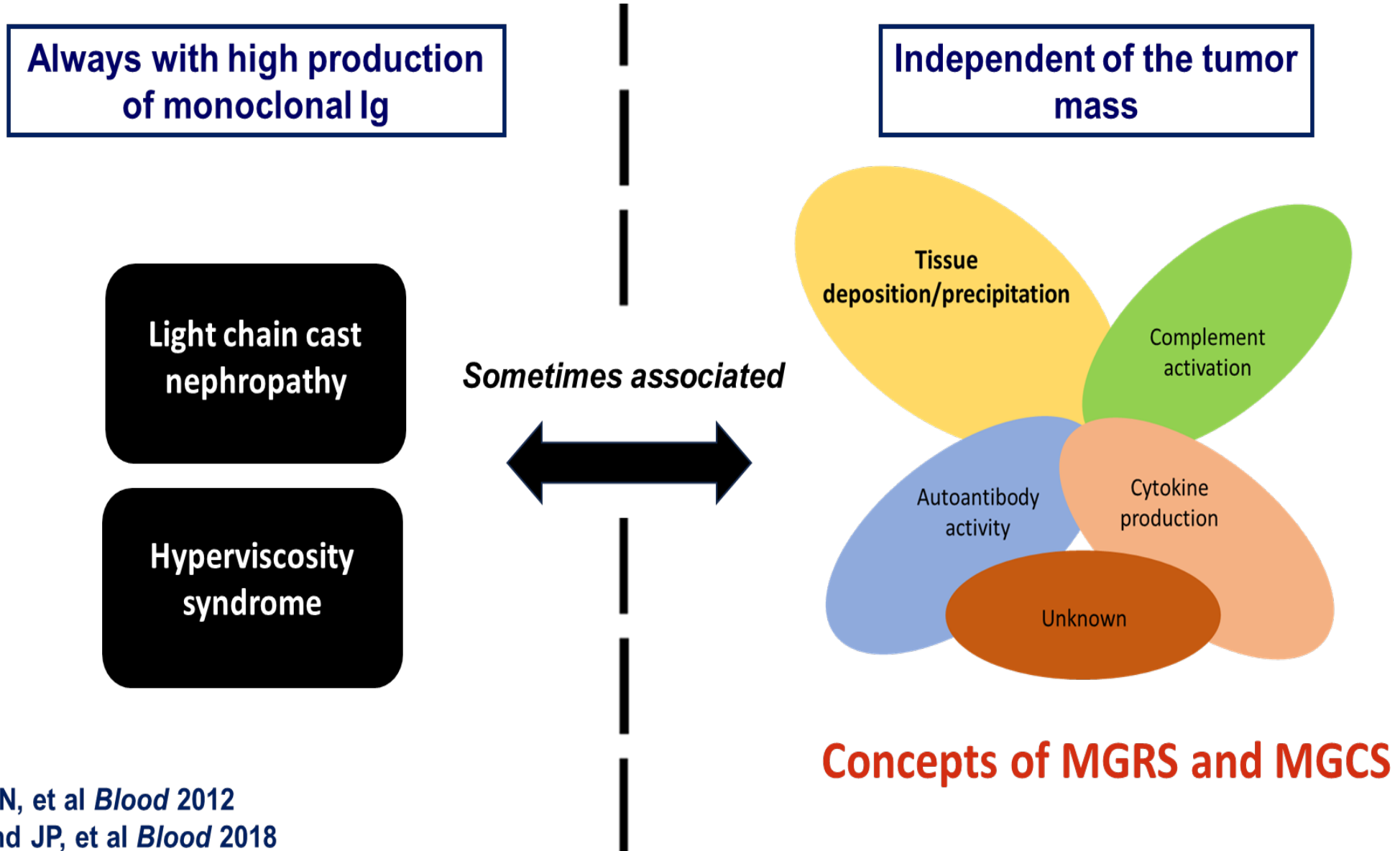
*Centre national de référence Amylose AL et
autres maladies par dépôts d'Ig monoclonales*



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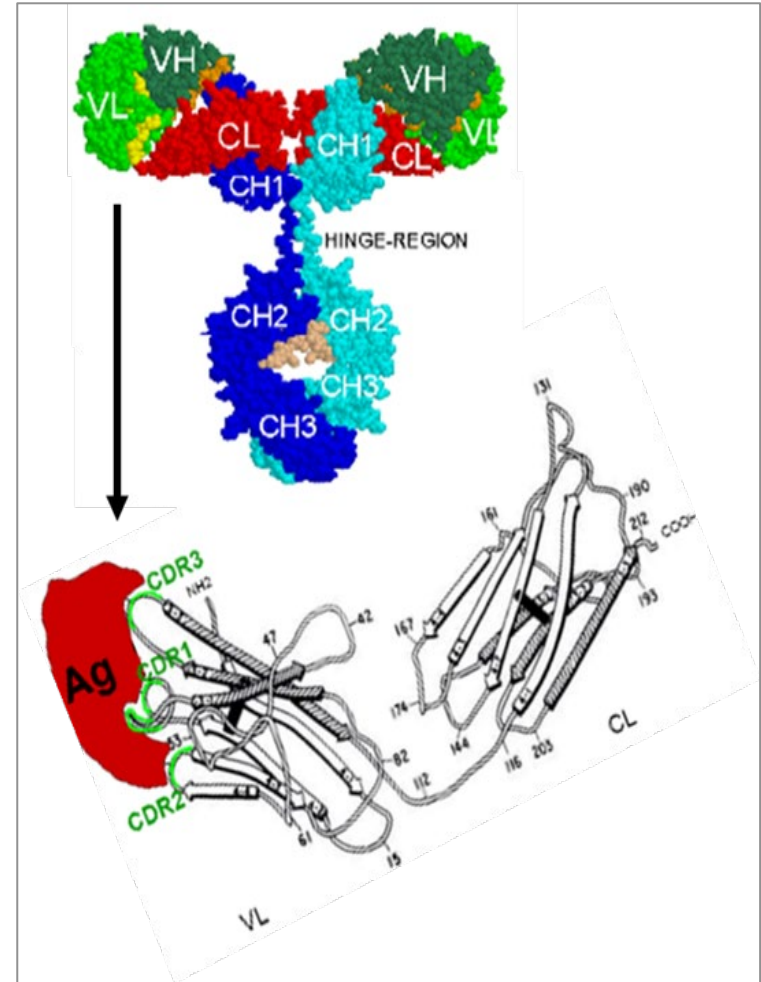


Renal Toxicity of Monoclonal Immunoglobulins



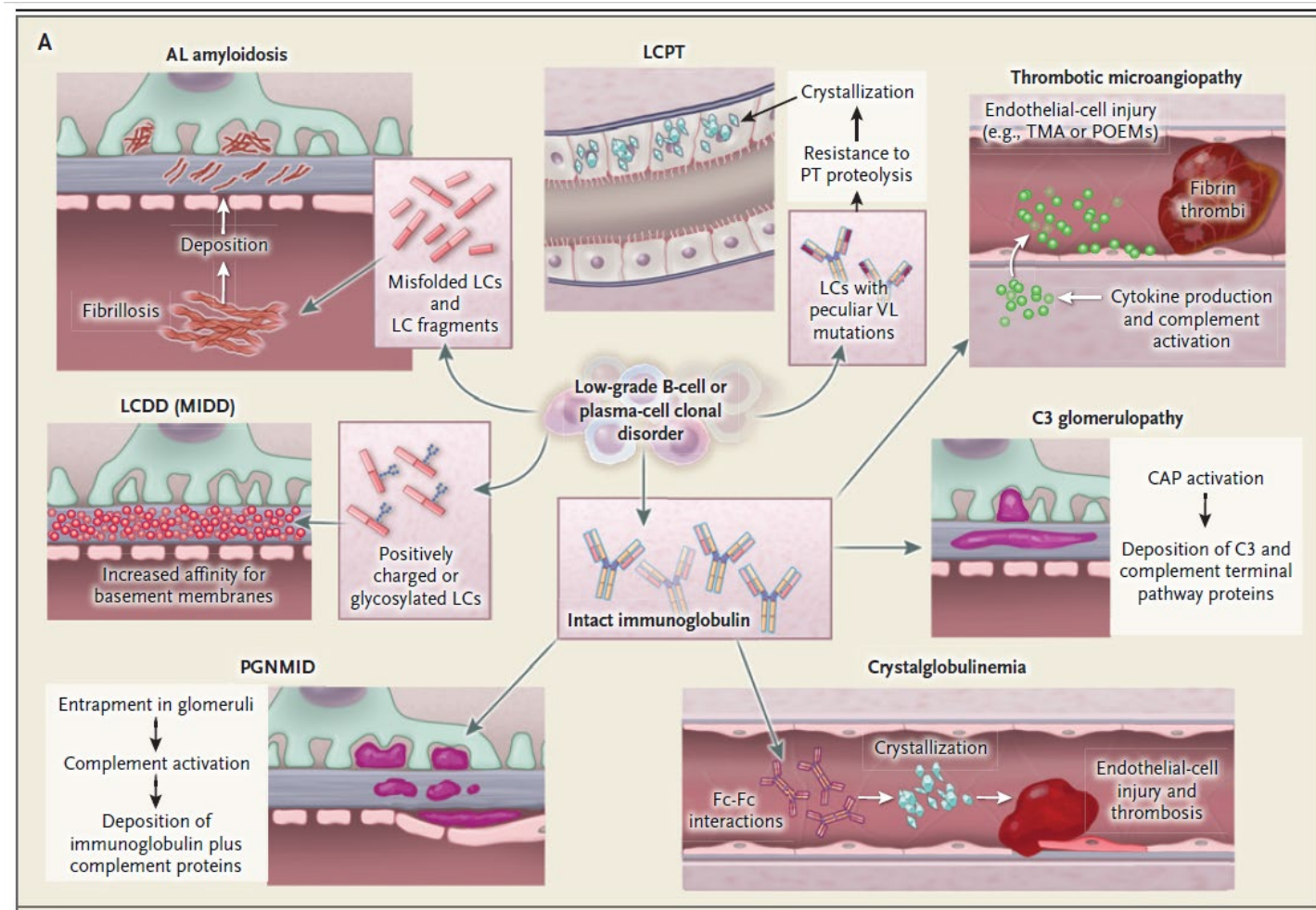
Renal Toxicity of Monoclonal Immunoglobulins

- **Intrinsic property** : not all monoclonal Ig are nephrotoxic
- **Mechanisms of nephrotoxicity** :
 - Direct : tissue deposition
 - Indirect : complement activation, autoantibody activity, cytokine production ...
- **Driven by molecular characteristics of the variable domain** (somatic hypermutations ++)
- **Independent of the tumor mass** (exception : LC cast nephropathy)

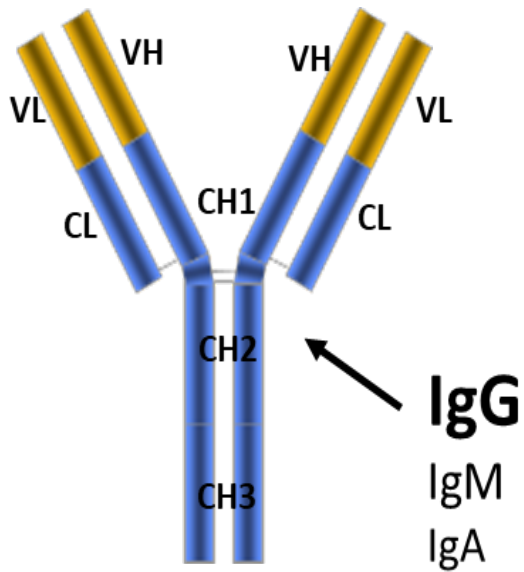


Mécanismes de toxicité rénale des Ig monoclonales au cours des MGRS

- Toxicité liée aux propriétés intrinsèques de l'Ig monoclonale (++) domaine variable)
- Indépendant de la masse tumorale (≠NCM)



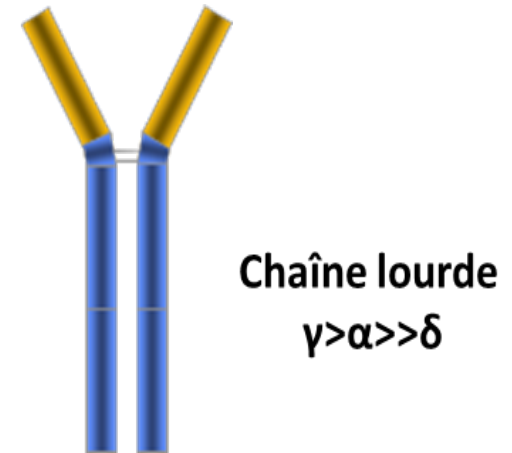
Immunoglobulines monoclonales au cours des MGRS



- *Cryoglobulinémie*
- *PGNMID*
- *ITG (GOMMID)*
- *GPC3, MAT*



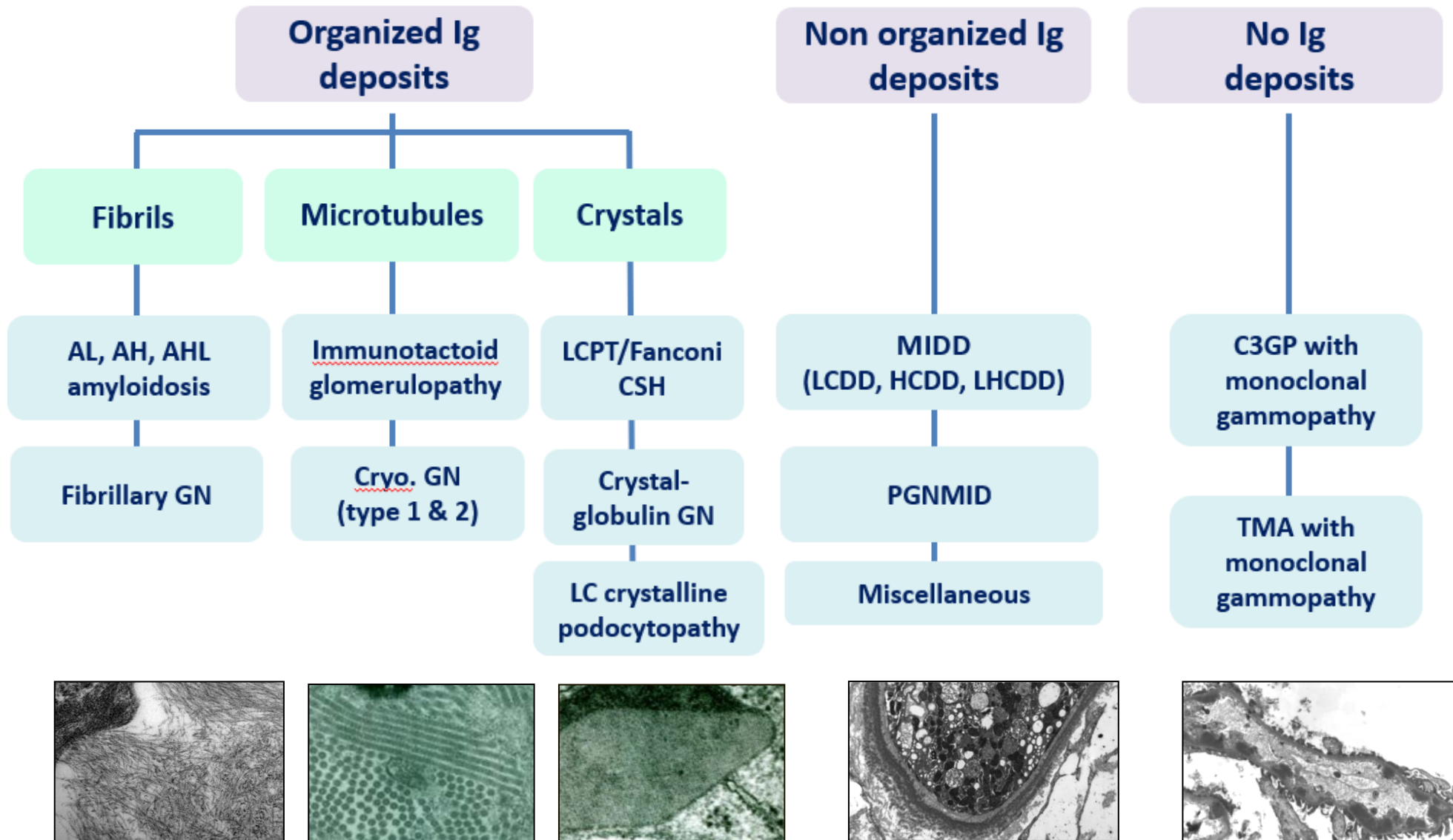
- *Amylose AL*
- *LCDD (Randall)*
- *Fanconi*
- *PGNMID*



- *Amylose AH*
- *HCDD (Randall)*

- *LHCDD (Randall)*
- *Amylose AHL*

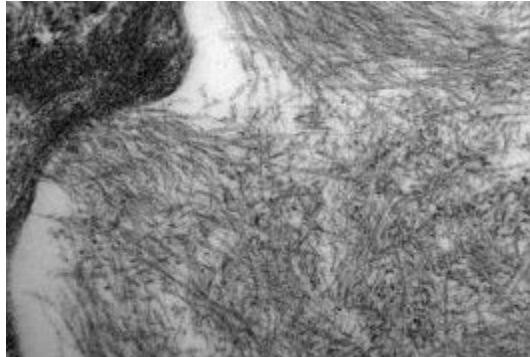
IKMG classification of MGRS-related renal diseases



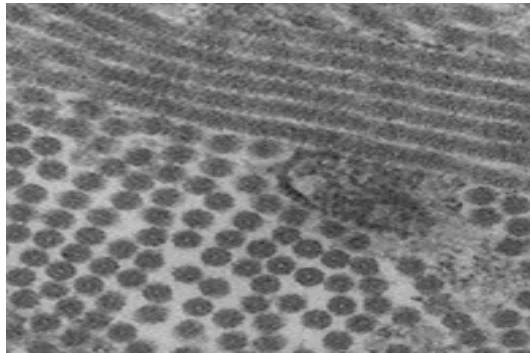
Glomérulopathies associées aux Ig monoclonales

Dépôts organisés

Amylose AL
(ou AH)



GN
immunotactöide
(=GOMMID)

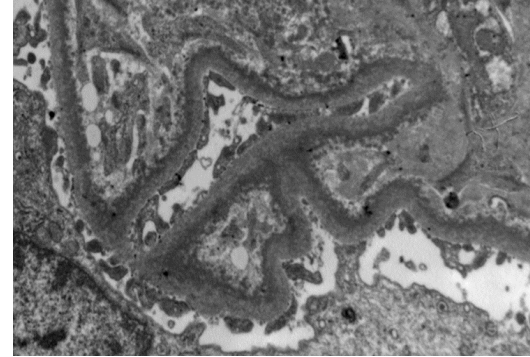


Cryo type I
Cryo type II

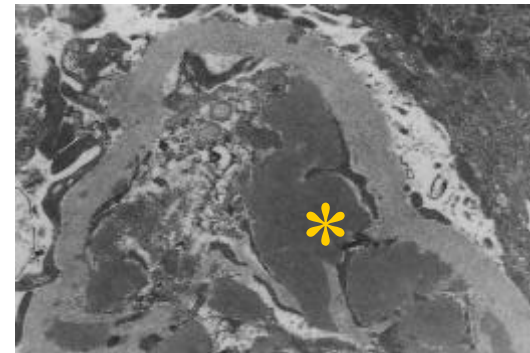


Dépôts non organisés

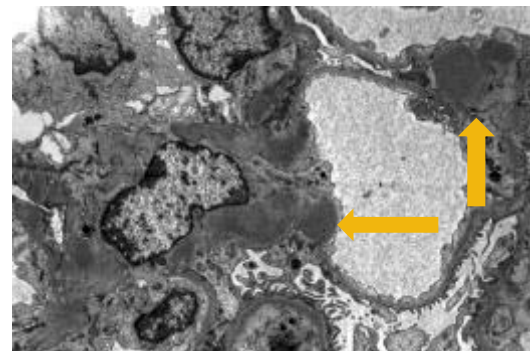
MIDD
(Randall)



PGNMID
(non-Randall)



GN à dépôts de
C3



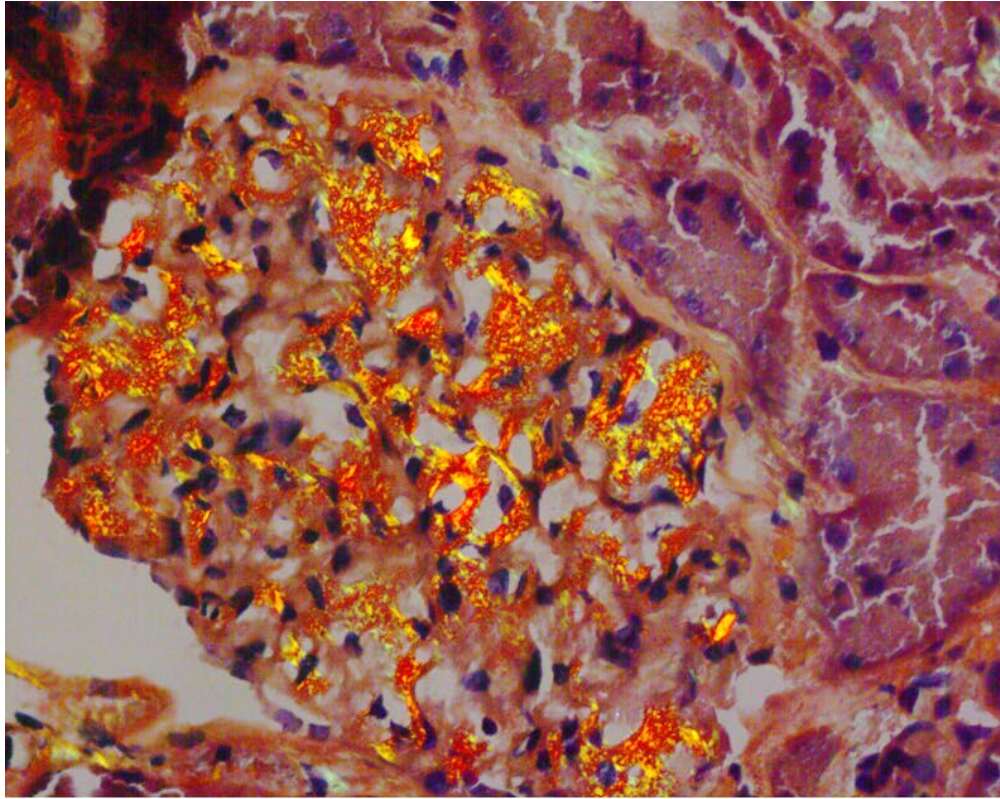
Cas clinique 1

- Mr A. 69 ans, caucasien, hospitalisé pour exploration d'une protéinurie glomérulaire (3,5 g/g, albuminémie = 35 g/l), sans hématurie ni augmentation de créatininémie
- Ancien manutentionnaire BTP à la retraite
- Absence d'antécédents familiaux de néphropathie
- Antécédents:
 - ✓ Hypertension artérielle.
 - ✓ Fibrillation auriculaire sous anticoagulation
 - ✓ **Lymphome B folliculaire inguinal traité il y a 2 ans par radiothérapie, en surveillance tous les 6 mois. Considéré en rémission**
 - ✓ Polyneuropathie d'étiologie indéterminée
 - ✓ Syndrome d'apnées du sommeil appareillé

Cas clinique 1

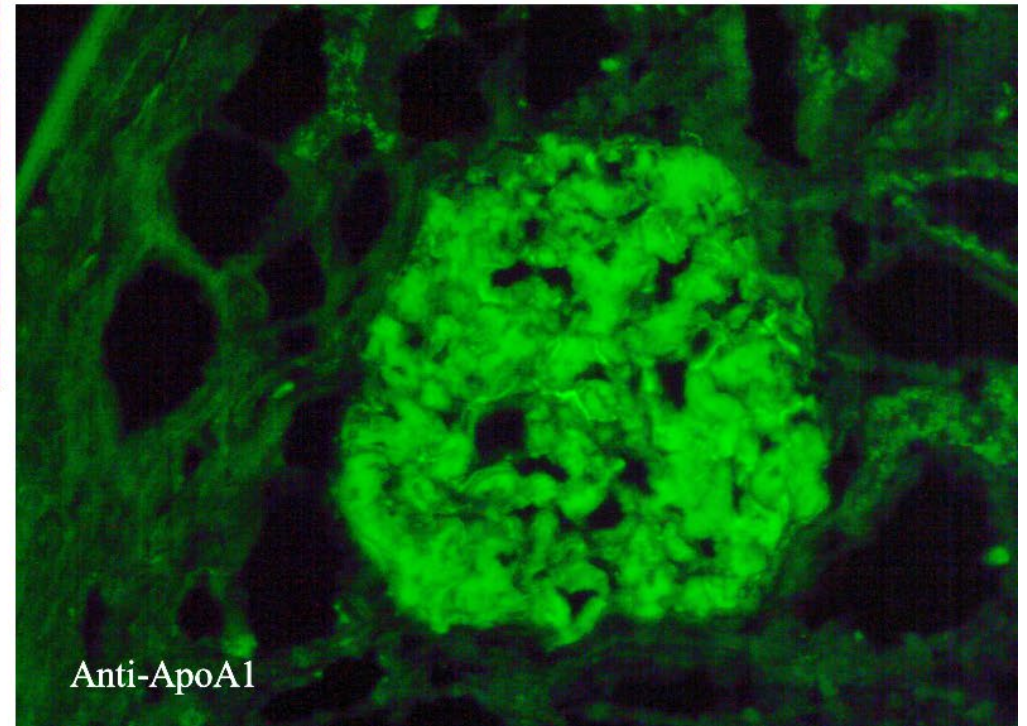
- Bilan immunologique : IgGκ <1g/l, κ=37 mg/l, λ=21 mg/l (κ/λ= 1,73)
- Myélogramme: plasmocyte 1%, CMF = absence de clone plasmocytaire ou lymphocytaire
- BGSA + graisse abdominale négative

Biopsie rénale : amylose glomérulaire isolée.
Aucun marquage en IF avec les anticorps anti-κ, anti-λ, anti-γ, anti-α, anti-μ et anti-SAA



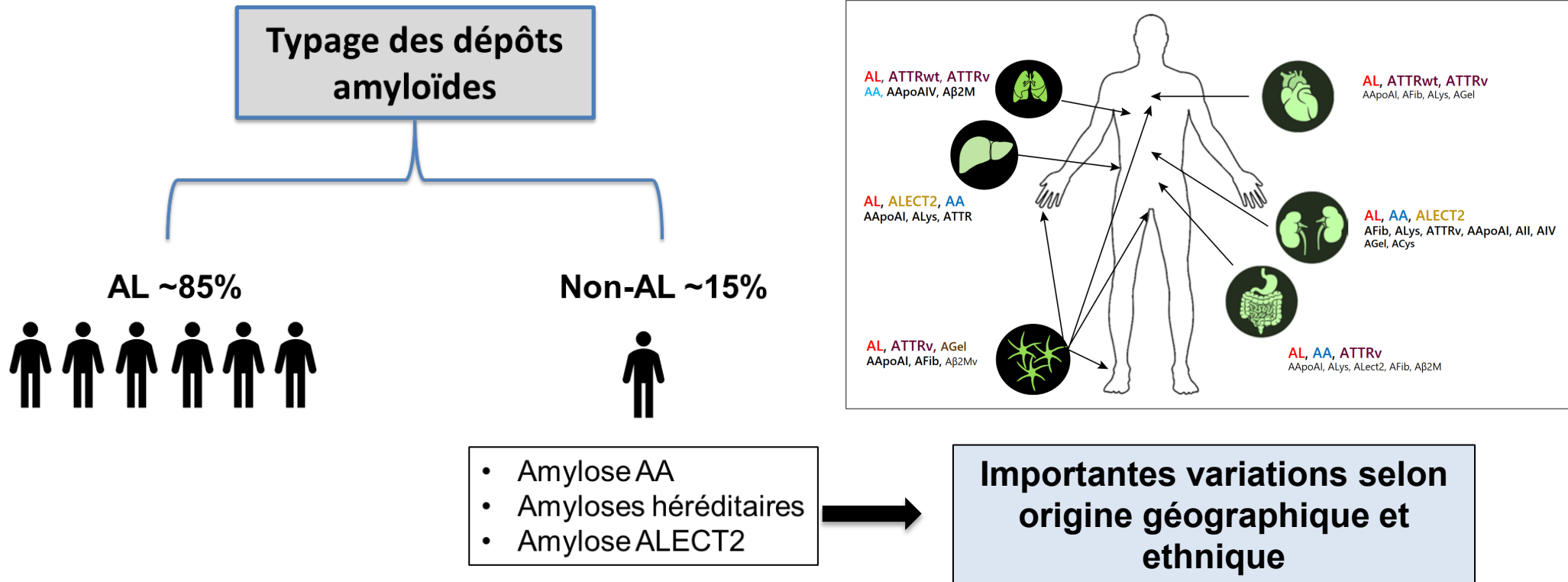
Panel utilisé:
anti-kappa, anti-lambda, anti-gamma,
anti-alpha, anti-mu, anti-SSA,
anti-chaîne α fibrinogène, anti-TTR,
anti-ApoA1, anti-ApoA2

***Amylose ApoA1 ?
=> Analyse génétique***



Anti-ApoA1

Amyloses avec atteinte rénale



Atteintes extra-rénales ?

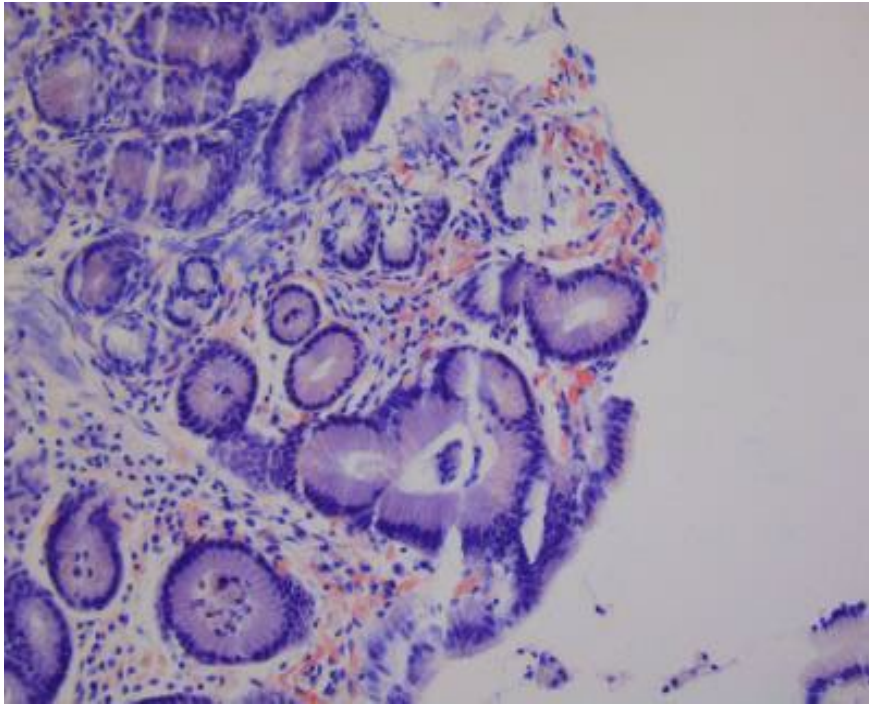
- **Explorations cardiaques :**
 - ✓ Troponine à 21 ng/l, NTproBNP à 915 ng/l
 - ✓ ETT : HVG concentrique avec SIVd 15 nm, FEVG 68%, altération de la fonction systolique longitudinale (GLS - 14%)
 - ✓ Holter ECG : FA lente permanente sans autres anomalies
- **ENMG:** polyneuropathie axonale sévère sensitivo-motrice longueur dépendante; atteinte des nerfs médians au canal carpien bilatéral
- Bilan hépatique normal
- Examen cutané, ORL et ophtalmologique sans particularités

6 mois plus tard...

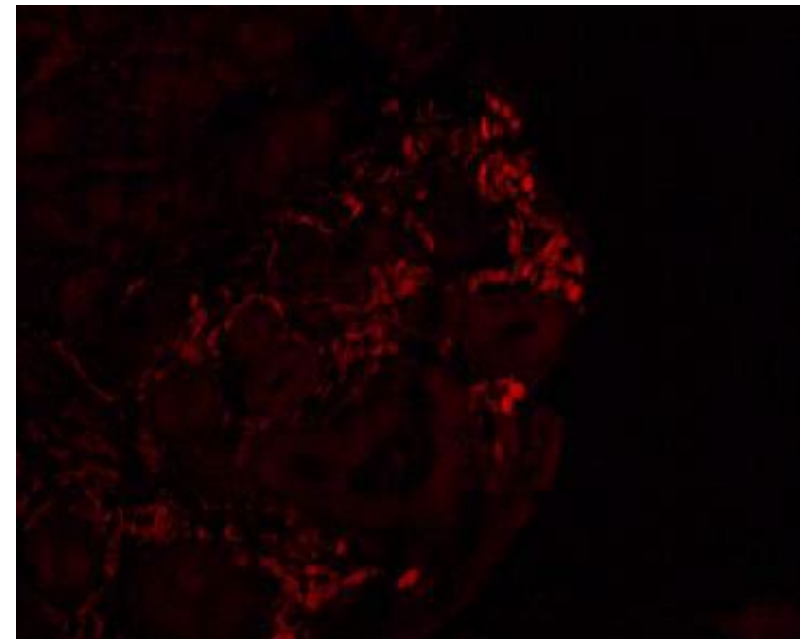
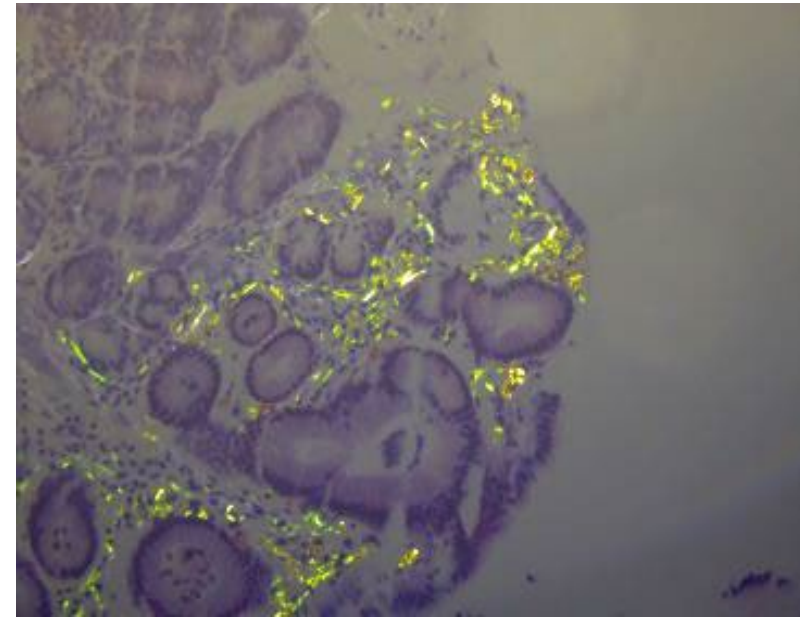
- Altération de l'état général avec dyspnée de moindre effort + OMI
- Apparition diarrhée
- Génétique toujours en attente

=> nouvelle hospitalisation

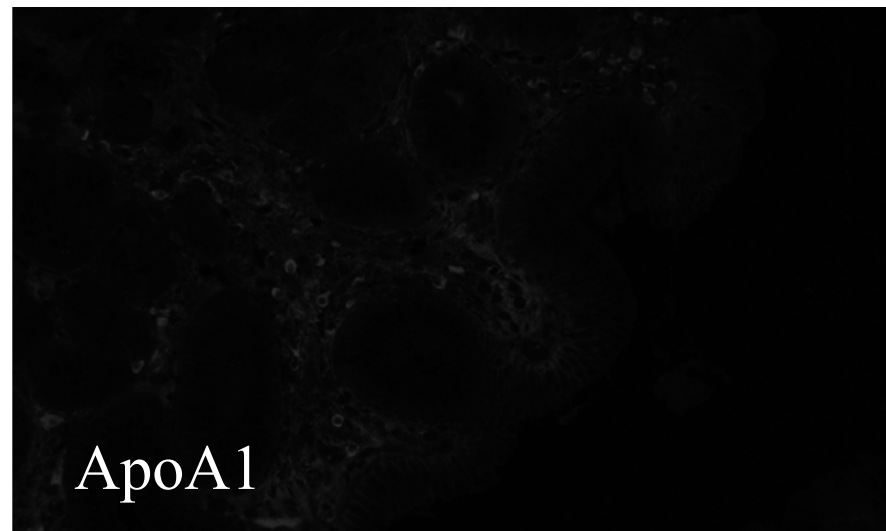
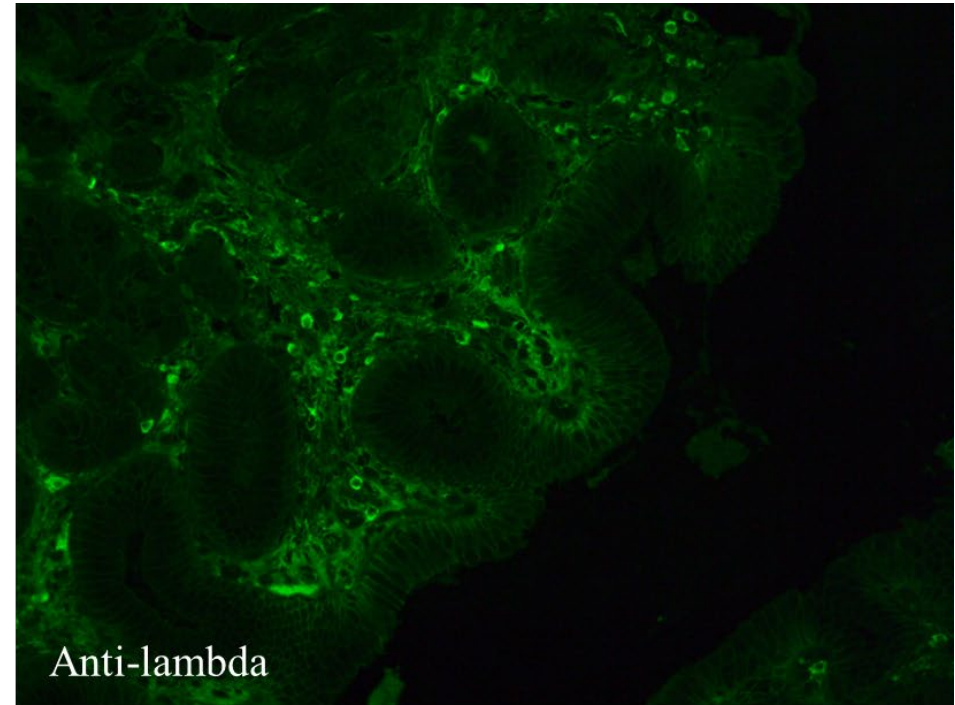
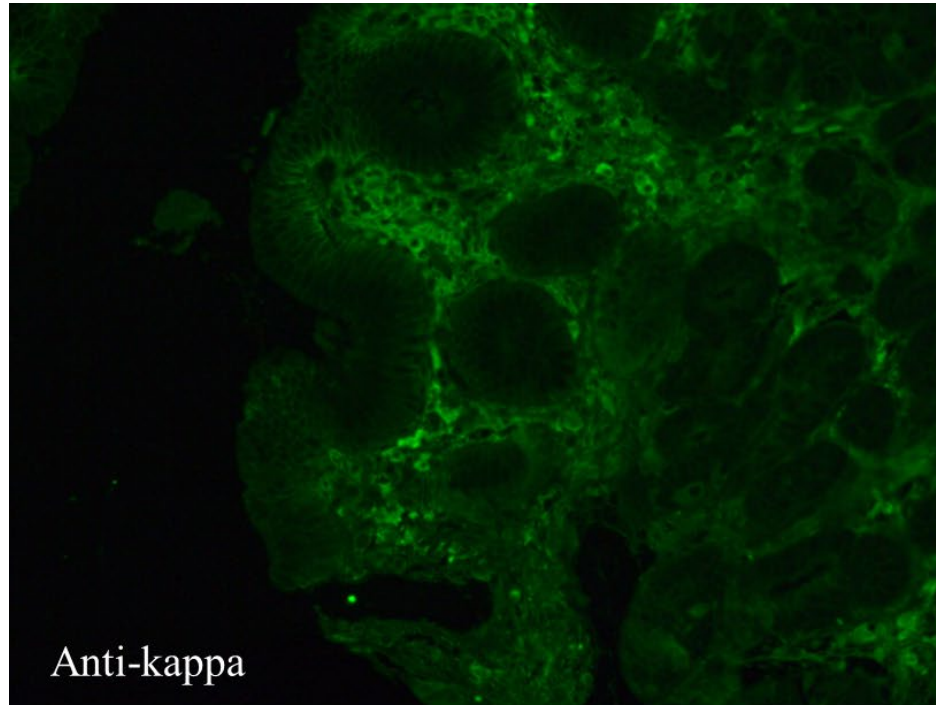
Bilan endoscopique



Biopsie gastrique



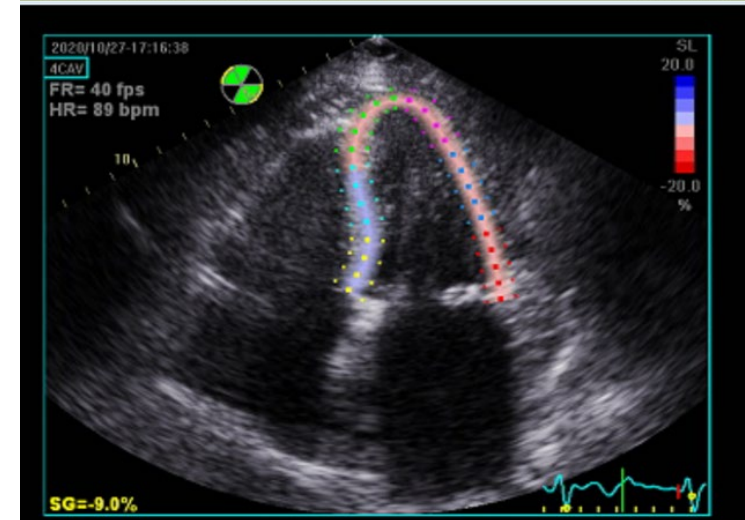
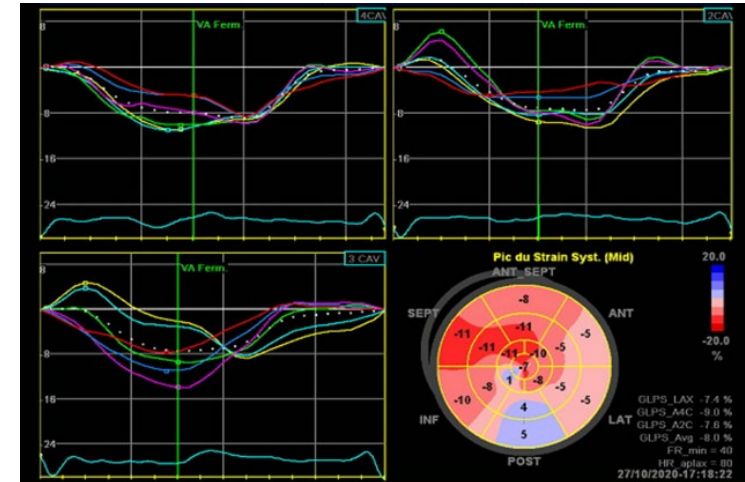
Nouvelle tentative de typage des dépôts



Evolution des paramètres cardiaques



6 mois



Réévaluation hématologique

- Pic IgG kappa stable 1 g/l mais majoration des chaînes légères kappa à 92 mg/l (contre 37 mg/l il y a 6 mois), dFLC = 68 (contre 16)
- Myélogramme: 1% de plasmocytes sans clone lymphocytaire ou plasmocytaire en CMF
- BOM : pas d'infiltrat lymphomateux, RC négatif

Attention aux pièges dans la recherche de gammopathie monoclonale

- **Présence d'une Ig monoclonale ?**

- Pourcentage sans protéine monoclonale (en couplant FLC, immunofixation sang et urine) <1%

- 1/592 patients série récente (*Muchtar E, et al. Mayo Clin Proc 2019*)
- **mais problèmes de sensibilité de détection selon les techniques utilisées**

- **ATTENTION: patients avec FLC normales**

- ~10%
- Formes généralement moins évolutives
- Atteinte rénale isolée fréquente
- **Interprétation selon le niveau de fonction rénale**

Intérêt des
nouvelles
méthodes?

www.kidney-international.org

technical notes

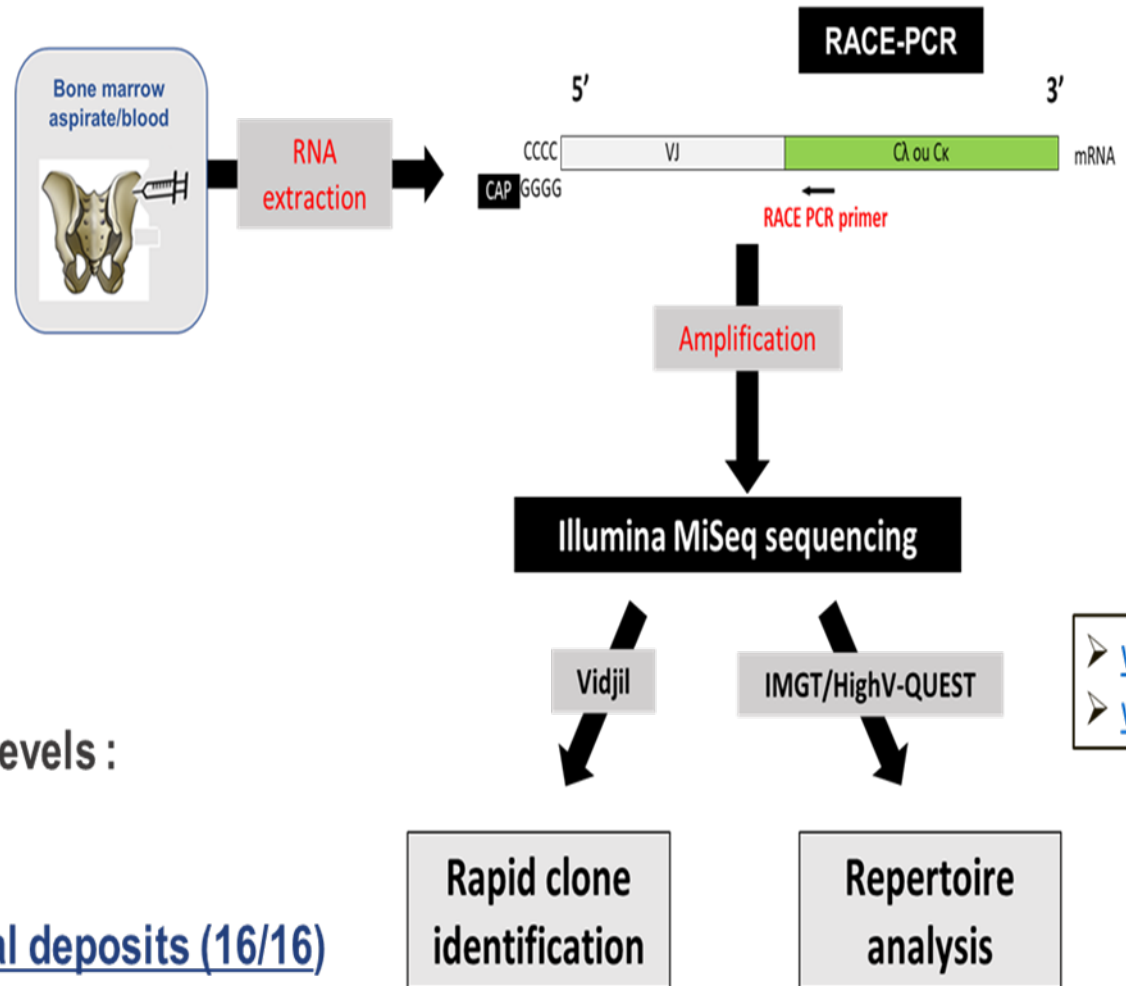
RNA-based immunoglobulin repertoire sequencing is a new tool for the management of monoclonal gammopathy of renal (kidney) significance

Vincent Javague^{1,2,9,10}, Virginie Pascal^{2,3,9,10}, Sébastien Bender^{2,9}, Sarah Nasraddine³, Mathilde Dargelos^{1,9}, Mehdi Alizadeh⁴, Alexis Saintamand⁵, Matthieu Filloux³, Paco Derouault⁶, Sabrina Bouyer⁷, Estelle Desport^{1,9}, Arnaud Jaccard^{2,8,9}, Frank Bridoux^{1,2,9}, Michel Cogne^{2,5,9} and Christophe Sirac^{2,9}

RNA-based Ig repertoire sequencing : a new tool for managing MGRS

RACE-RepSeq :

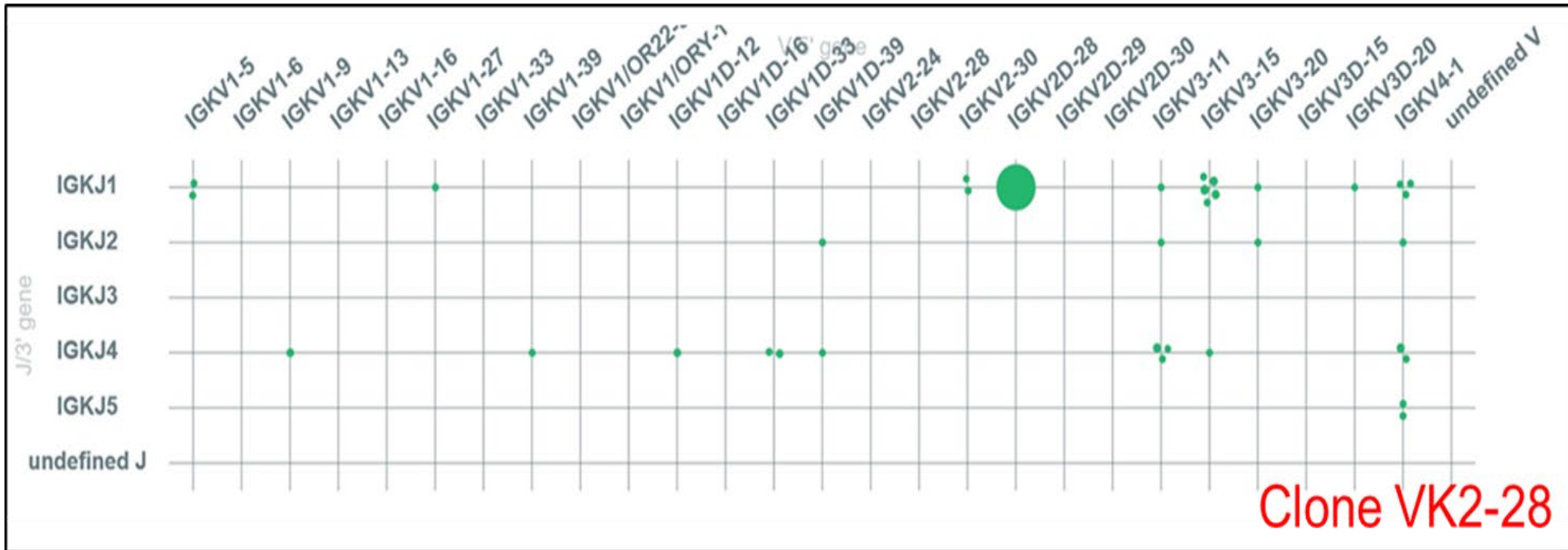
- High-throughput sequencing assay from bone marrow/blood mRNA encoding immunoglobulins
- Provides full-length V(D)J sequence of the monoclonal Ig and the dominant immunoglobulin repertoire



16 MGRS patients with low MIg/FLC levels :

- Negative bone marrow IHC (11/11)
- Negative flow cytometry (7/12)
- RACE RepSeq: clone matching renal deposits (16/16)

Séquençage des immunoglobulines par RACE-RepSeq chez Mr A.

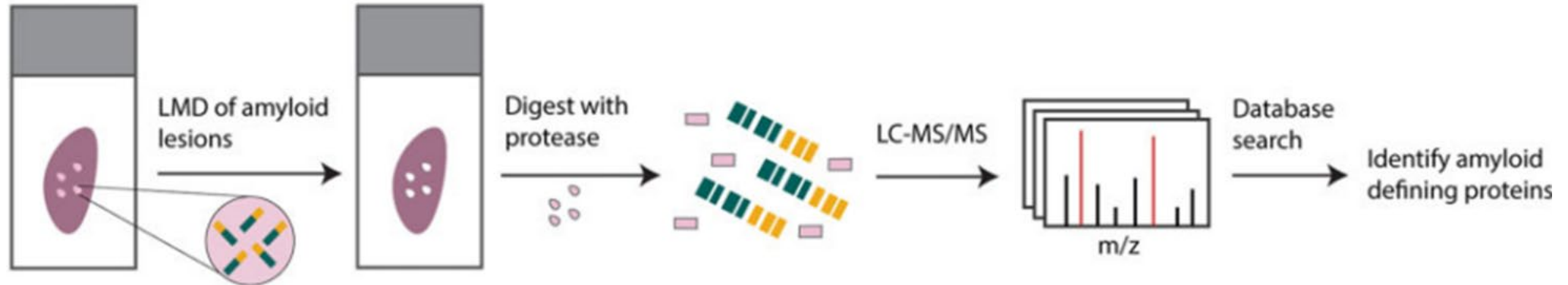


Chaîne légère Kappa tronquée d'une partie du domaine constant pouvant expliquer l'absence de marquage en IF sur la PBR

Improving the Diagnostic Performance of Kidney Biopsy in MGRS

- **Tandem mass spectrometry after laser microdissection (LMD/LC-MS)**

- Performed on paraffin-embedded tissue, salvage technique when inconclusive IF
- High diagnostic accuracy (>90%) in renal amyloidosis



Vrana JA, et al. Blood 2009; Leung N, et al. Nat Rev Nephrol. 2019

Amyloid Protein ID, Par, LC MS/MS

Case Number CR-20-75340

Interpretation

MCR

Stomach, specimen for amyloid typing (20-0g-170B; 2/25/2020): Involved by amyloidosis, consistent with AL (kappa)-type.

A Congo red stain was performed on paraffin sections of the specimen. Congo red-positive amyloid deposits are present.

Liquid chromatography tandem mass spectrometry (LC MS/MS) was performed on peptides extracted from Congo red-positive, microdissected areas of the paraffin-embedded specimen.

LC MS/MS detected a peptide profile consistent with AL (kappa)-type amyloid deposition.

These findings support the diagnosis of amyloidosis and are consistent with AL (kappa)-type amyloid deposition.

Comment: In this anatomic site and with the observed histologic pattern of amyloid deposition, AL amyloidosis is usually associated with systemic disease due to an underlying clonal plasma cell proliferation/B-cell lymphoma. Correlation of the mass spectrometry findings with the clinical and laboratory features is recommended.

If there are any questions about the analysis or the diagnosis in this case, please call the Department of Laboratory Medicine and Pathology, Mayo Clinic Laboratories at 1-800-533-1710.

Material Received

MCR

A. 20CNR163 / 20/0g170B: Gastric biopsy
1 block

Report electronically signed by

MCR

Ellen D. McPhail, M.D.

Seen in consultation with: Matthew Howard, M.D. and Linda N. Dao, M.D. 3-5343

Disclaimer

MCR

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

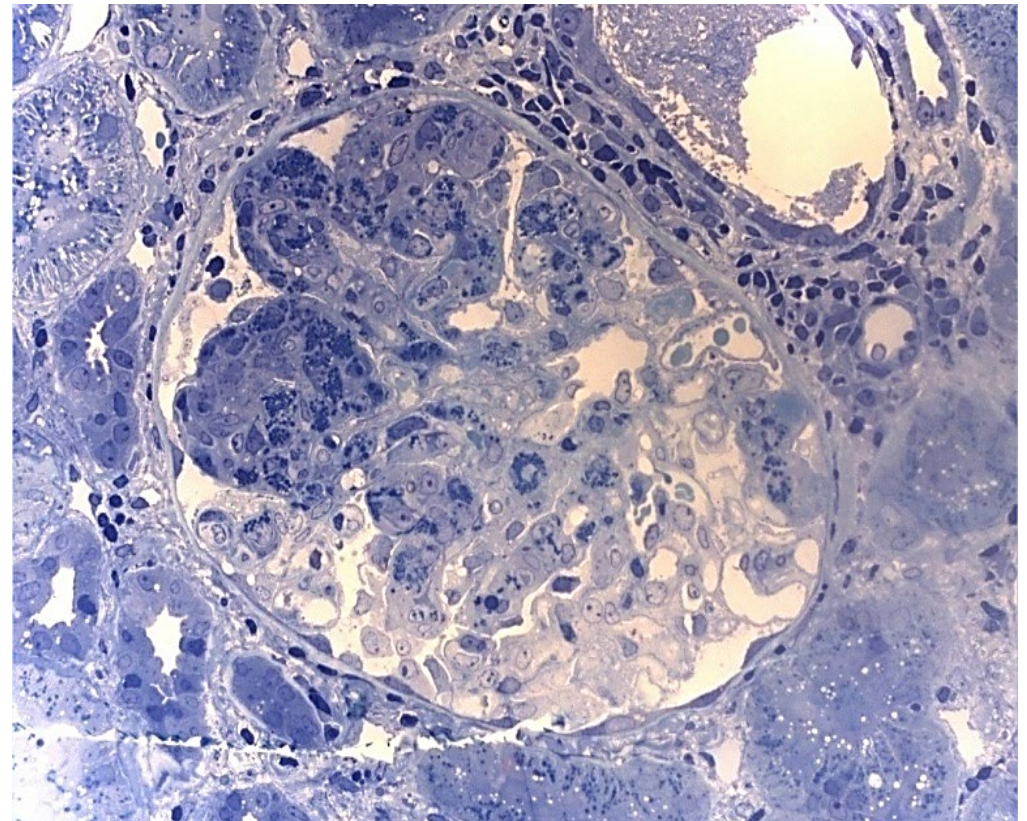
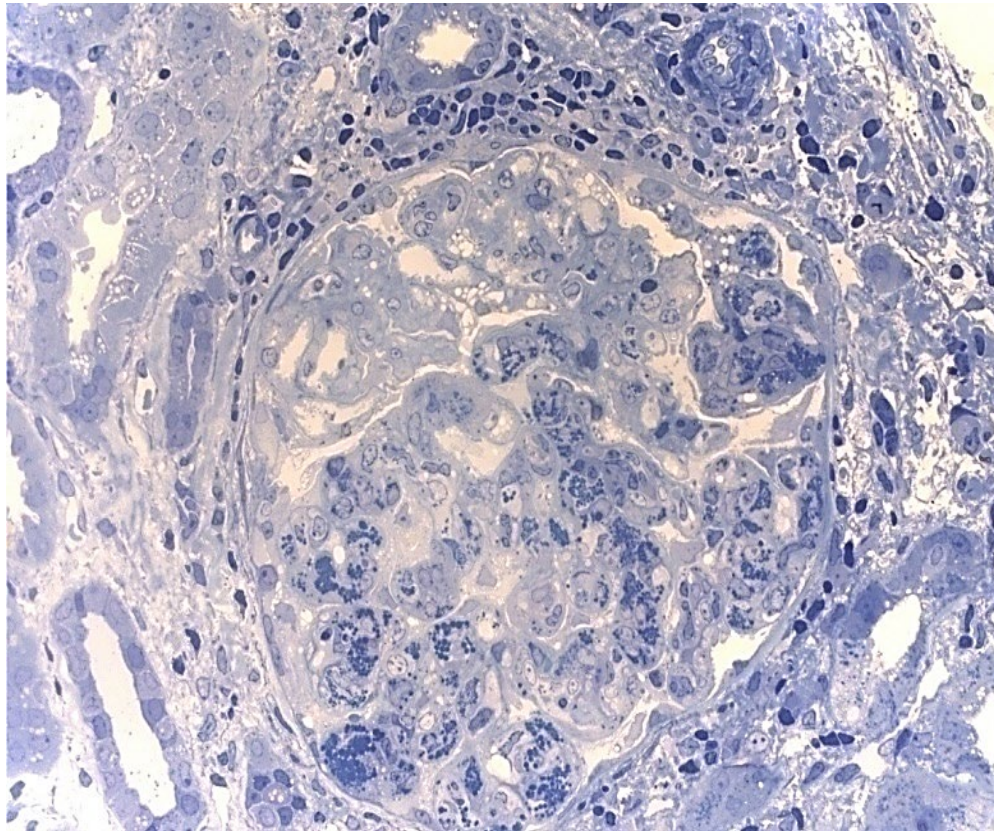
Difficultés du diagnostic histologique des amyloses

- **Interprétation du rouge Congo**
 - *Faux négatifs : épaisseur de coupe insuffisante, certaines amyloses peu congophiles (TTR)*
 - *Faux positifs : collagène*
- **Certaines amyloses débutantes peuvent se présenter avec des glomérules optiquement normaux et IF douteuse/négative (place de la ME +++)**
- **Actuellement aucune technique ne permet un typage certain dans 100% des cas**

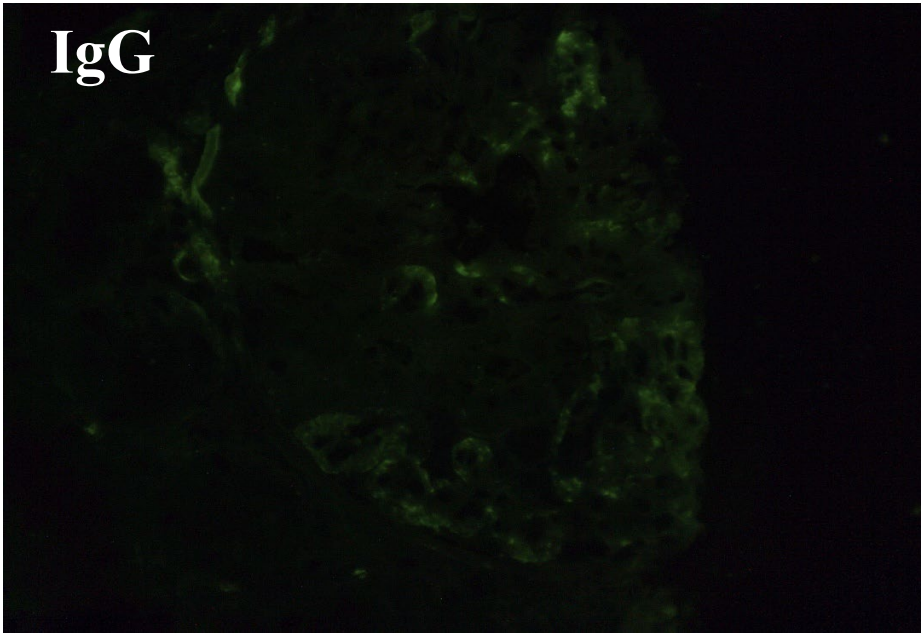
Importance de la corrélation anatomo-clinique +++

Cas clinique 2

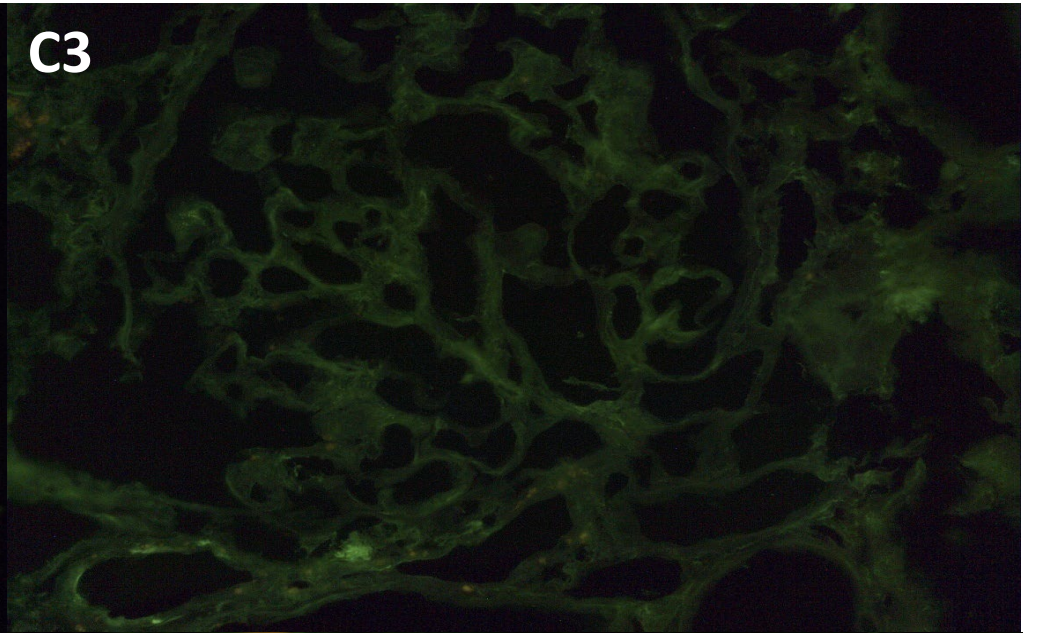
**Patiente âgée de 61 ans, gammapathie monoclonale IgG kappa.
Tableau de syndrome néphritique, créatininémie = 159 $\mu\text{mol/l}$**



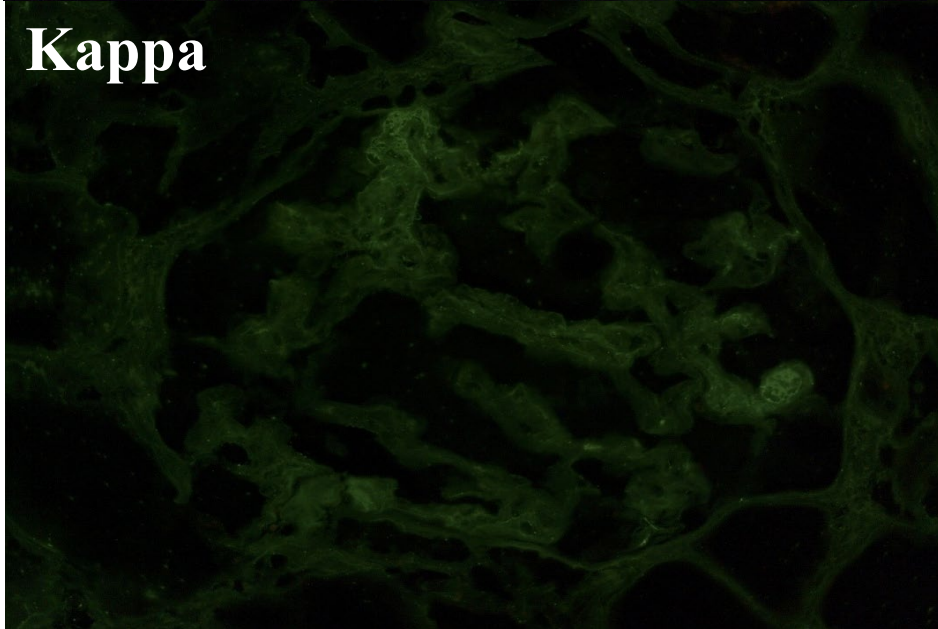
IgG



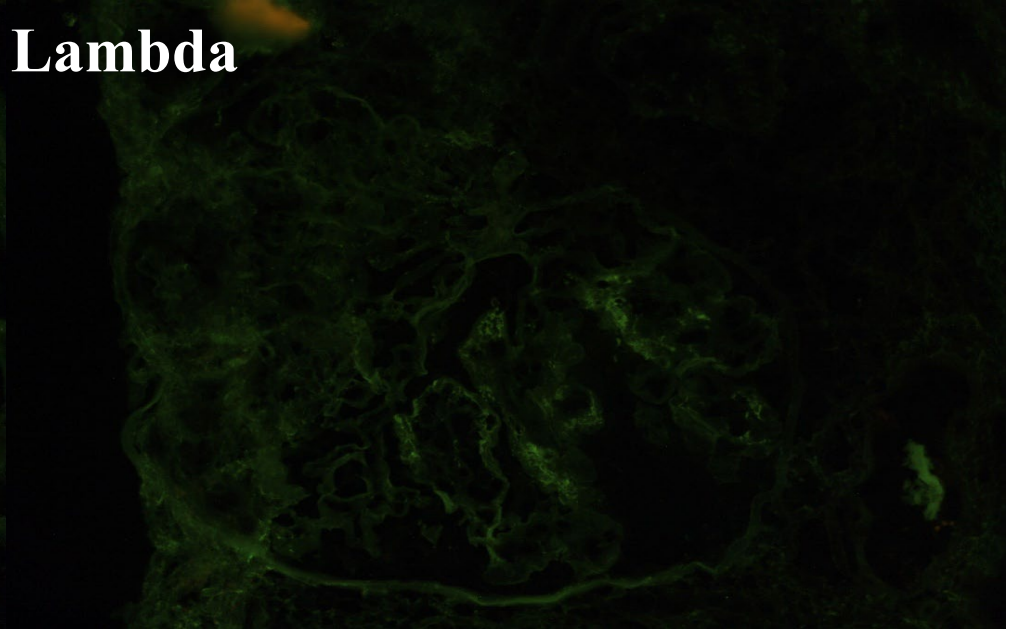
C3

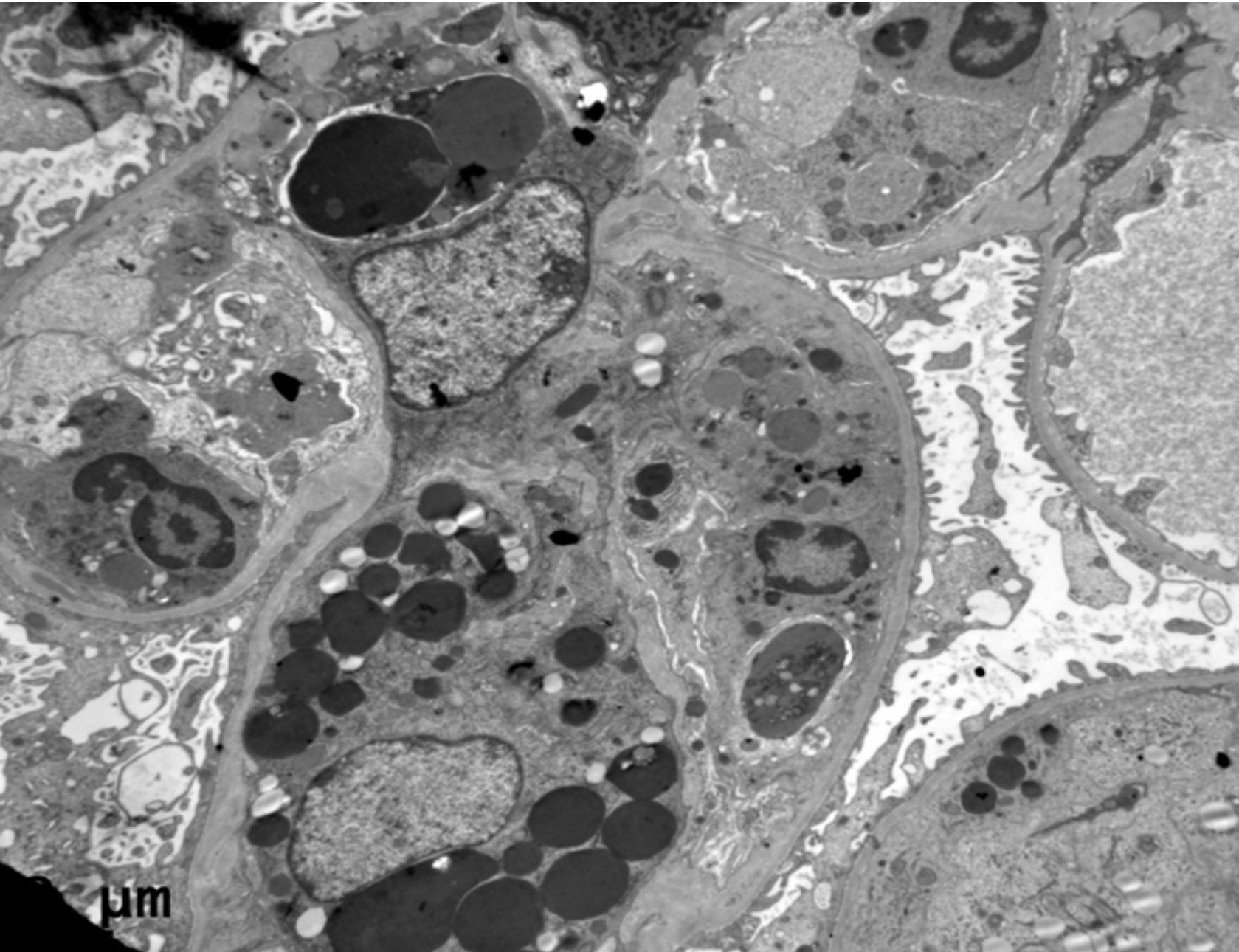


Kappa

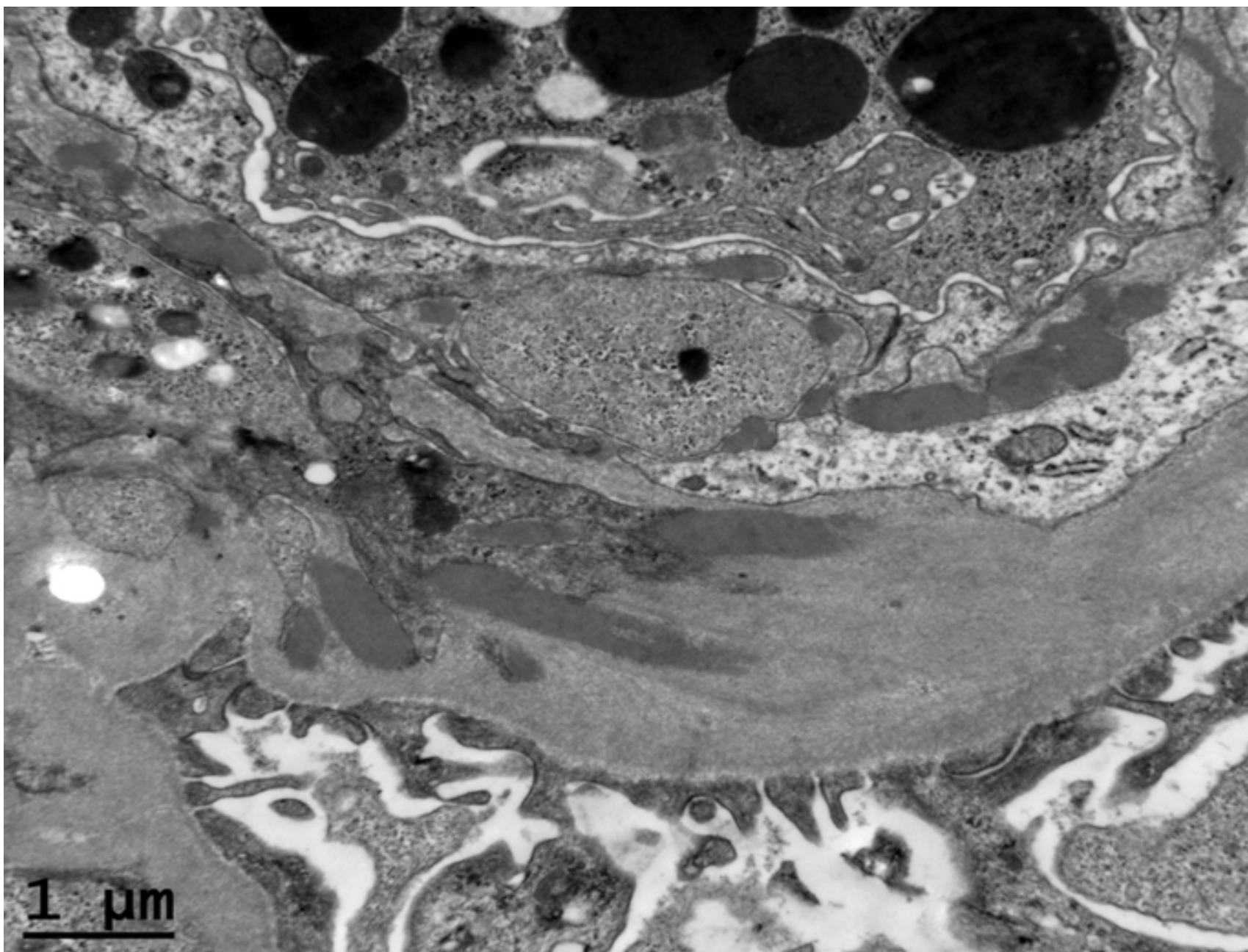


Lambda

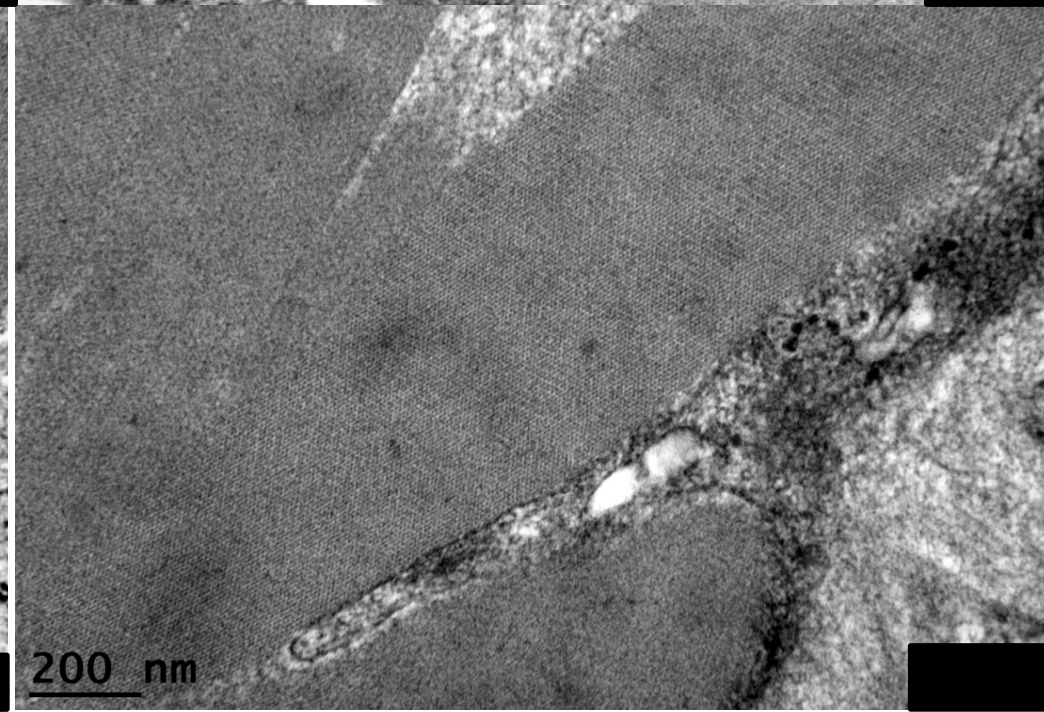
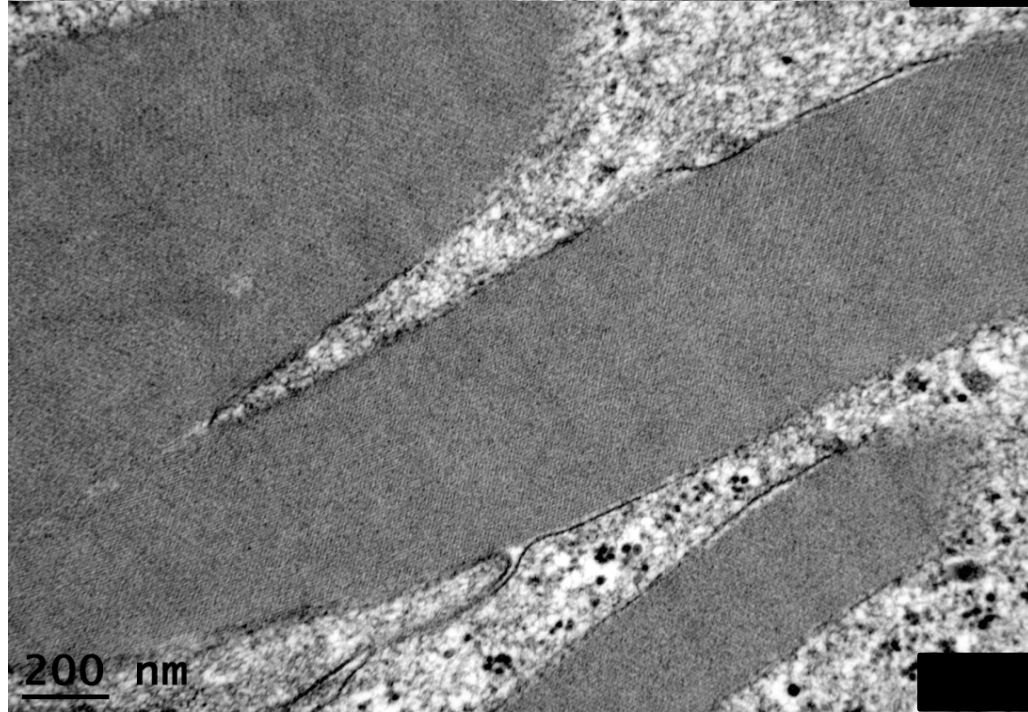
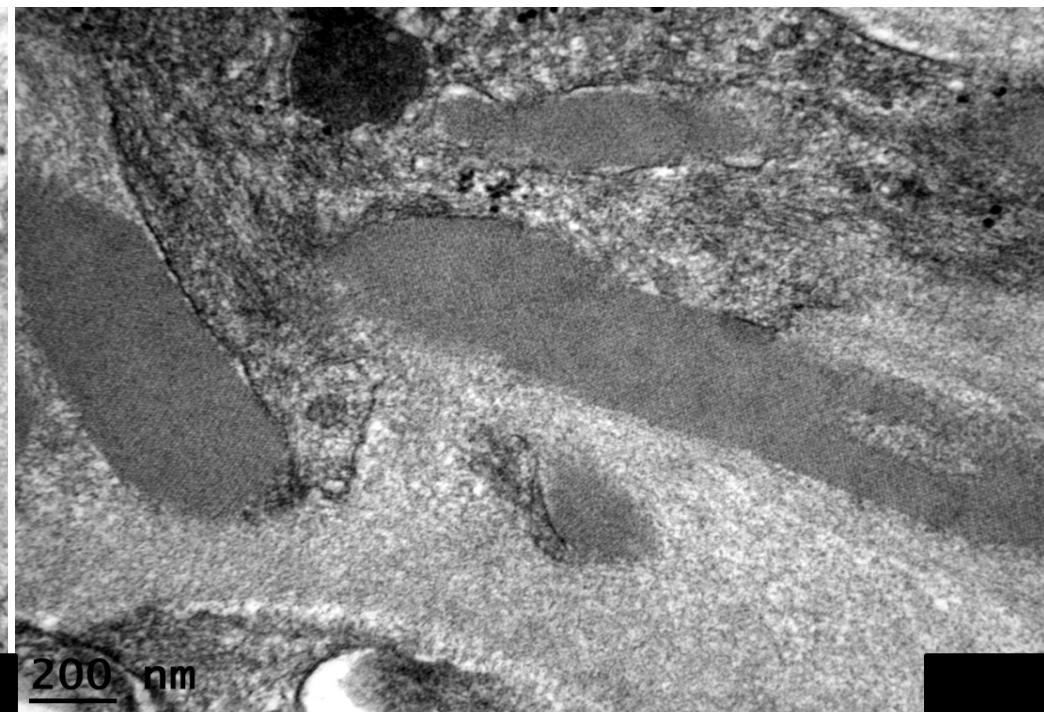
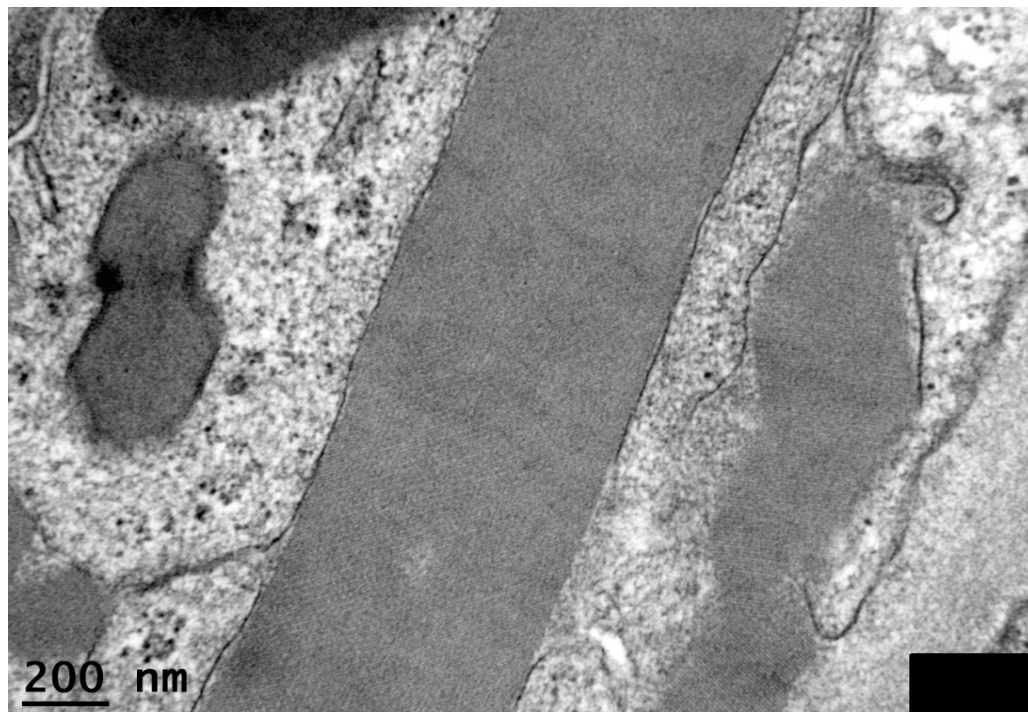


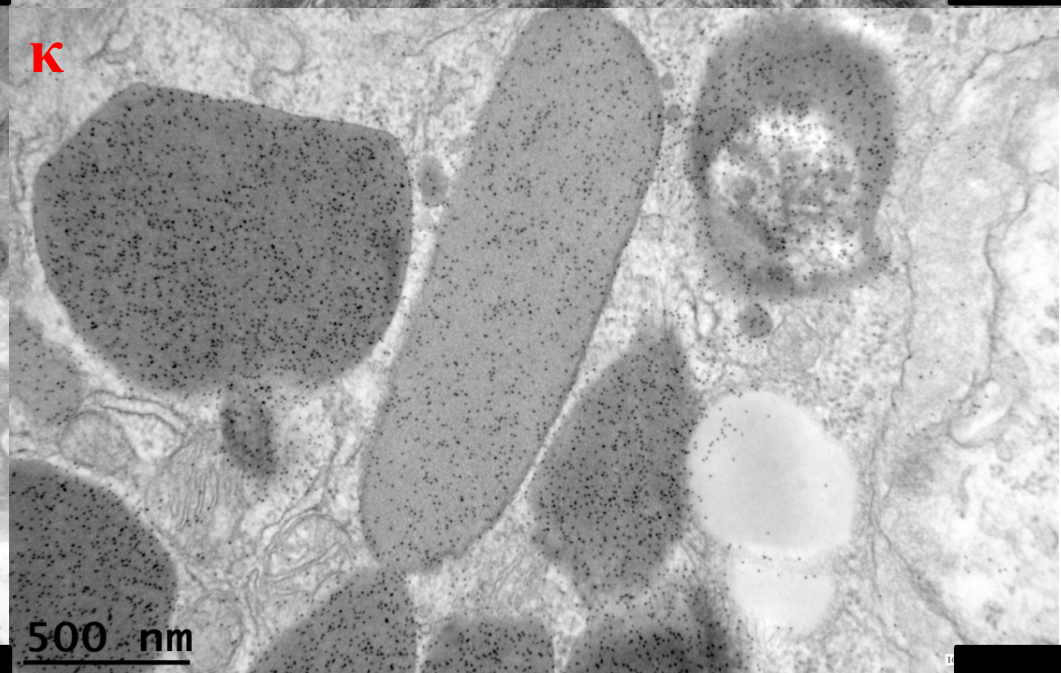
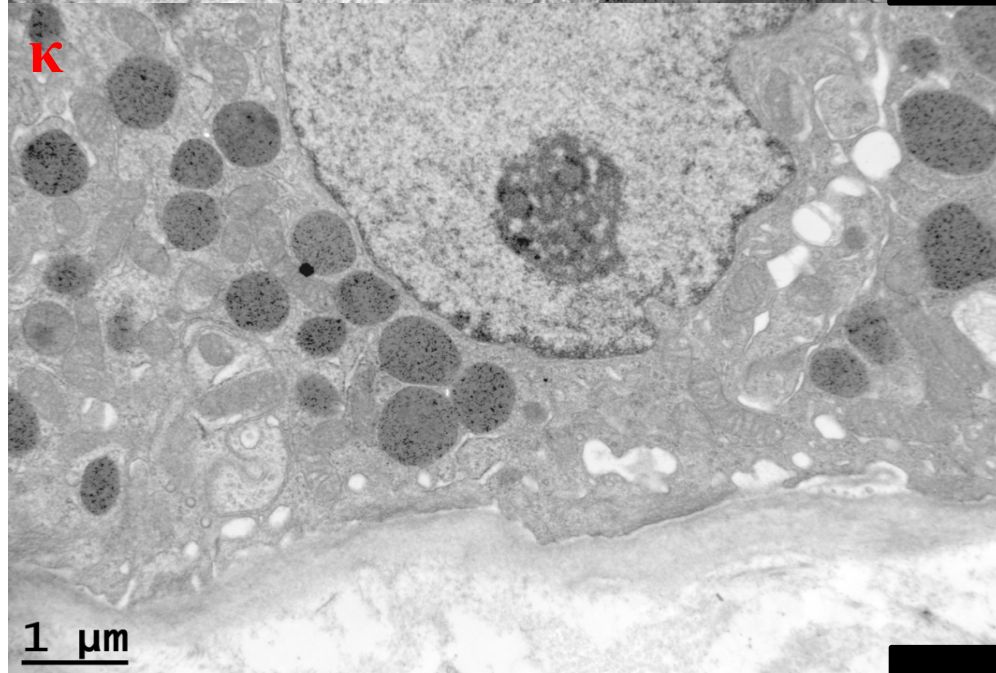
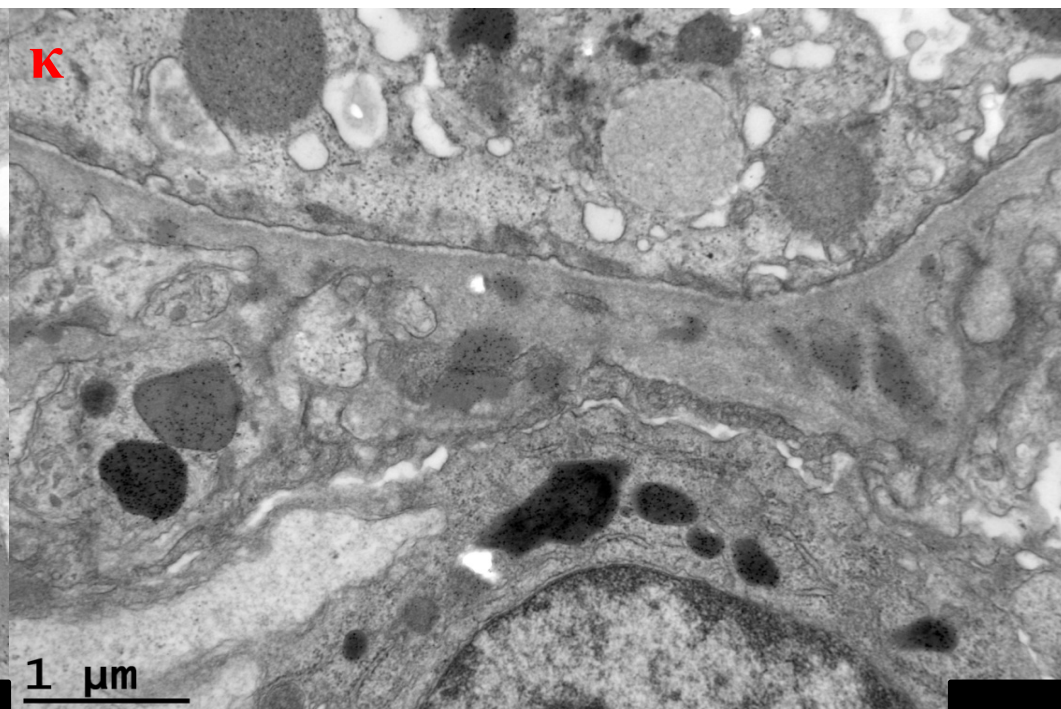
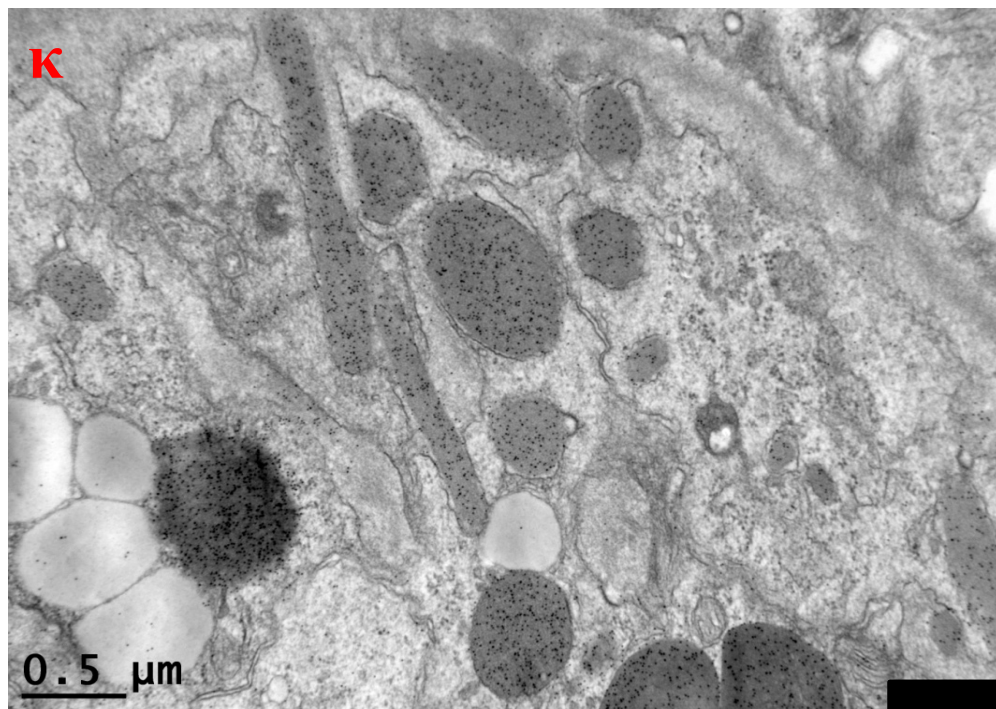


ME x 15 000



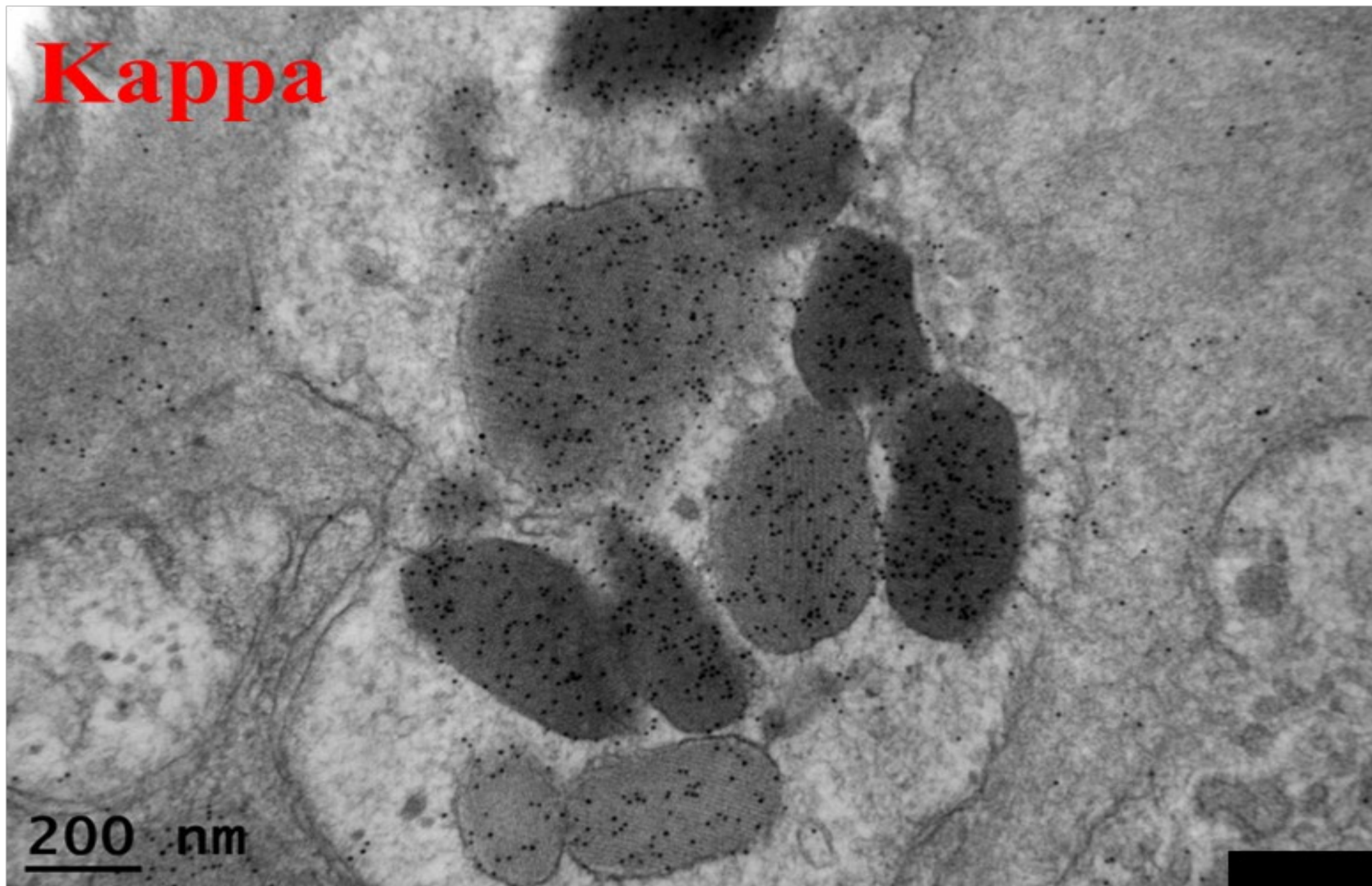
ME x 30 000





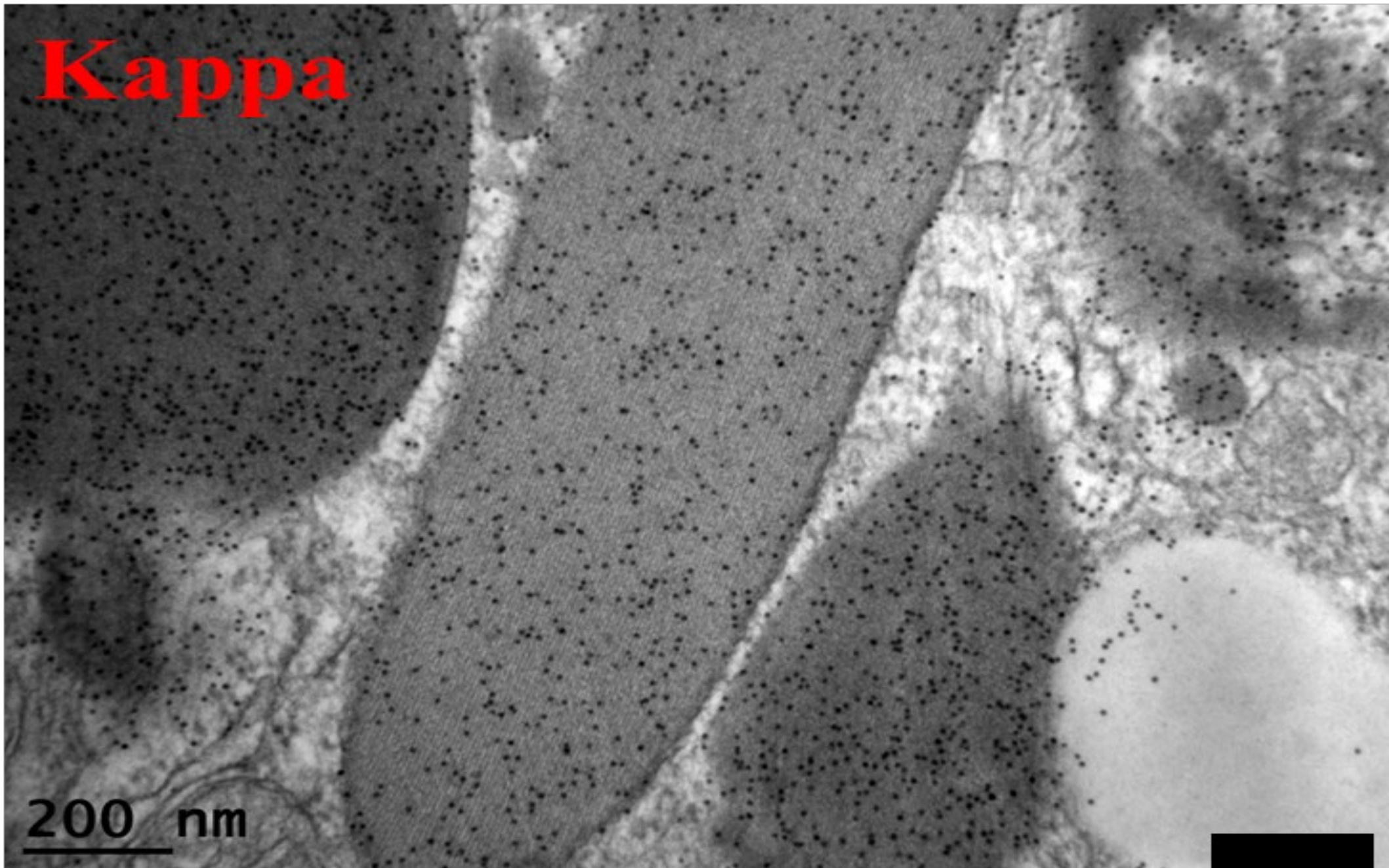
Kappa

200 nm

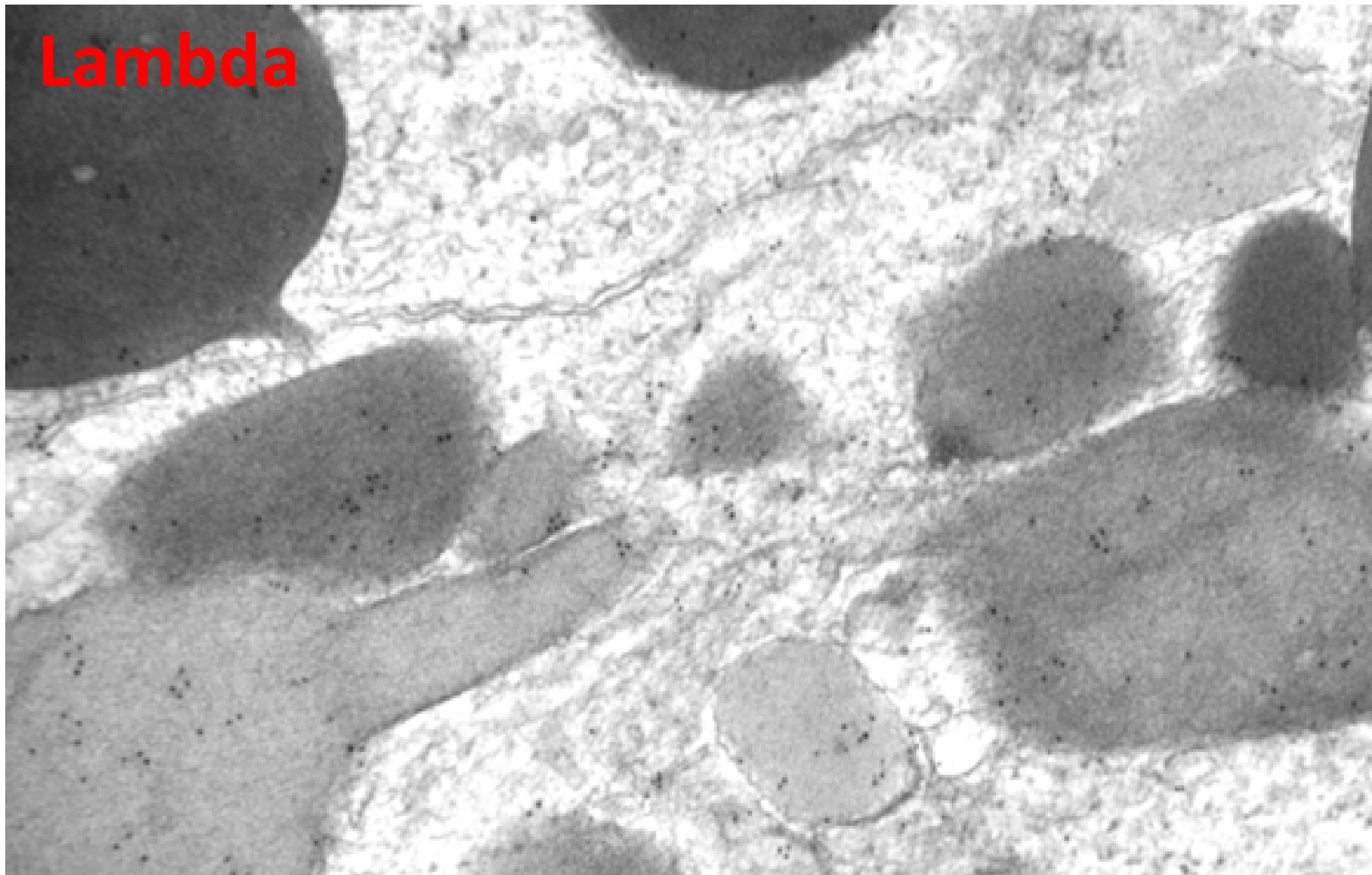


Kappa

200 nm



Lambda



IgG

=> Diagnostic de
Cristalcryo IgG kappa

*Absence de cryoglobulinémie
détectable*

μm

Cryoglobulinemic Glomerulonephritis

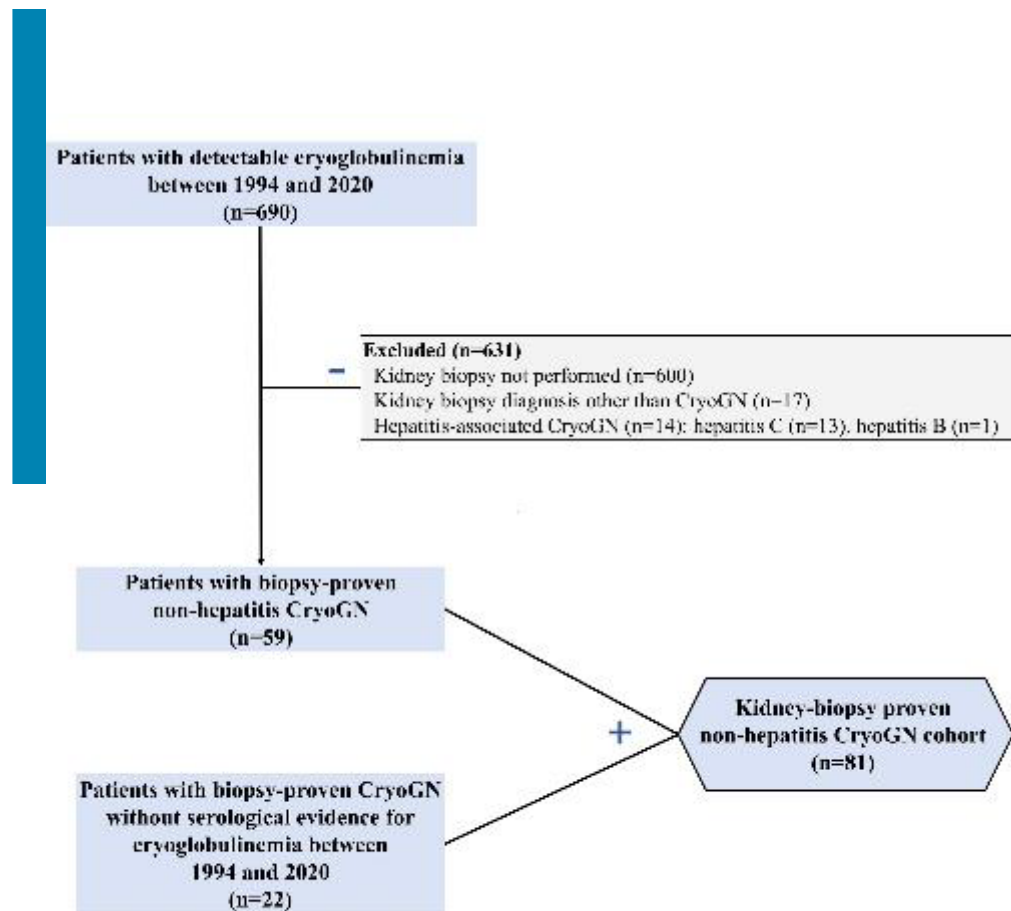
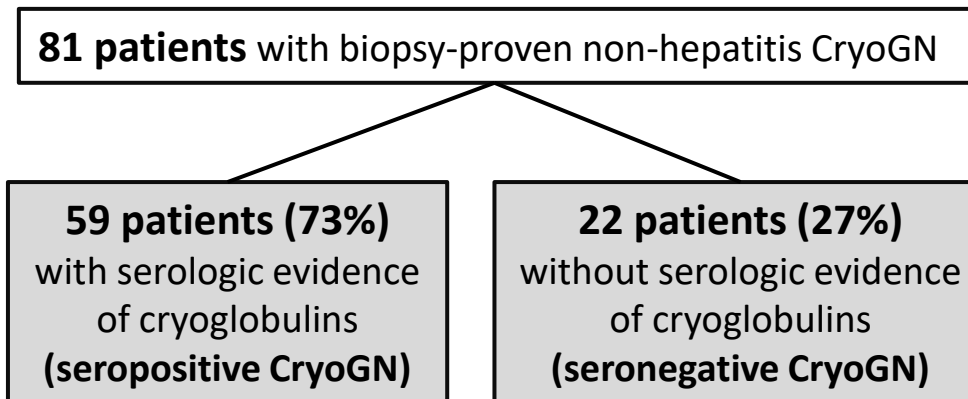
www.kidney-international.org

clinical investigation

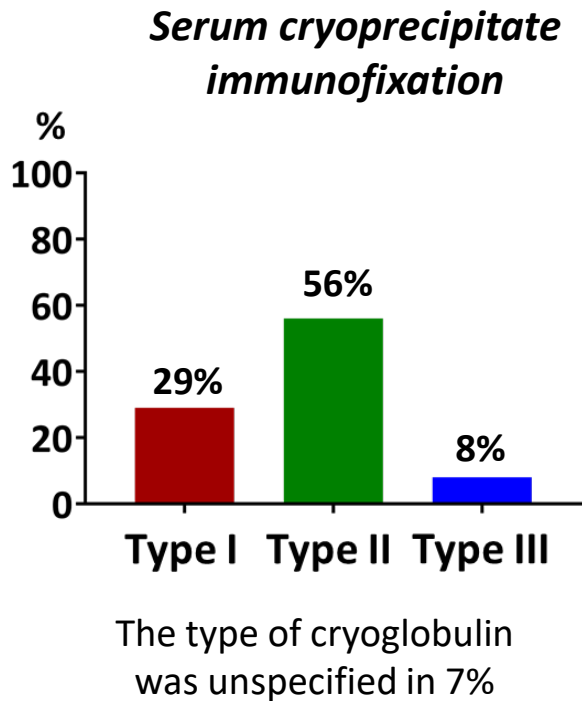
The characteristics of seronegative and seropositive non-hepatitis-associated cryoglobulinemic glomerulonephritis

Vincent Javaugue^{1,2}, Anthony M. Valeri³, Insara Jaffer Sathick⁴, Samar M. Said⁵, Sibel Erdogan Damgard⁶, David L. Murray⁵, Tyler Klobucher¹, Nicole K. Andeen⁷, Sanjeev Sethi⁵, Fernando C. Fervenza¹, Nelson Leung^{1,8} and Samih H. Nasr⁵

¹Nephrology and Hypertension, Department of Medicine, Mayo Clinic, Rochester, Minnesota, USA; ²Department of Nephrology, Centre Hospitalier Universitaire, Université de Poitiers, Poitiers, France; ³Division of Nephrology, Columbia University, College of Physicians and Surgeons, New York, New York, USA; ⁴Department of Medicine, Renal Service, Memorial Sloan Kettering Cancer Center, New York, New York, USA; ⁵Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA; ⁶Microscopy and Cell Analysis Core, Mayo Clinic, Rochester, Minnesota, USA; ⁷Department of Pathology, Oregon Health & Science University, Portland, Oregon, USA; and ⁸Hematology, Department of Medicine, Mayo Clinic, Rochester, Minnesota, USA

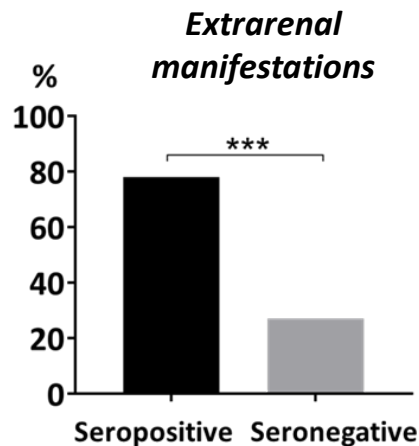


Seropositive Cryoglobulinemic Glomerulonephritis



	Seropositive type I CryoGN (N=17)	Seropositive type II CryoGN (N=33)	P value
Clinical characteristics			
Median age, years	61 (43-82)	65 (29-84)	0.94
Median creatinine, mg/dl	1.5 (0.9-3.8)	1.5 (0.6-5.8)	0.84
Median proteinuria, g/24h	3.7 (0.1-19.4)	2.2 (0.1-10)	0.26
Low C3 level	11/15 (73%)	18/31 (58%)	0.35
Low C4 level	9/13 (69%)	27/32 (84%)	0.41
Low C3 and C4 levels	7/13 (54%)	17/32 (53%)	1.00
Positive rheumatoid factor	3/12 (25%)	27/33 (82%)	<0.001
Positive SPEP/SIF	16/17 (94%)	24/33 (73%)	0.13
Positive UPEP/UIF	14/17 (82%)	10/24 (42%)	0.012
Paraprotein on SIF/UIF or MASS-Fix	17/17 (100%)	26/33 (79%)	0.079
Abnormal serum free light chain ratio	7/12 (58%)	16/21 (76%)	0.43
Underlying clone			0.18
Lymphocytic clone	11/16 (69%)	15/29 (52%)	
Plasmacytic clone	3/16 (19%)	3/29 (10%)	
Unknown	2/16 (12%)	11/29 (38%)	
Extrarenal manifestations	13/17 (76%)	28/33 (85%)	0.47

Seronegative Cryoglobulinemic Glomerulonephritis



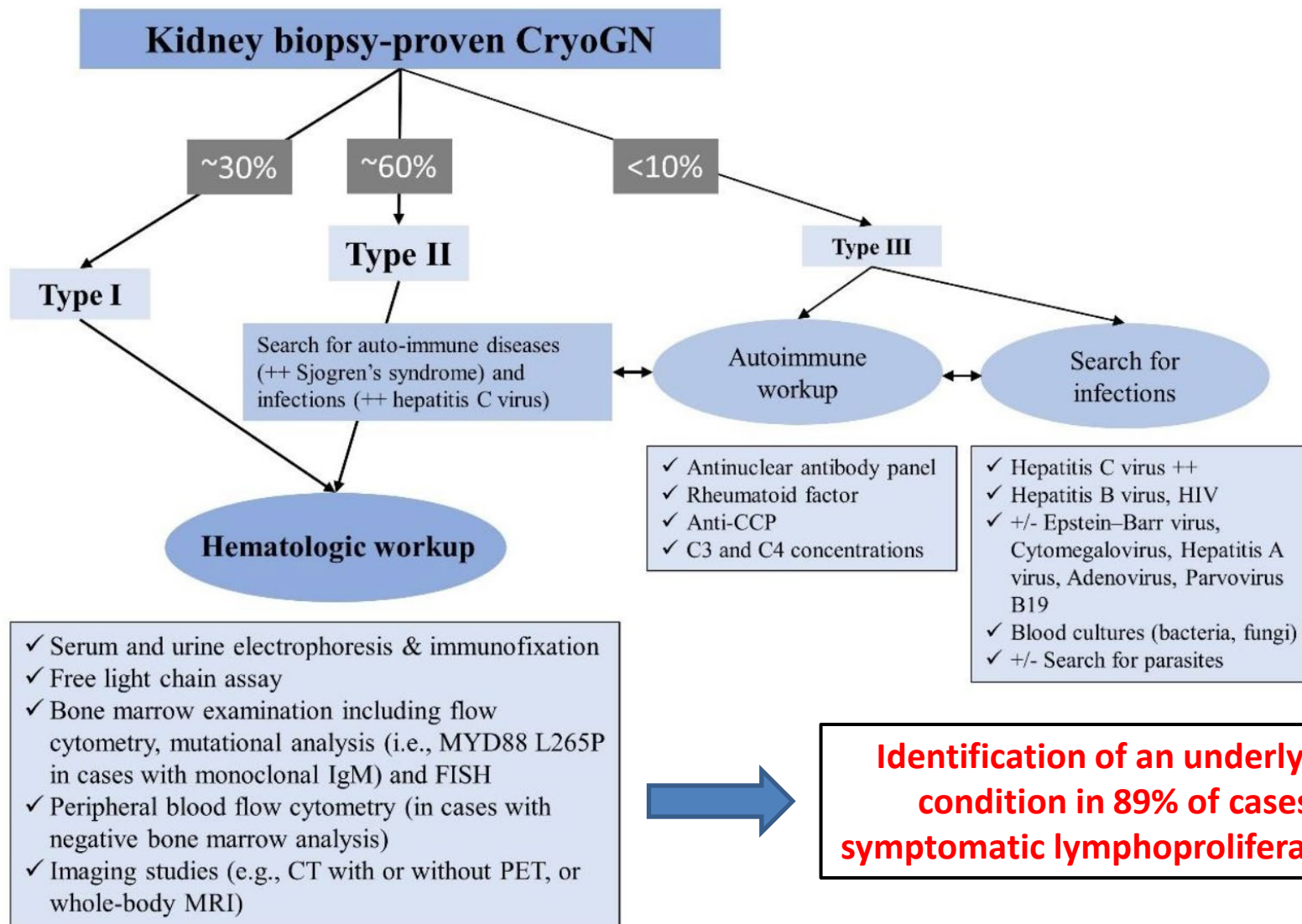
Extrarenal manifestations
(mostly of skin) less common in seronegative vs. seropositive CryoGN (27% vs. 78%)

- Intrinsic physiochemical properties of monoclonal Igs with precipitation only in glomeruli?

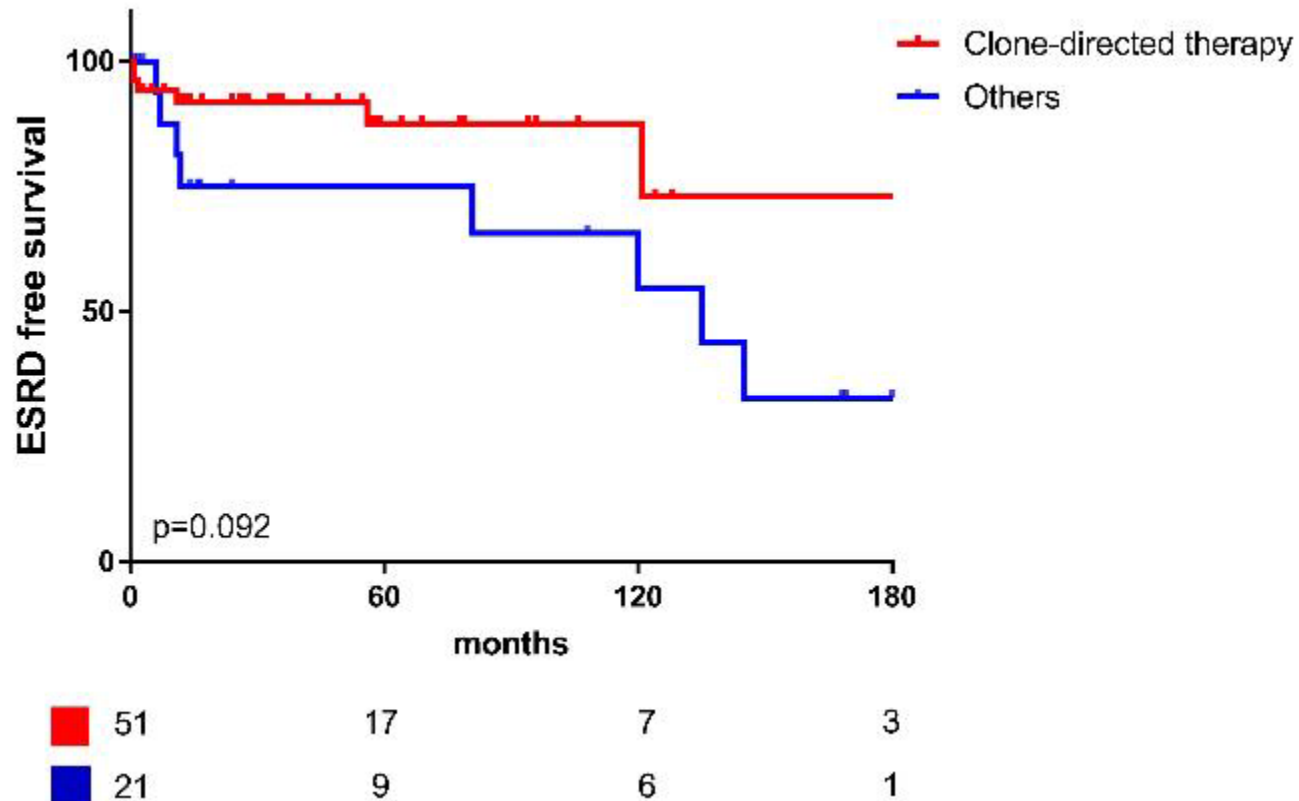
Characteristic	Seropositive CryoGN (n = 59)	Seronegative CryoGN (n = 22)	P value
Positive rheumatoid factor	33/53 (62)	3/18 (17)	<0.001
Positive SPEP/SIF	44/58 (76)	16/22 (73)	0.769
Positive UPEP/UIF	28/47 (60)	12/20 (60)	1.000
Abnormal serum free light chain ratio	27/42 (64)	3/21 (14)	<0.001
Monoclonal protein class			
μ	31/46 (67)	5/16 (31)	0.01
γ	7/46 (15)	6/16 (38)	
α	0	1/16 (6)	
Monoclonal light chain isotype			
κ	33/47 (70) ^a	6/17 (35)	0.02
λ	6/47 (13)	7/17 (41) ^b	
Biclonal gammopathy	8/46 (18)	4/16 (25)	0.726
Underlying clone			
Lymphocytic	32/54 (59)	6/18 (33)	0.149
Plasmacytic	7/54 (13)	4/18 (22)	
Unknown	15/54 (28) ^c	8/18 (45)	

- Most seronegative cases had monotypic (i.e., type I cryoglobulin) deposits
- Despite the absence of detectable cryoglobulin, blood and/or urine immunofixation allowed identification of a monoclonal Ig in ~80% of cases

Kidney-biopsy proven cryoglobulinemic GN workup



Renal prognosis in type I/II CryoGN

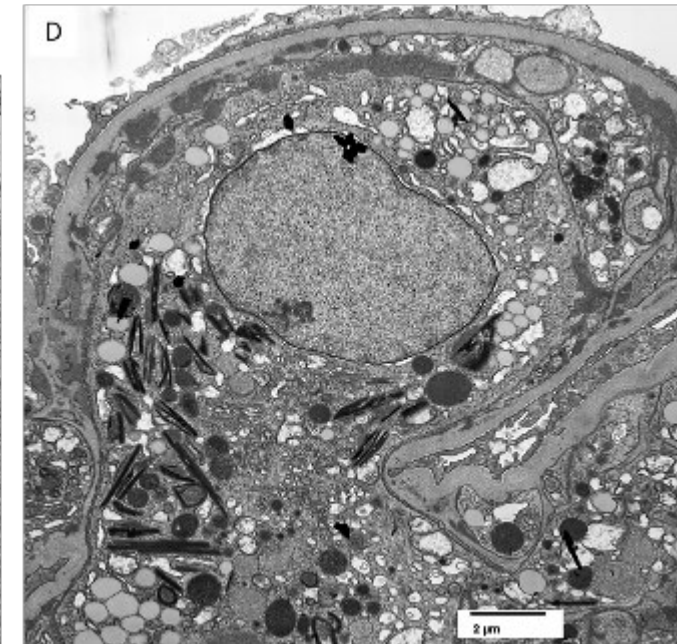
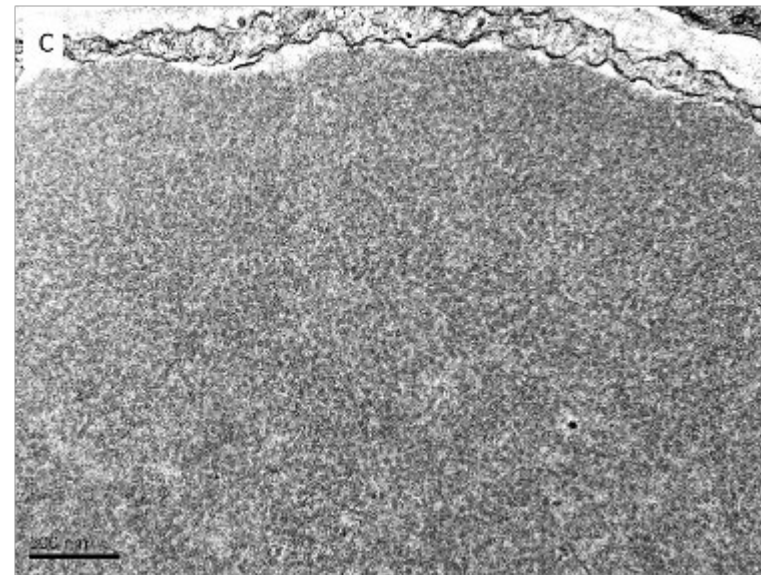
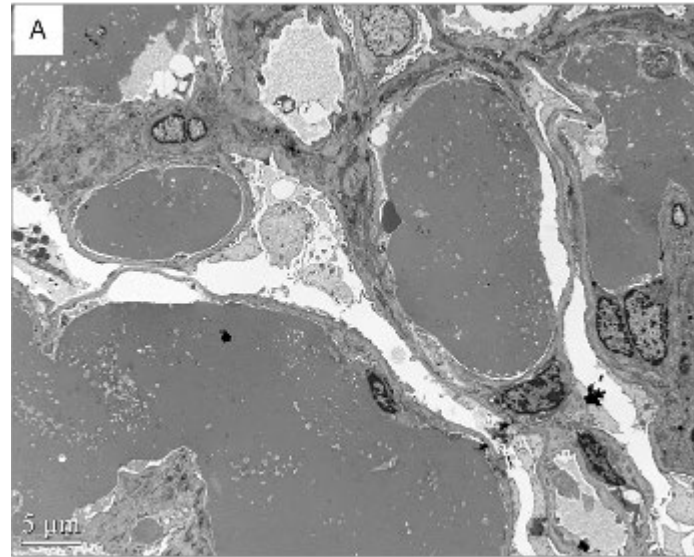


➤ After clone-directed therapy, 76% of patients had partial or complete remission

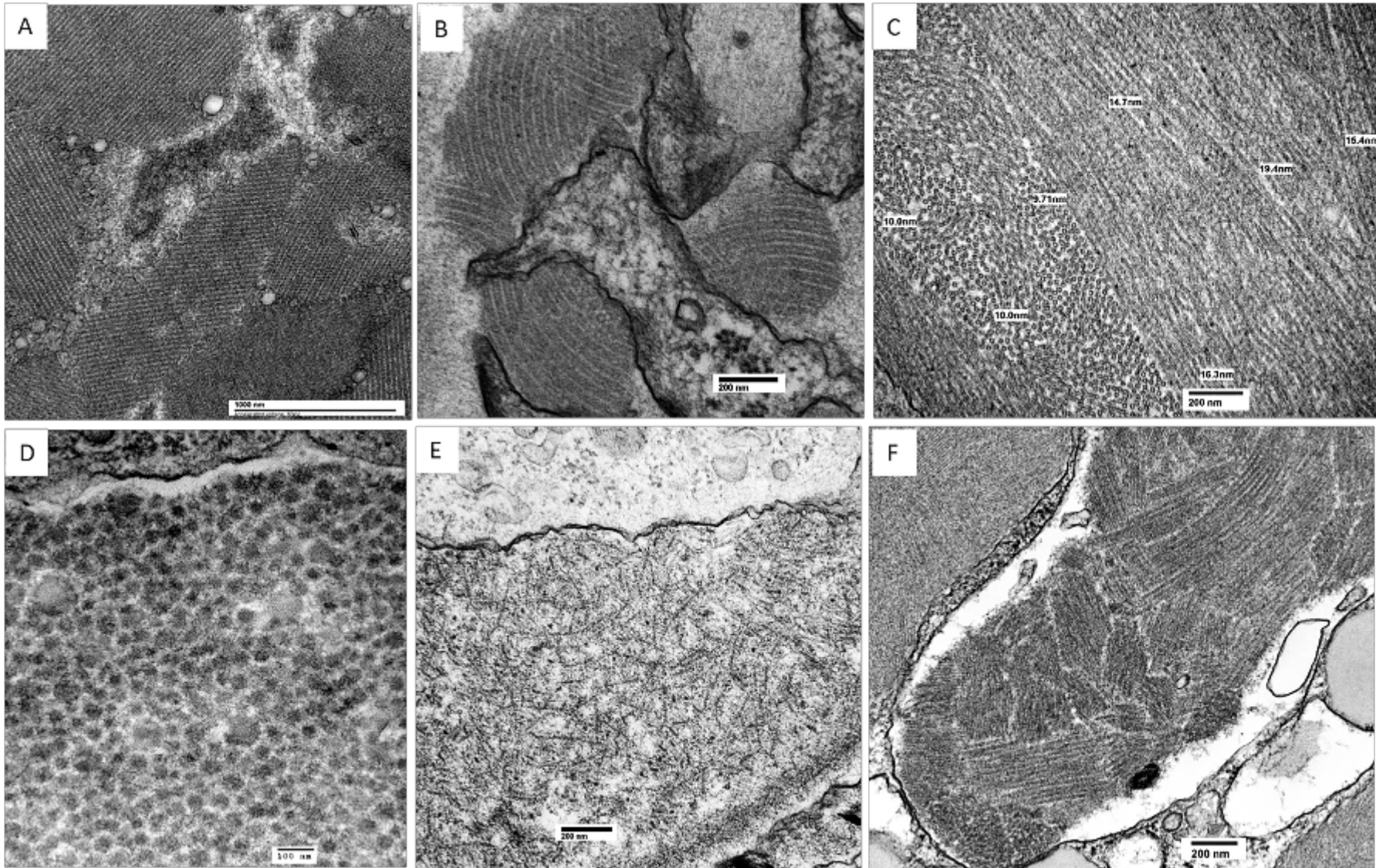
Javaugue V, et al. *Kidney Int.* 2022

Microscopie électronique au cours des GN cryoglobulinémiques

- A. Obstruction des capillaires glomérulaires par « cryo-plugs ».
- B. Afflux de macrophages avec de nombreux phagolysosomes denses.
- C. Dépôts glomérulaires organisés de cryo type II Aspect en “cocardes” et cylindres curvilignes avec limites floues.
- D. Cristaux dans le cytoplasme des macrophages au cours d’une crystalcryo IgGk

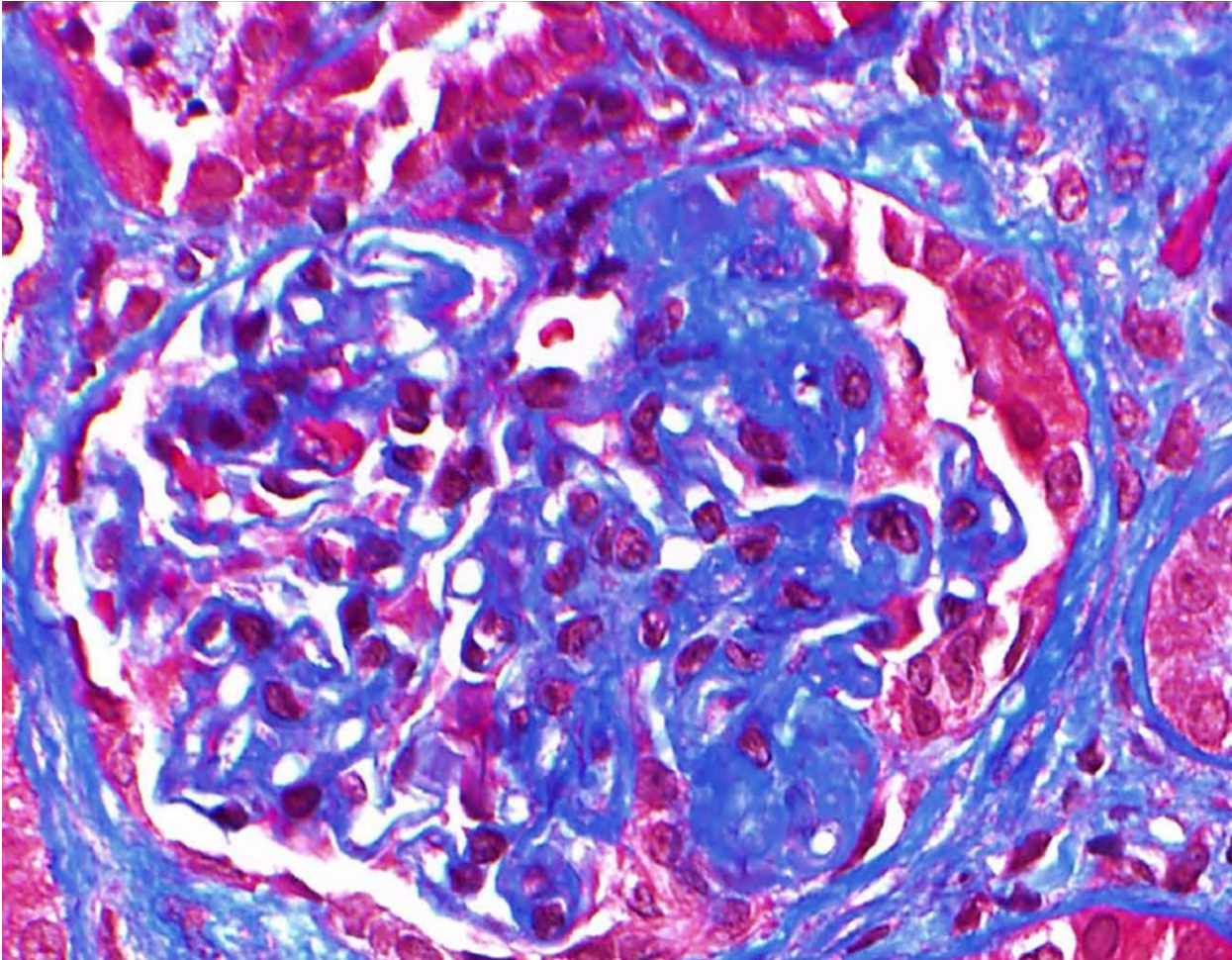


Microscopie électronique au cours des GN cryoglobulinémiques: autres substructures



Cas clinique 3

- Mr R., 58 ans, origine caucasienne, sans antécédents particuliers
- Poids = 68 kg, T = 1m75, IMC = 22 Kg/m²
- Découverte d'une protéinurie glomérulaire à 2,8 gr/24h (bilan d'HTA + OMI), albuminémie normale, créatinine = 90 µmol/l
- Pic IgG kappa (9 gr/l)



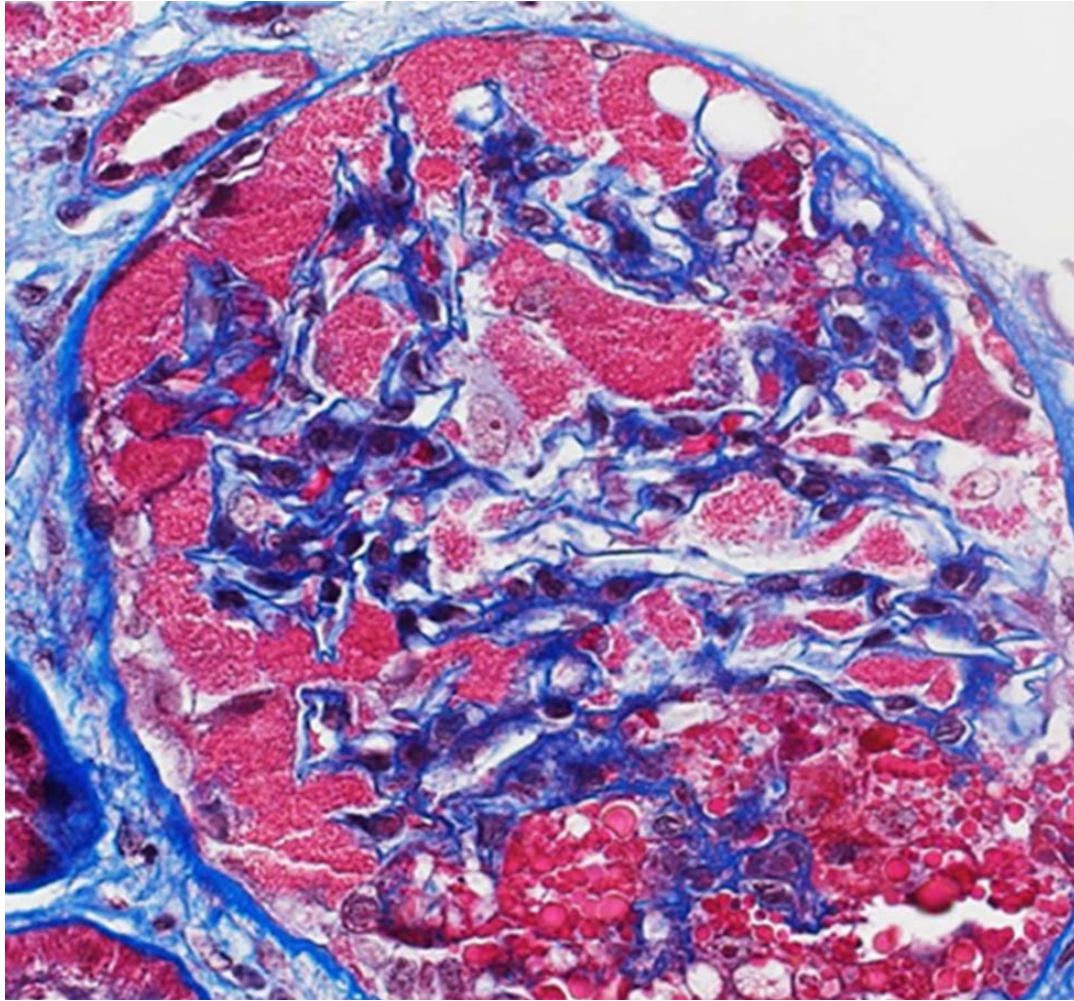
- HSF forme classique (20% des glomérules)
- IF négative

CAT: traitement symptomatique et surveillance

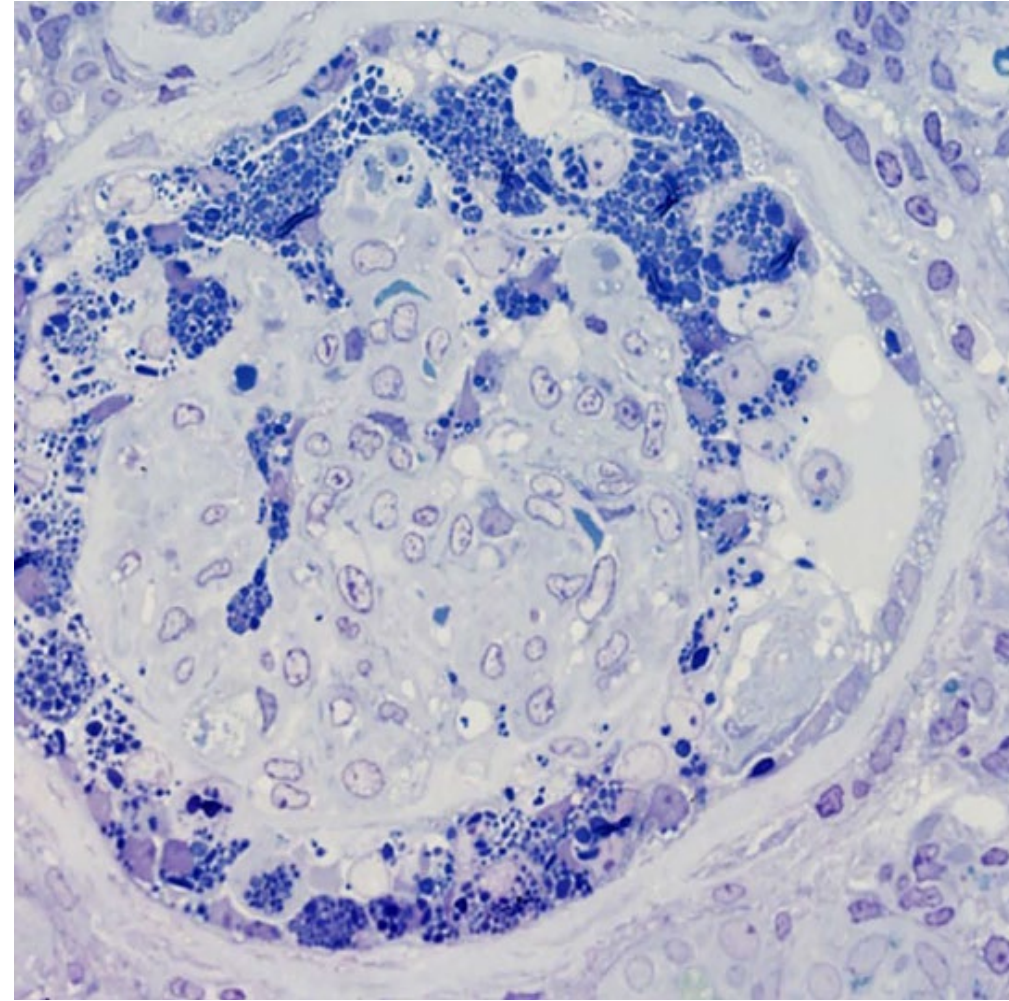
Six ans plus tard:

- créatinine 160 $\mu\text{mol/l}$, protéinurie >3 gr/24h, albuminémie 31 gr/l, pic IgG kappa stable (10 gr/l), excès de CL kappa à 120 mg/l

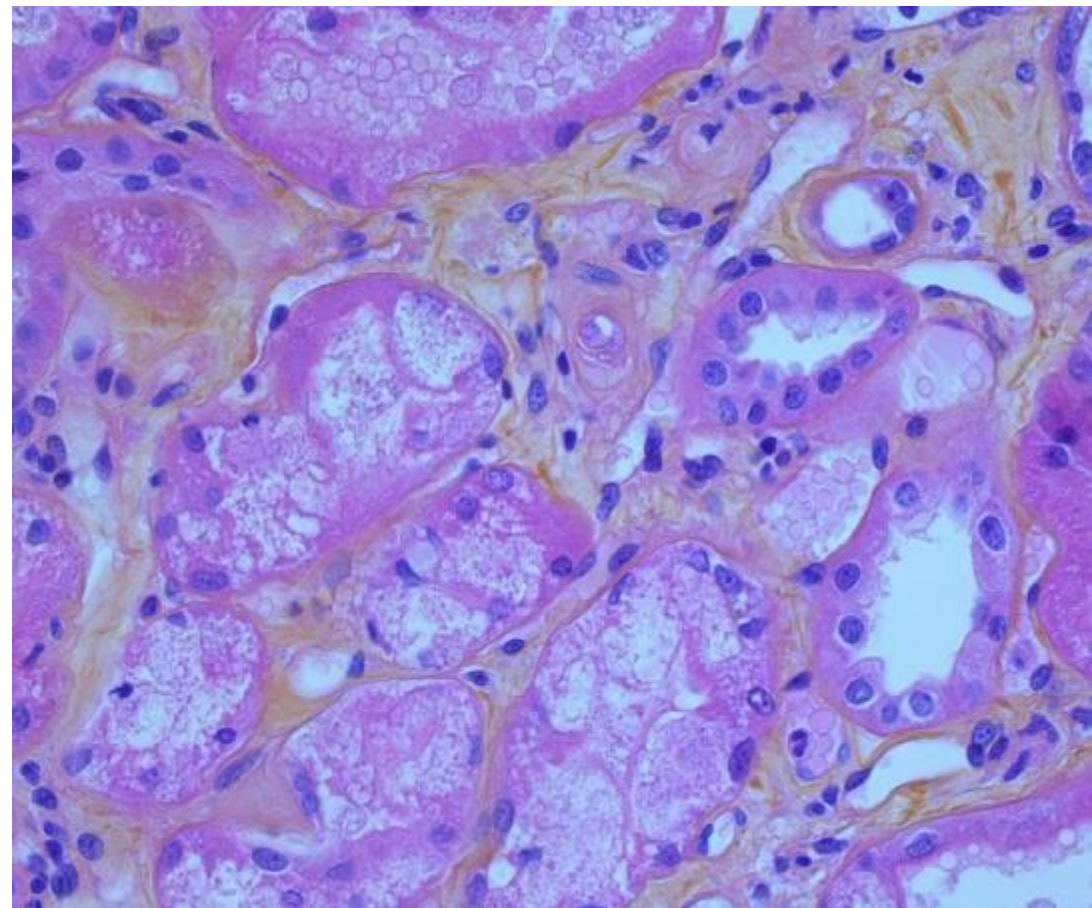
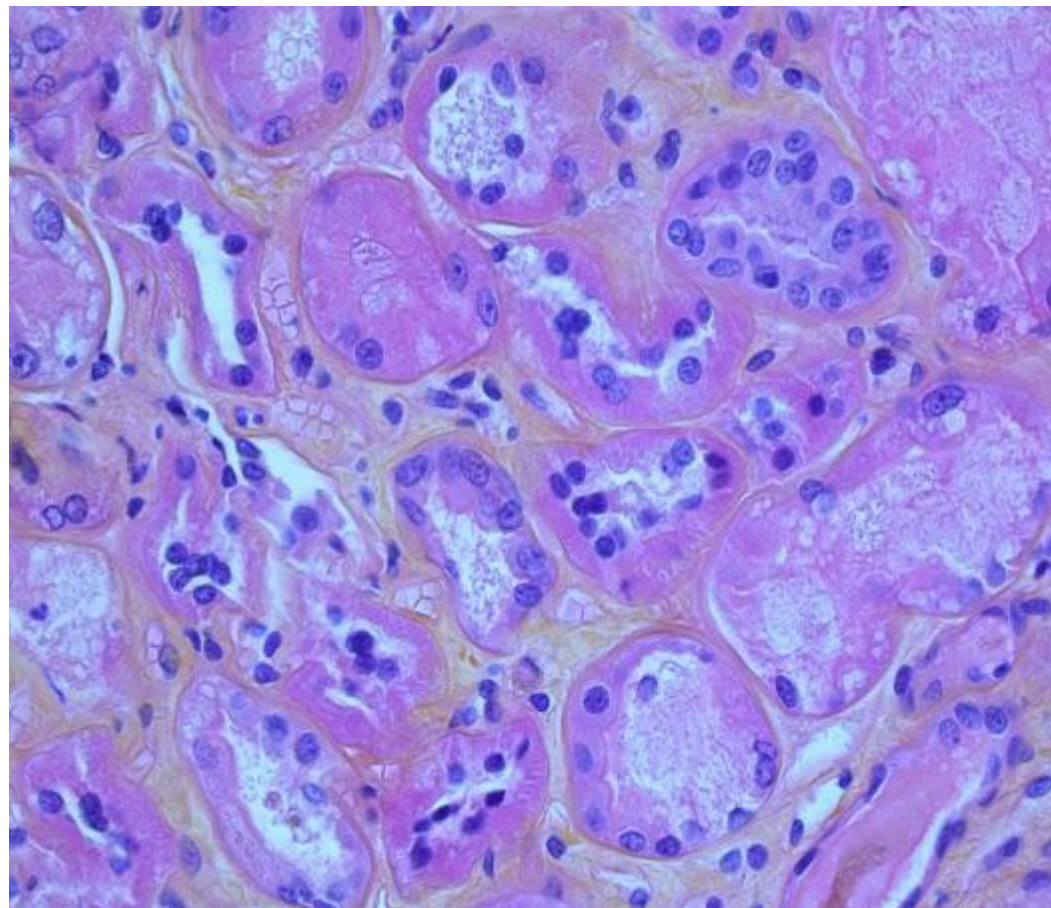
=> *nouvelle PBR*



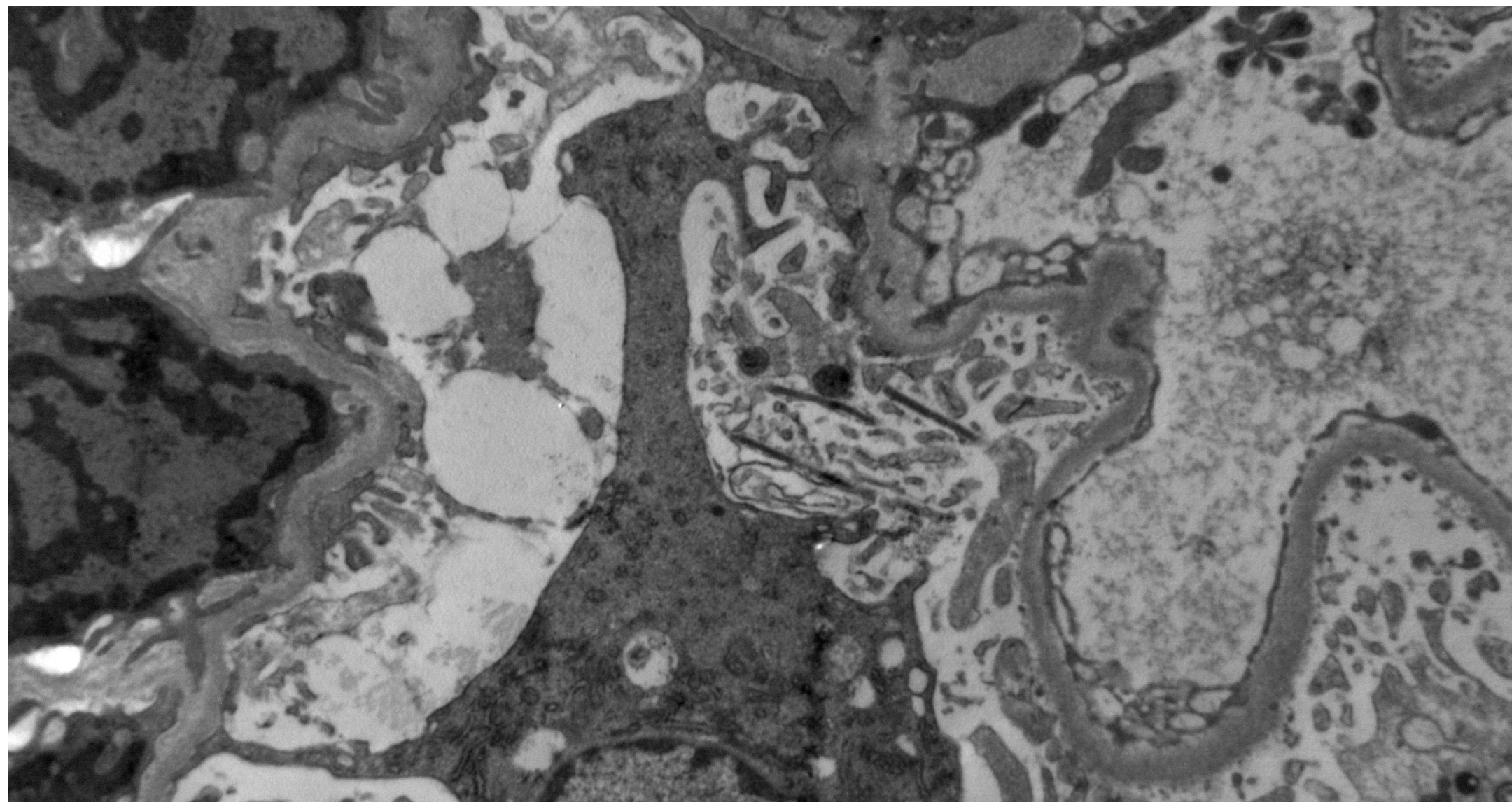
Trichrome

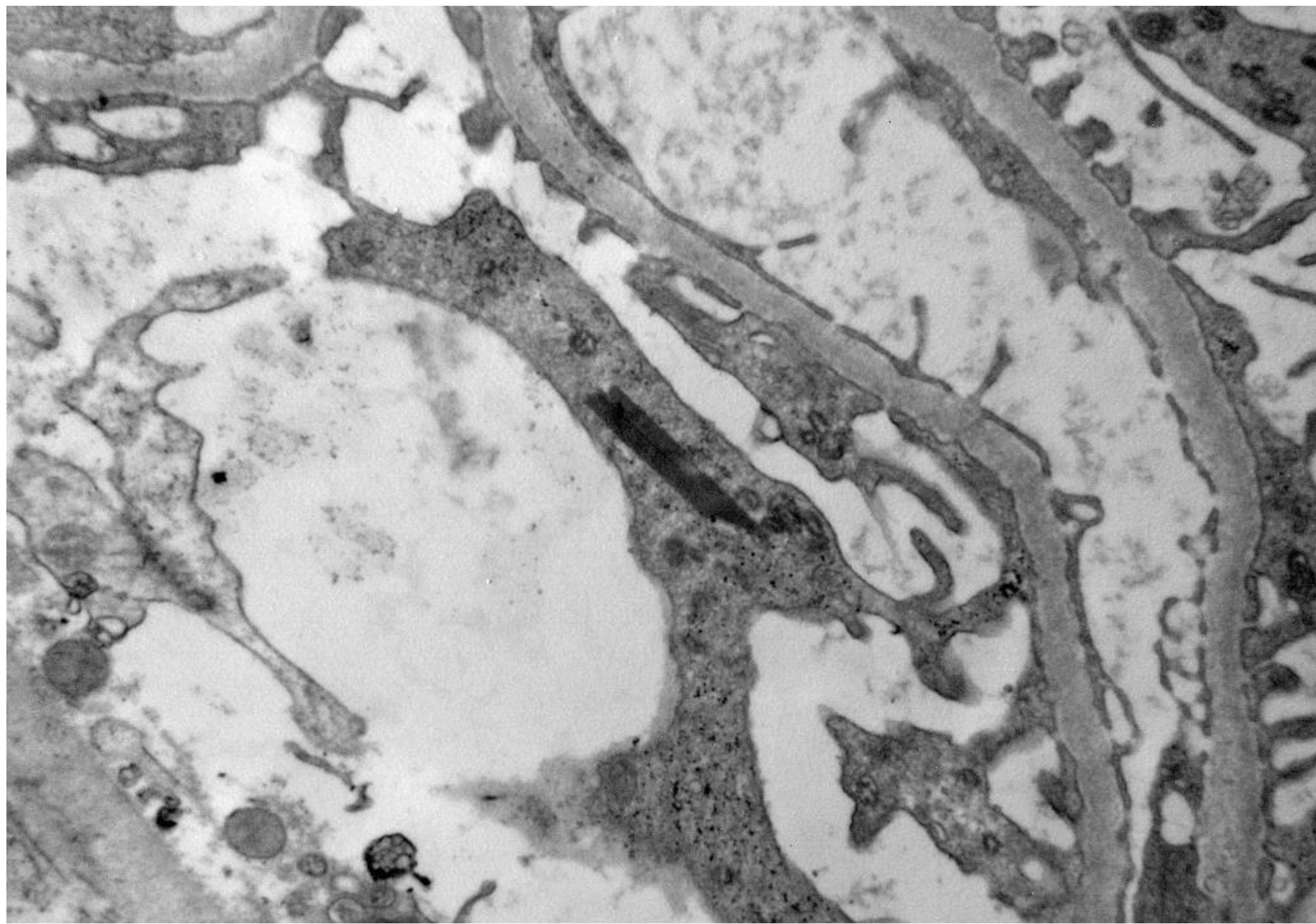


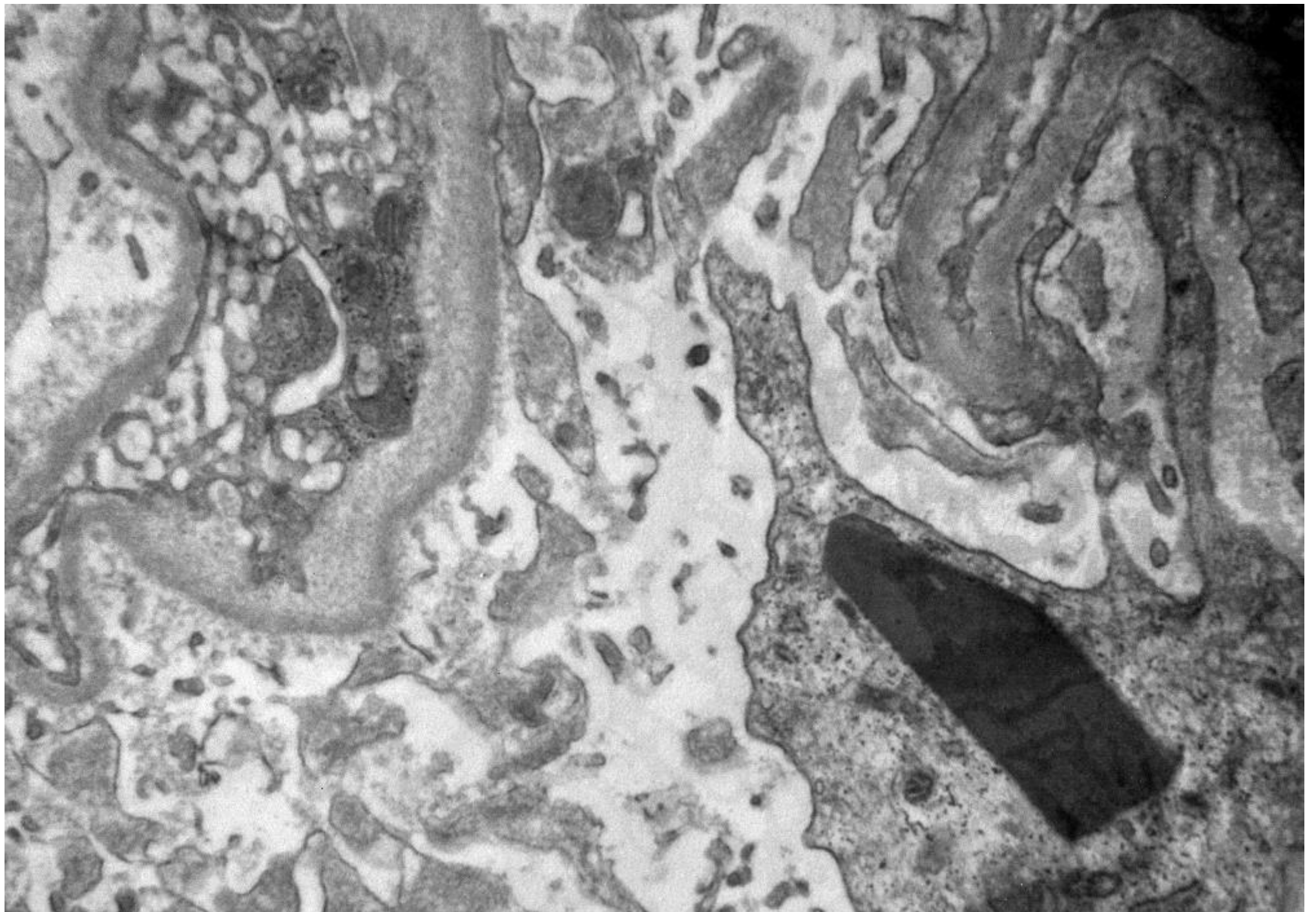
Bleu de Toluidine

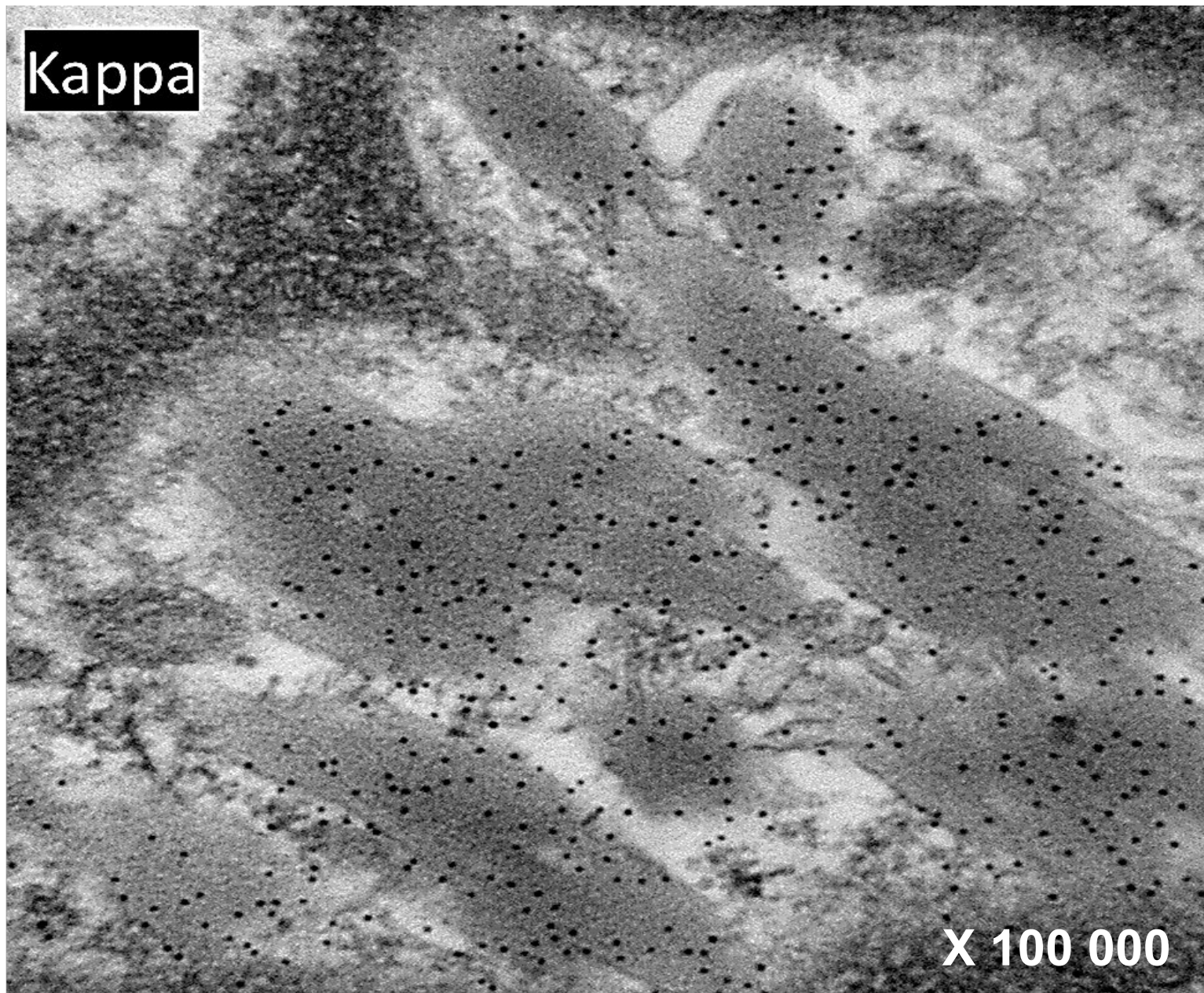


HES







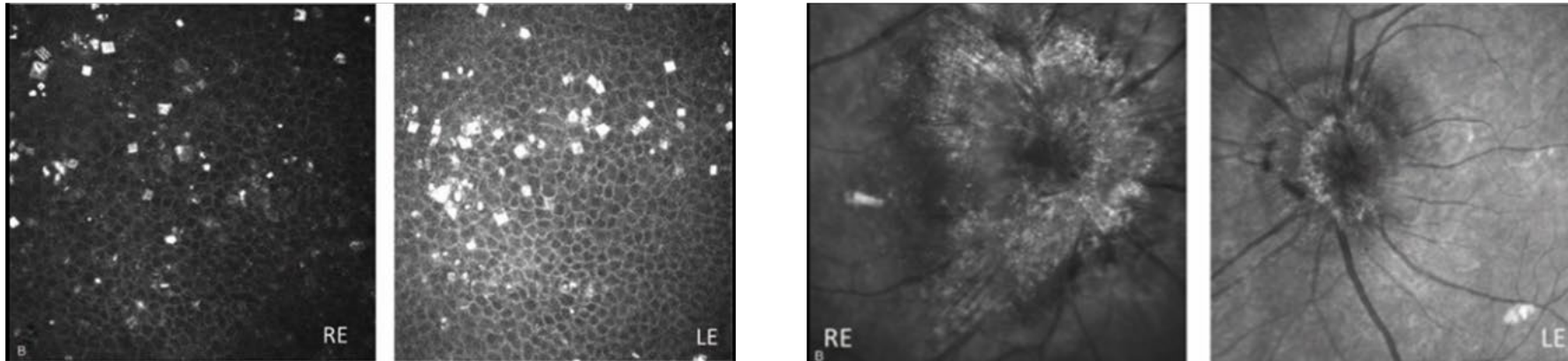


=> Podocytopathie microcristalline kappa

Podocytopathies microcristallines

Une entité récemment ajoutée au spectre des complications rénales associées aux MGRS

- Série de 25 patients (USA, n=20; France, n=5)
- IRC + protéinurie glomérulaire dans 79% des cas (créatininémie médiane = 167 $\mu\text{mol/l}$, DFG médian = 35 ml/min/1.73 m²)
- IRA dans 13% des cas (HSF collapsante dans 2 cas et infiltration plasmocytaire dans 1 cas)
- Syndrome de Fanconi dans 20% des cas
- Atteinte extra-rénale dans 7 cas (28%): **ophtalmologique** ++

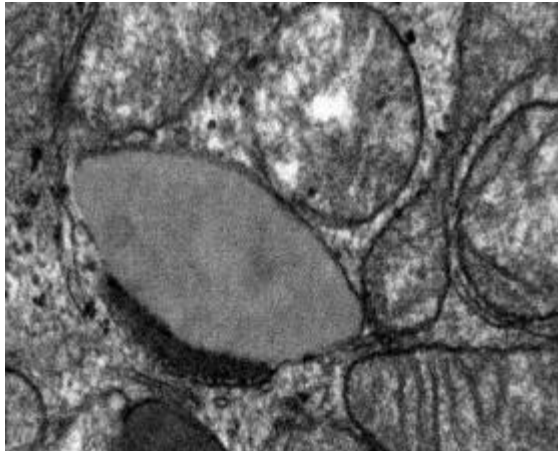


« Piège de cristal »

- ✓ Les inclusions cristallines peuvent être difficiles à voir en MO
- ✓ Etude en immunofluorescence sur coupe congelée parfois négative avec les anticorps anti-CL

ME + immunoGold

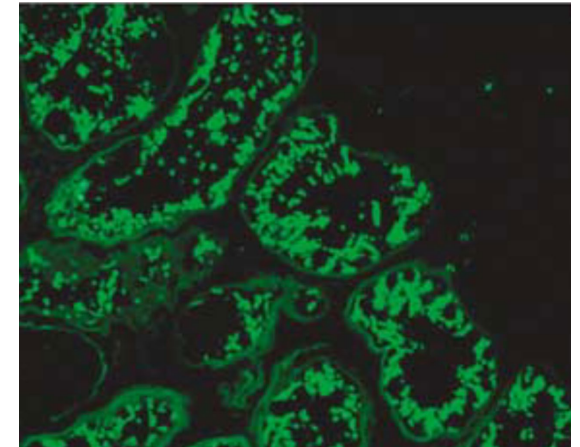
IF sur coupe paraffine



Fixation Gluta
(pour bonne
conservation des
structures cellulaires)



Fixation Formol
(ne fonctionne pas
si fixation dans
Bouin)



Nasr SH et al. Kidney Int. 2006

Cas clinique 4

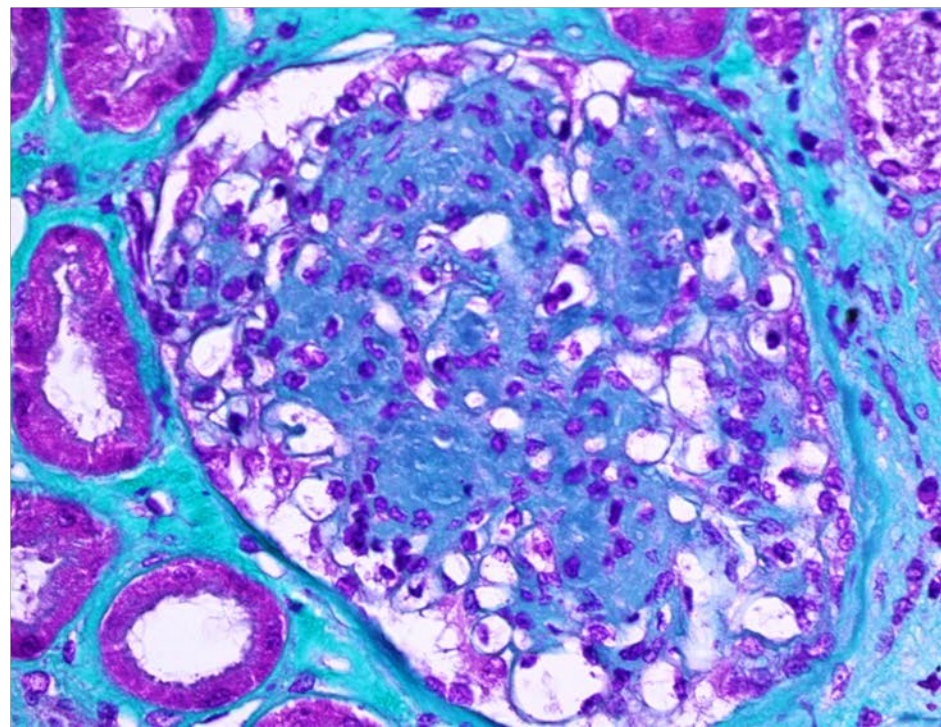
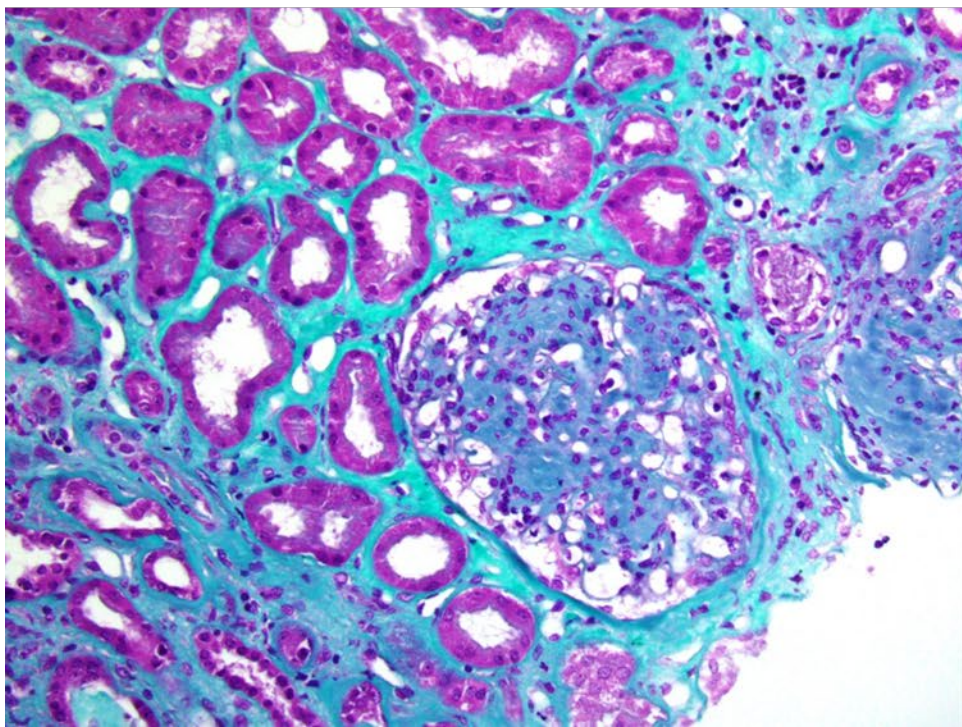
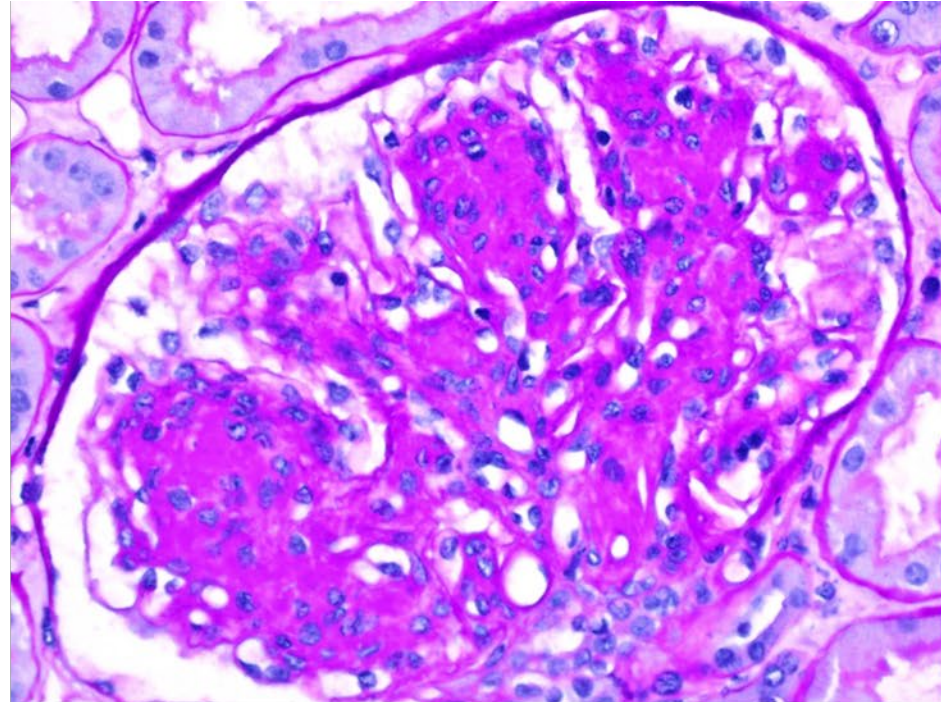
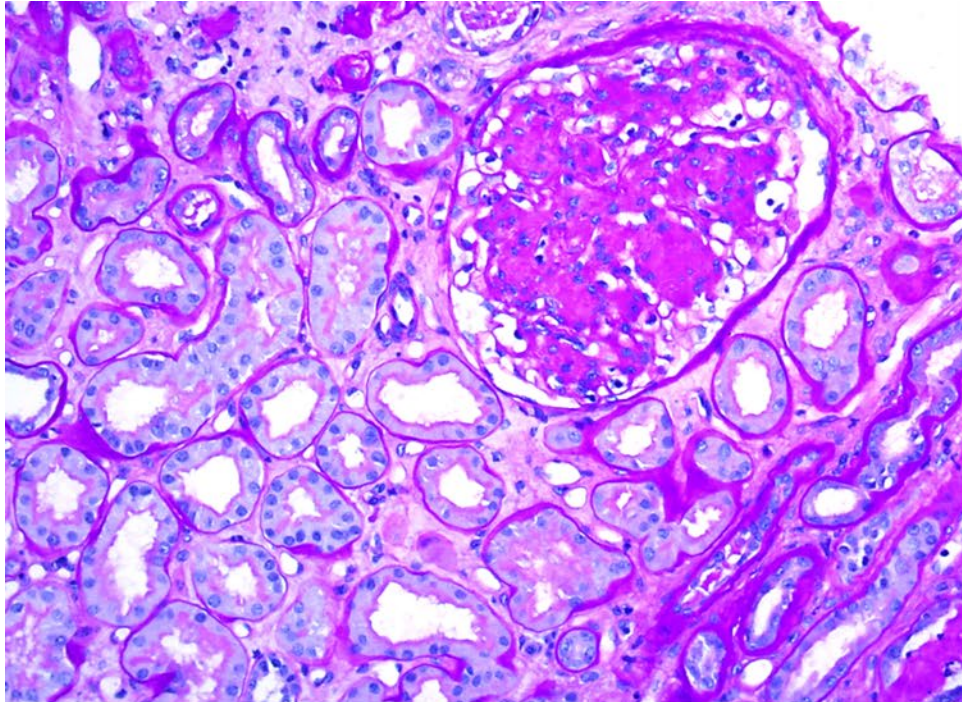
- ✓ A 58-year-old patient was referred for the evaluation of nephrotic syndrome and macroscopic hematuria. He has a past history of tobacco use (30 packs/year), atrial fibrillation, and severe hypertension.
- ✓ Medication: atenolol and aspirin.
- ✓ Clinical examination: Blood pressure was 190/90 mmHg. Diffuse lower limb edema and brownish total hematuria without clots, bilateral pleural effusion with crackles and wheezing. Physical examination was otherwise normal.

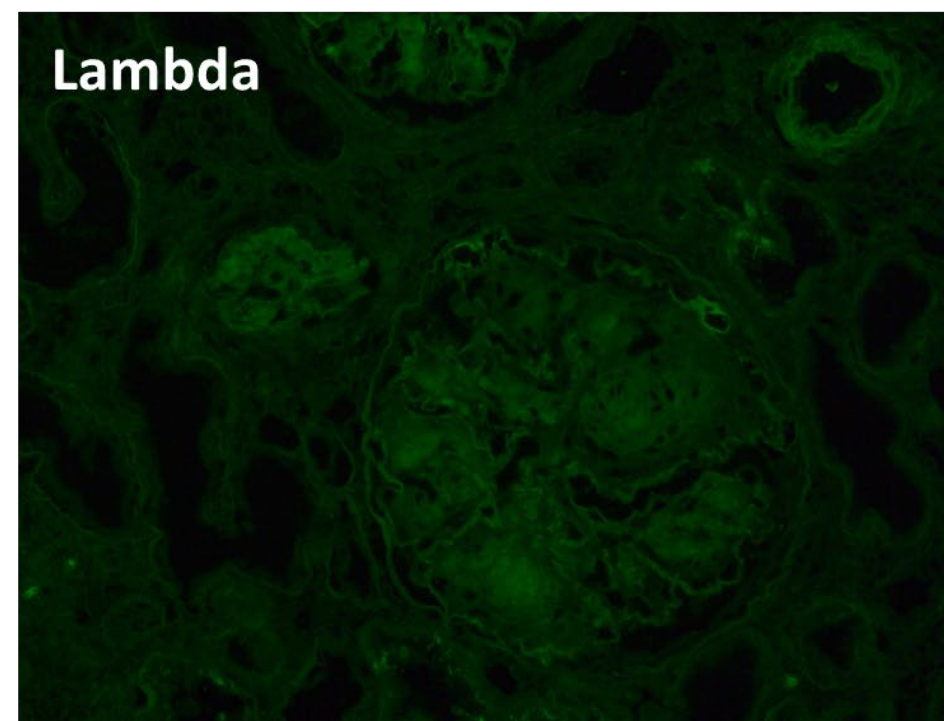
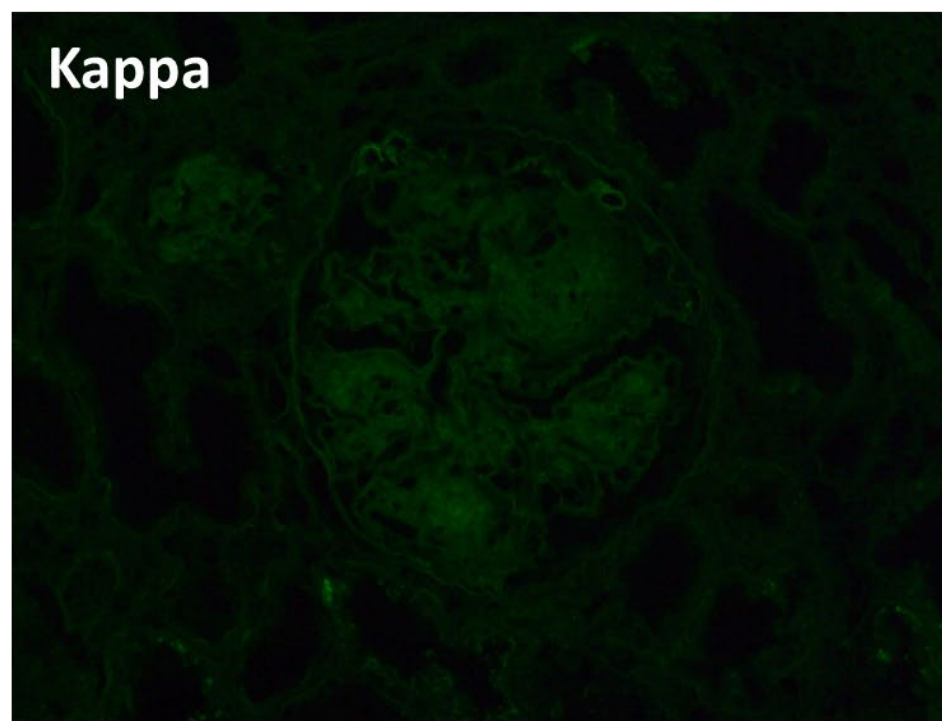
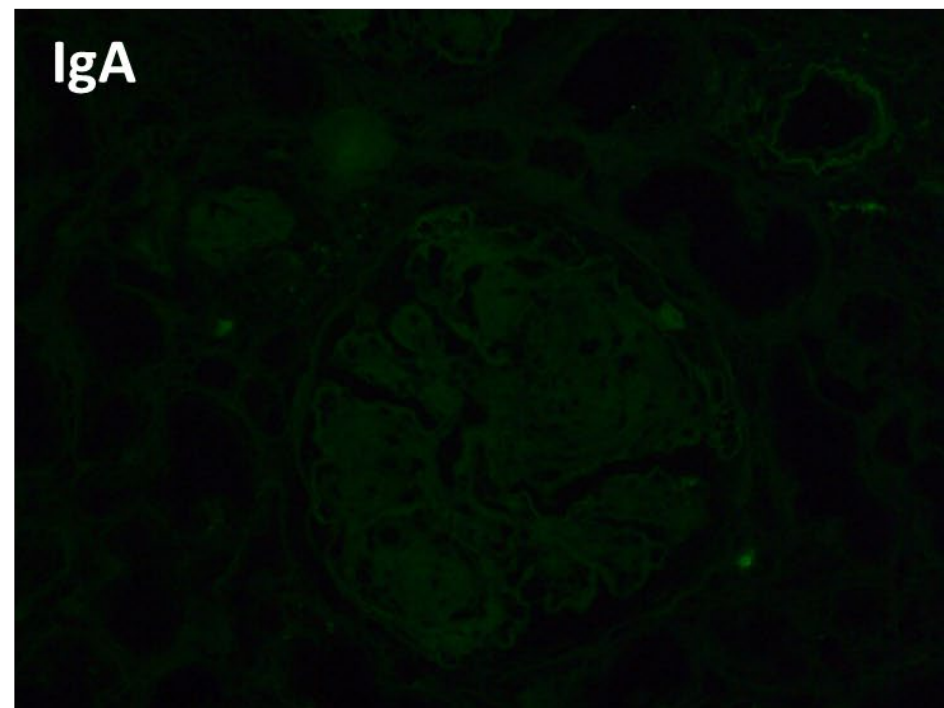
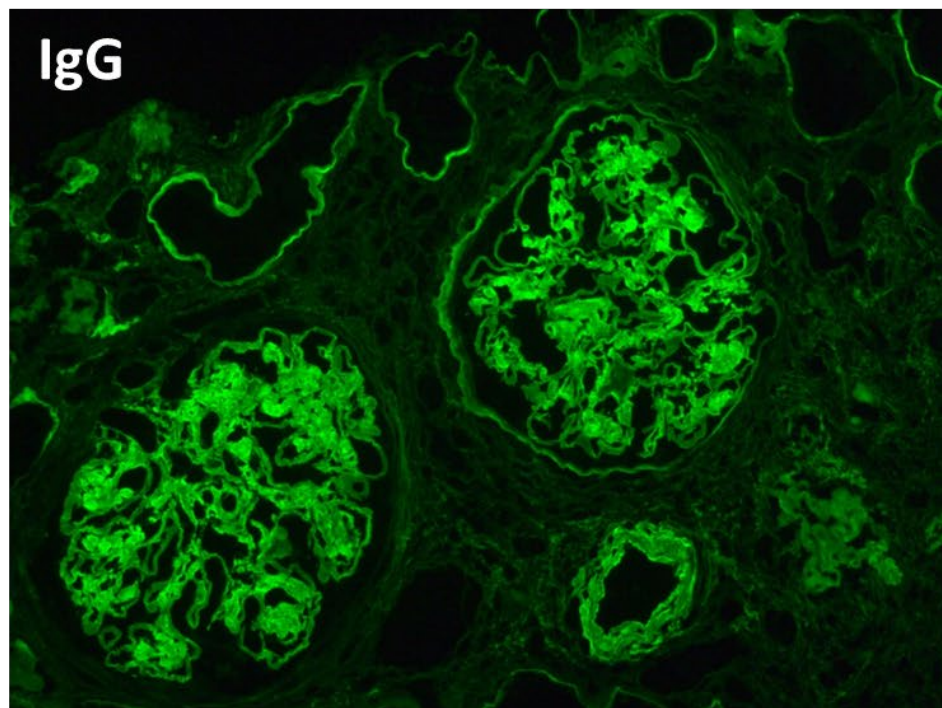
Biological analysis

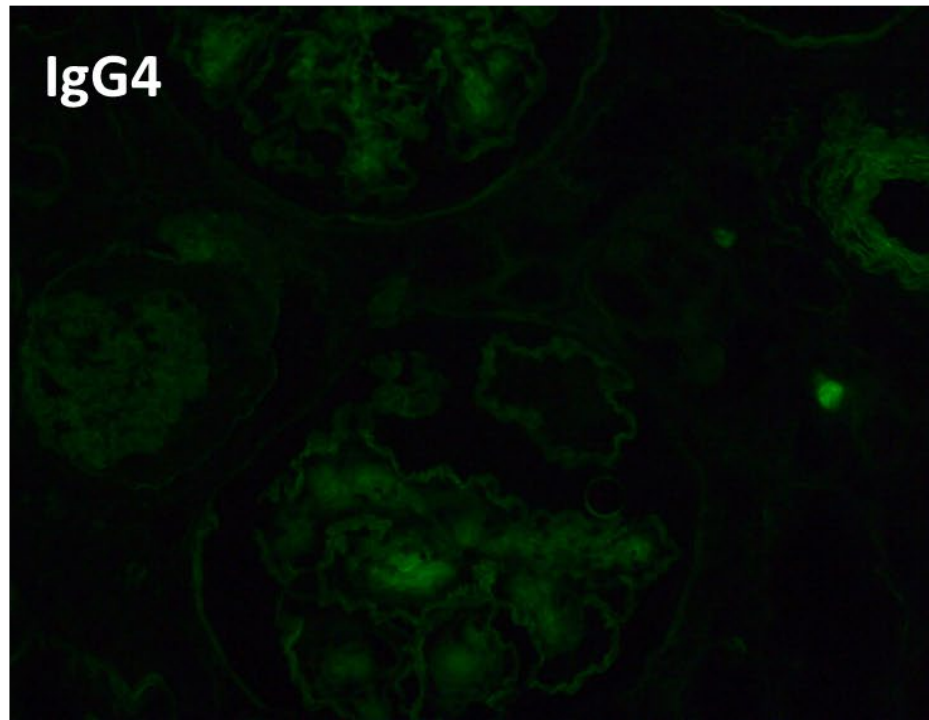
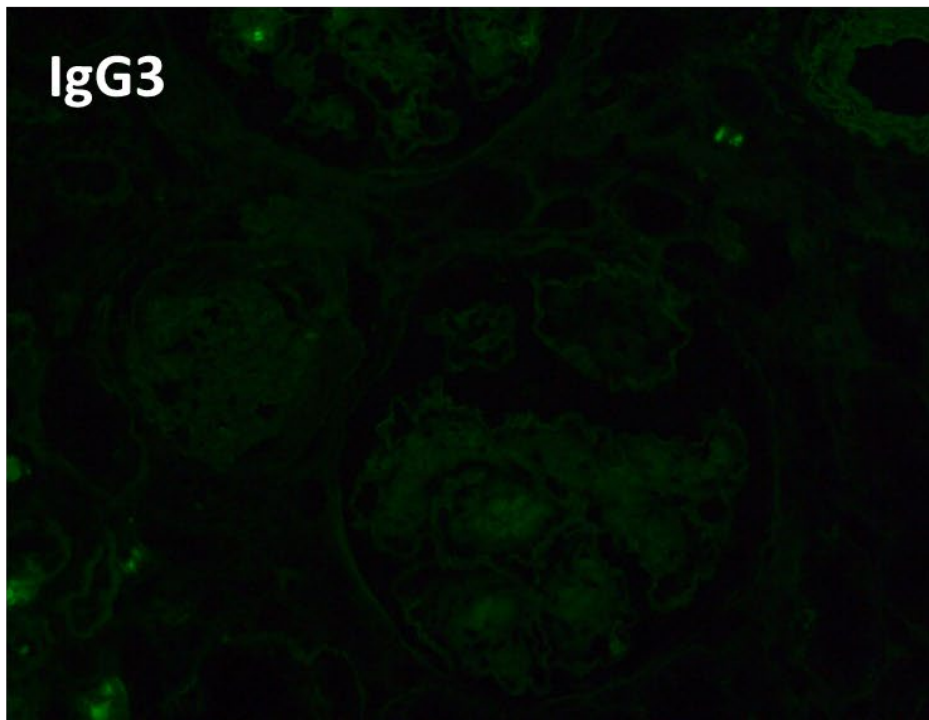
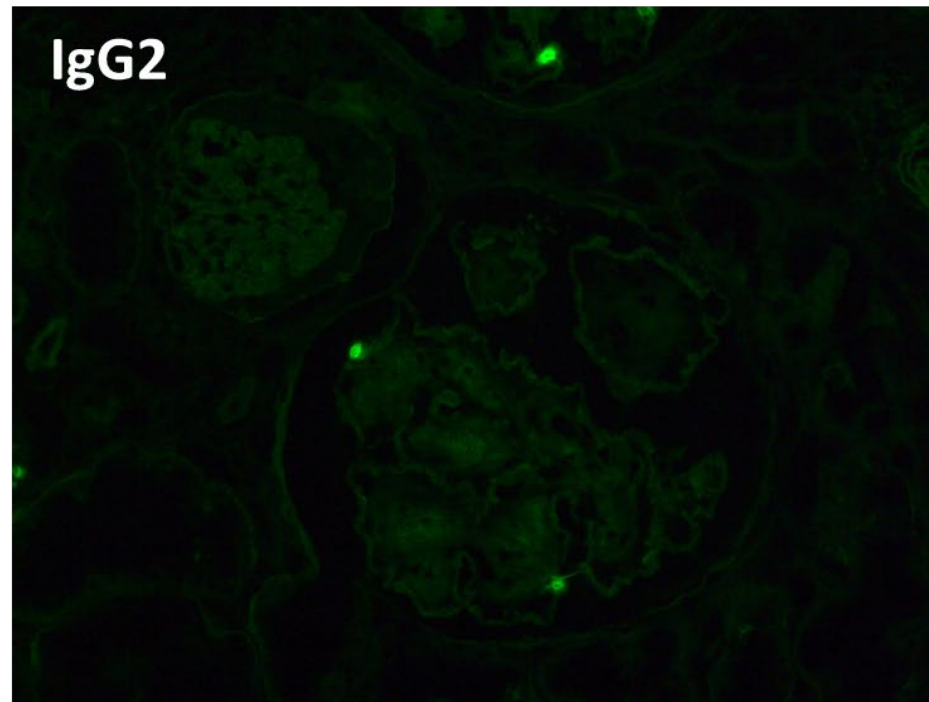
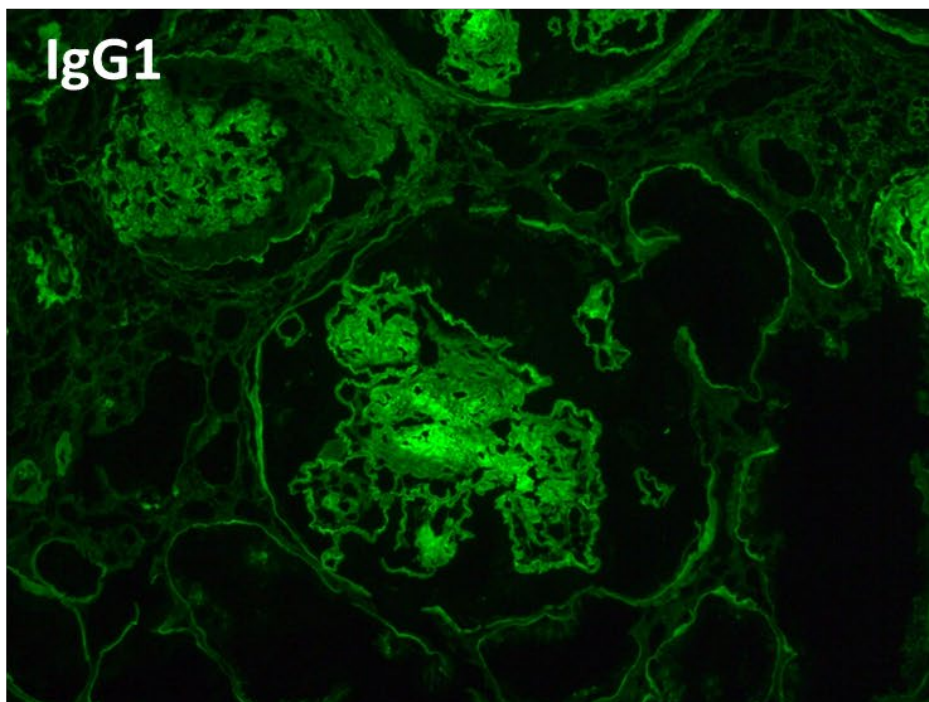
- ✓ Serum creatinine 1.8 mg/dl (eGFR 38 ml/min/1.73 m²), total proteins 58g/L, albumin 24 g/L, proteinuria 4.5g/24h predominantly composed of albumin (73%) and gross glomerular hematuria.
- ✓ Serum electrophoresis and immunofixation revealed a small serum IgG lambda (0.2g/dL). Serum free lambda light chains were 113 mg/l with kappa LC of 16 mg/L (kappa/lambda ratio 0.14, dFLC 97 mg/L). Urine electrophoresis and immunofixation did not detect any monoclonal component.
- ✓ Bone marrow smears revealed a small clonal lambda-positive plasma cell infiltration of 1%. A PET scan and MRI did not show evidence for lytic bone lesions.

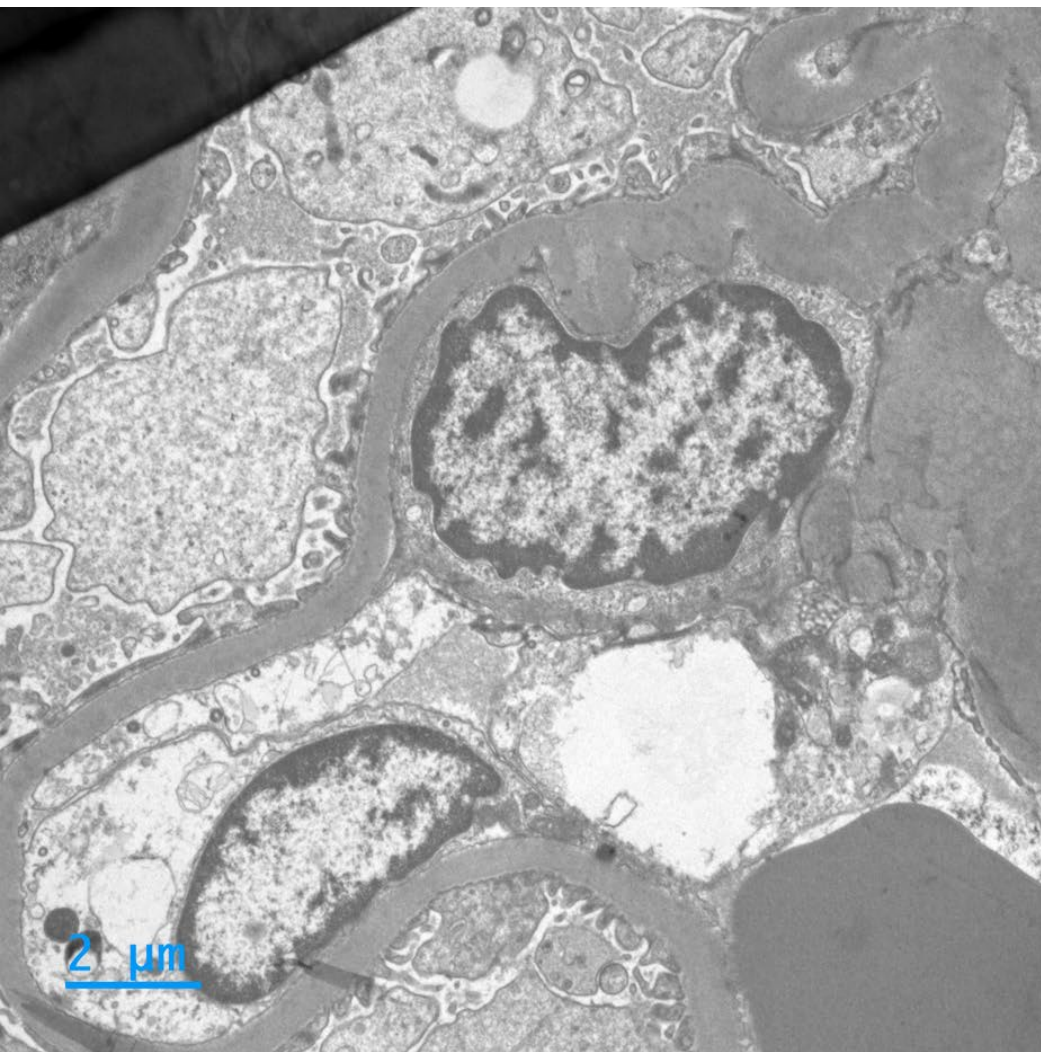
Biological analysis

- ✓ Immunologic tests (antinuclear and anti-DNA antibodies, rheumatoid factor, ANCA) and tests for hepatitis B, C and HV were negative.
- ✓ Hepatic tests and full blood count was normal except for mild anemia (95 g/L).
- ✓ Complement investigations showed low CH50 and C4 serum levels, with moderately decreased C3 level.

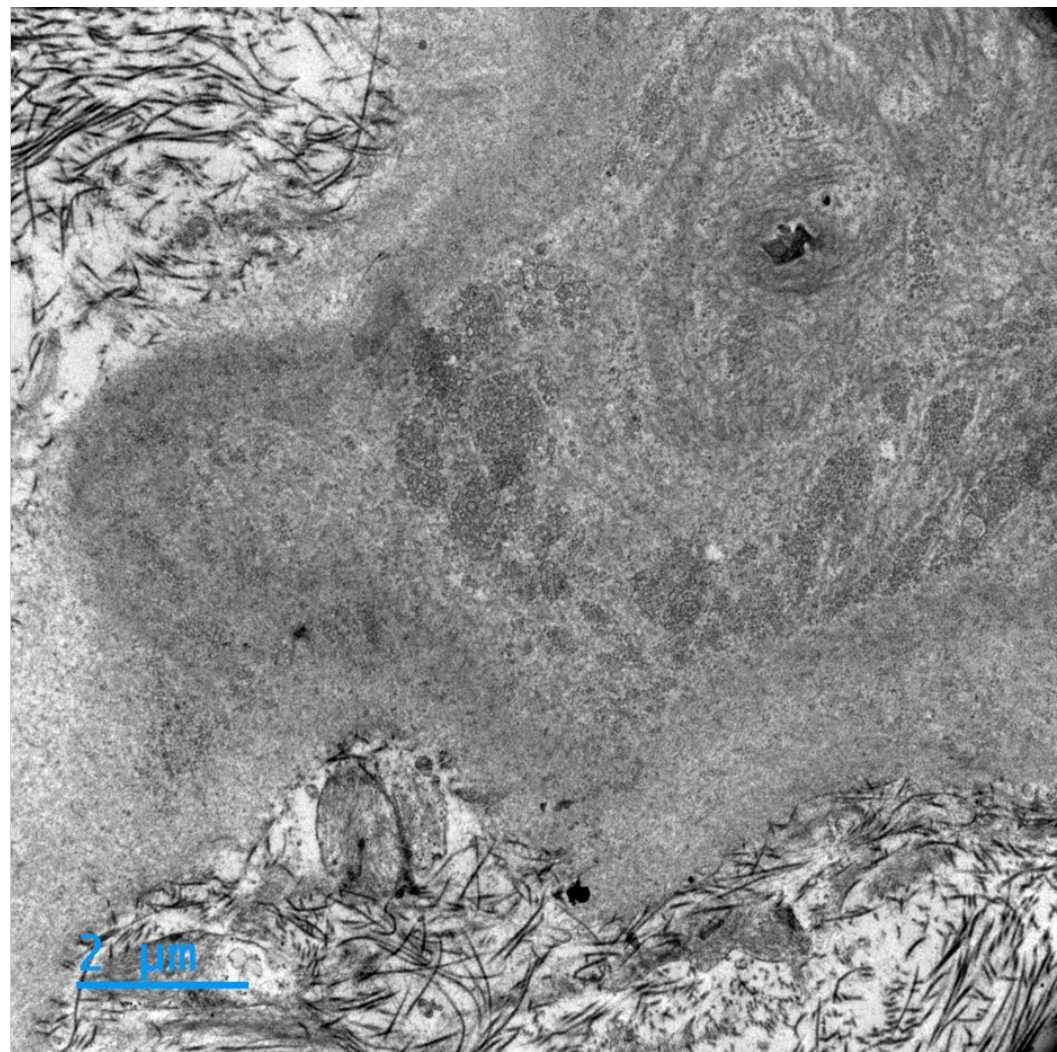




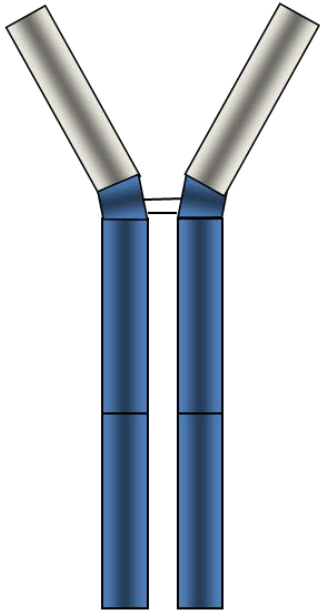




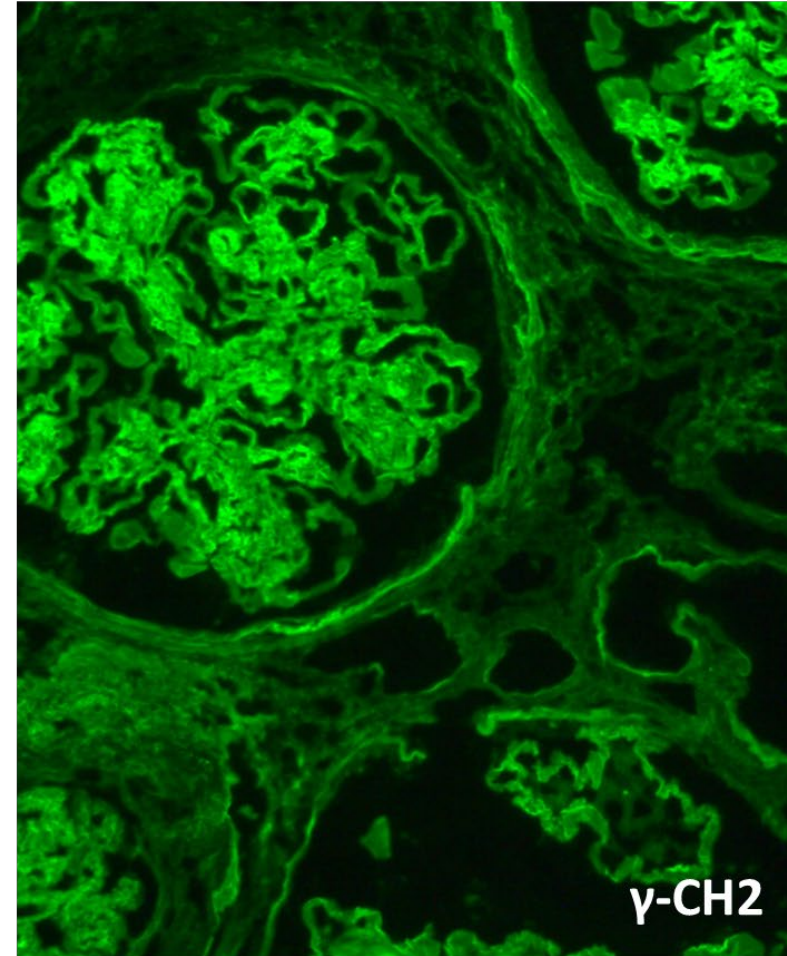
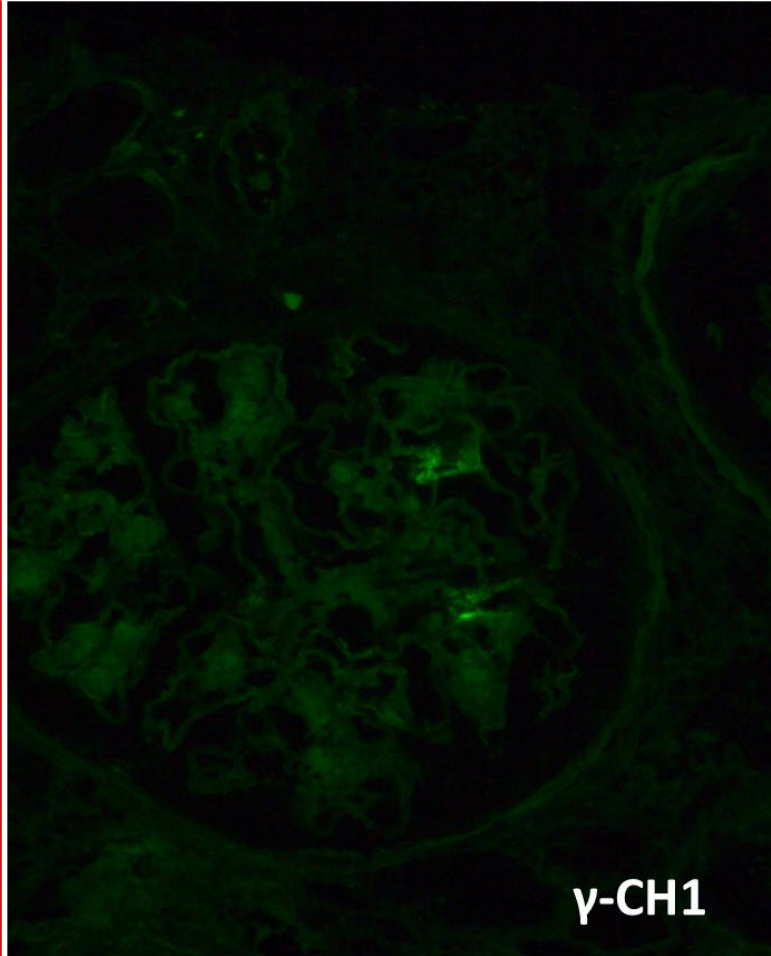
EM x 8000



EM x 10000



Truncated heavy chain
(CH1+/-CH2 deletion)



HCDD $\gamma 1$ with CH1 deletion

Unravelling the immunopathological mechanisms of heavy chain deposition disease with implications for clinical management

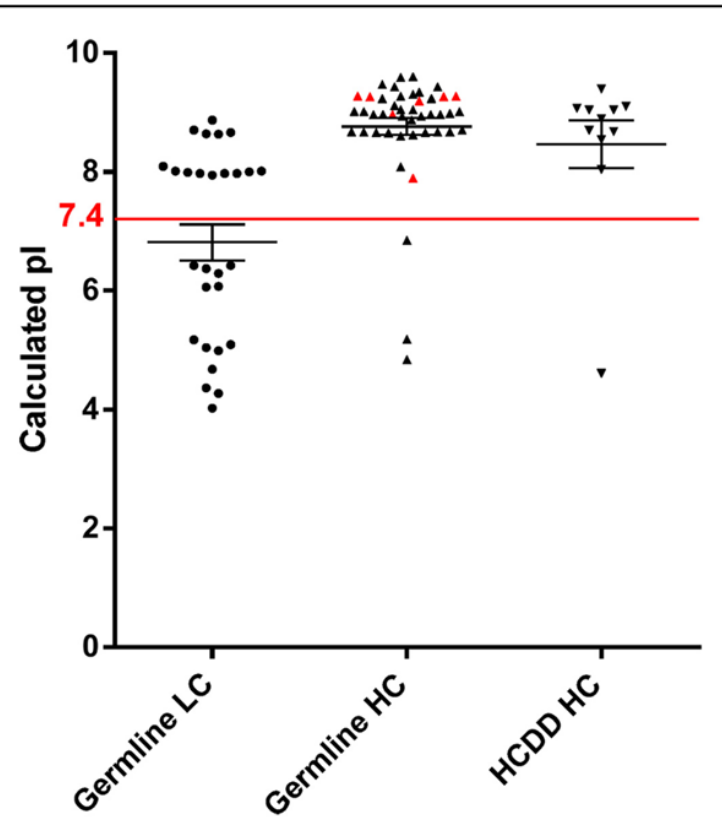


see commentary on page 272

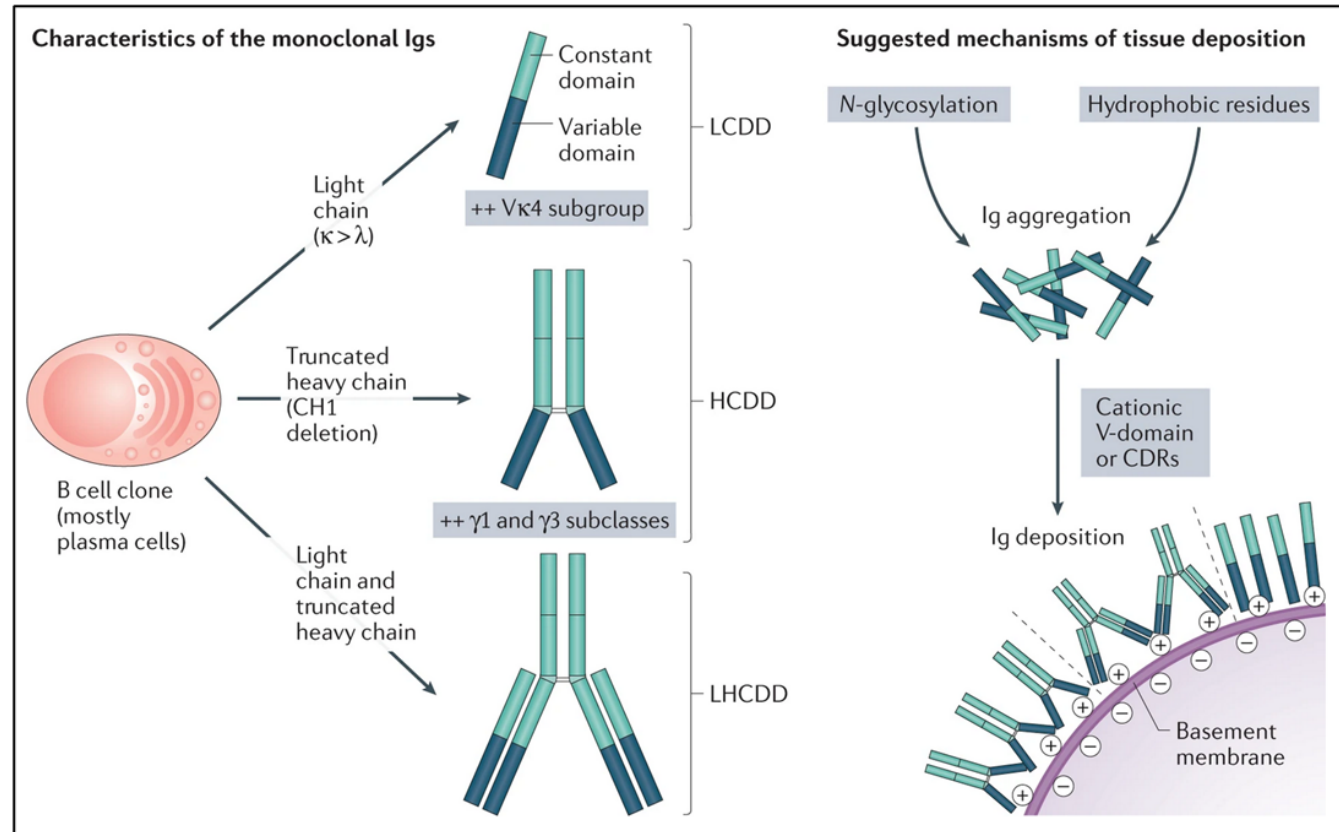
Frank Bridoux^{1,2,10}, Vincent Javaugue^{1,10}, Sébastien Bender², Fannie Leroy¹, Pierre Aucouturier³, Céline Debais-Delpech⁴, Jean-Michel Goujon⁴, Nathalie Quellard⁴, Amélie Bonaud², Marie Clavel², Patrick Trouillas^{5,6}, Florent Di Meo⁵, Jean-Marc Gombert⁷, Jean-Paul Fermand⁸, Arnaud Jaccard⁹, Michel Cogné², Guy Touchard¹ and Christophe Sirac²

- **HCDD** : deposited and circulating truncated heavy chain
(γ in most cases, uncommonly α and δ)
CH1 deletion (complete or partial) : 100%
Complement activation (classical pathway) in γ 1 and γ 3 HCDD : low CH50, C4, C3

Pathophysiology of HCDD (and MIDD)



Bridoux F, et al. *Kidney Int.* 2017



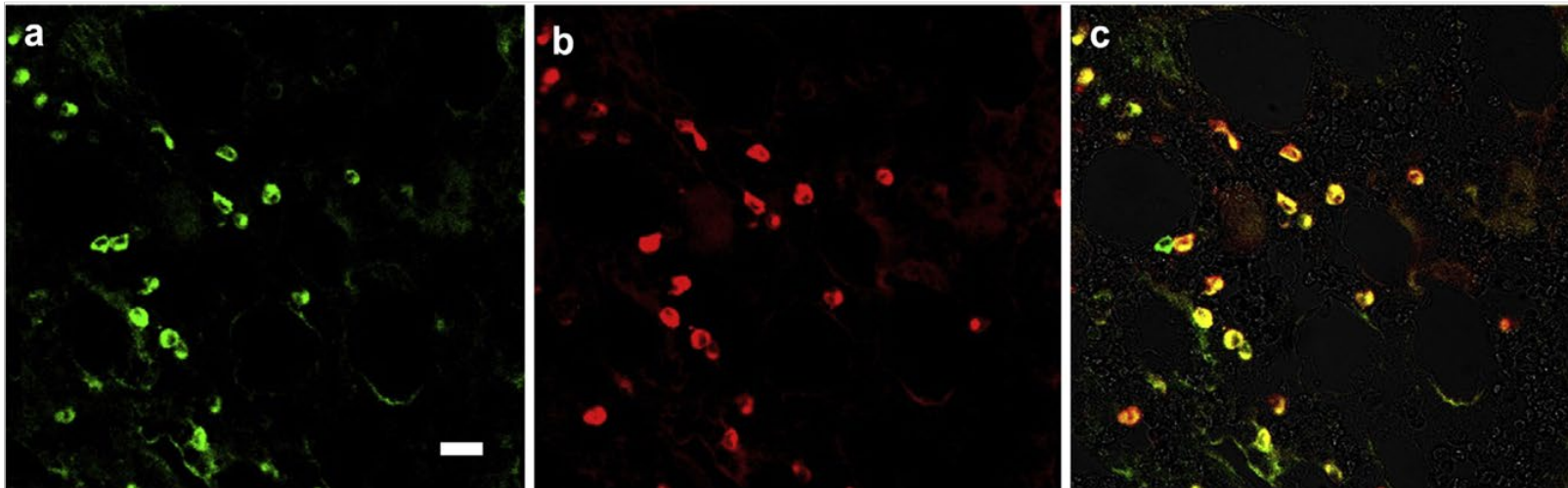
Bridoux F, et al. *Nat Rev Nephrol.* 2021

How to assess hematological response in HCDD ?

Anti- γ

Anti- λ

Merge

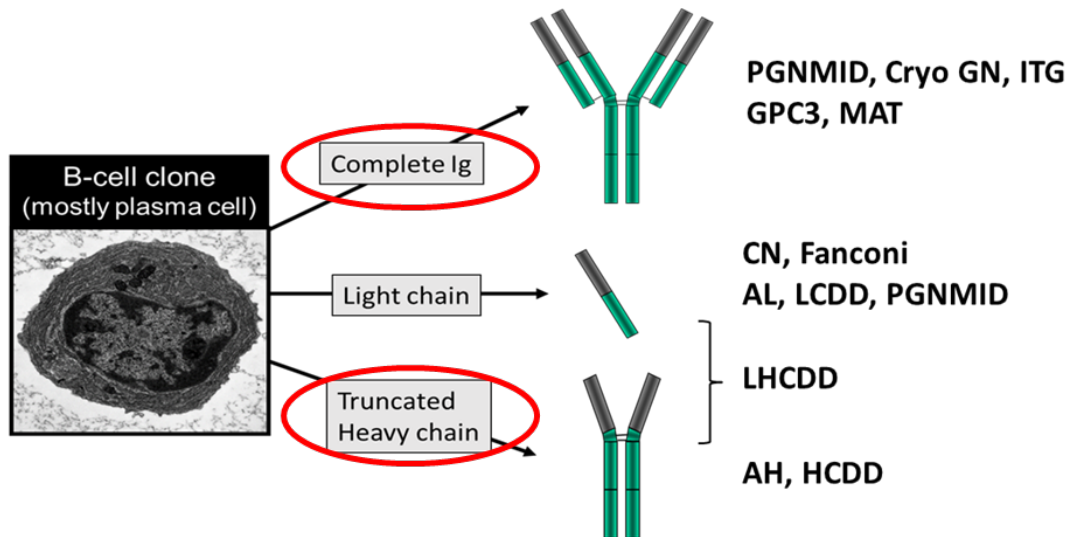


The toxic HC and FLC are produced by a single plasma cell clone

Bridoux F, et al. *Kidney Int.* 2017

Complement abnormalities (classical pathway) in MGRS-related renal disorders

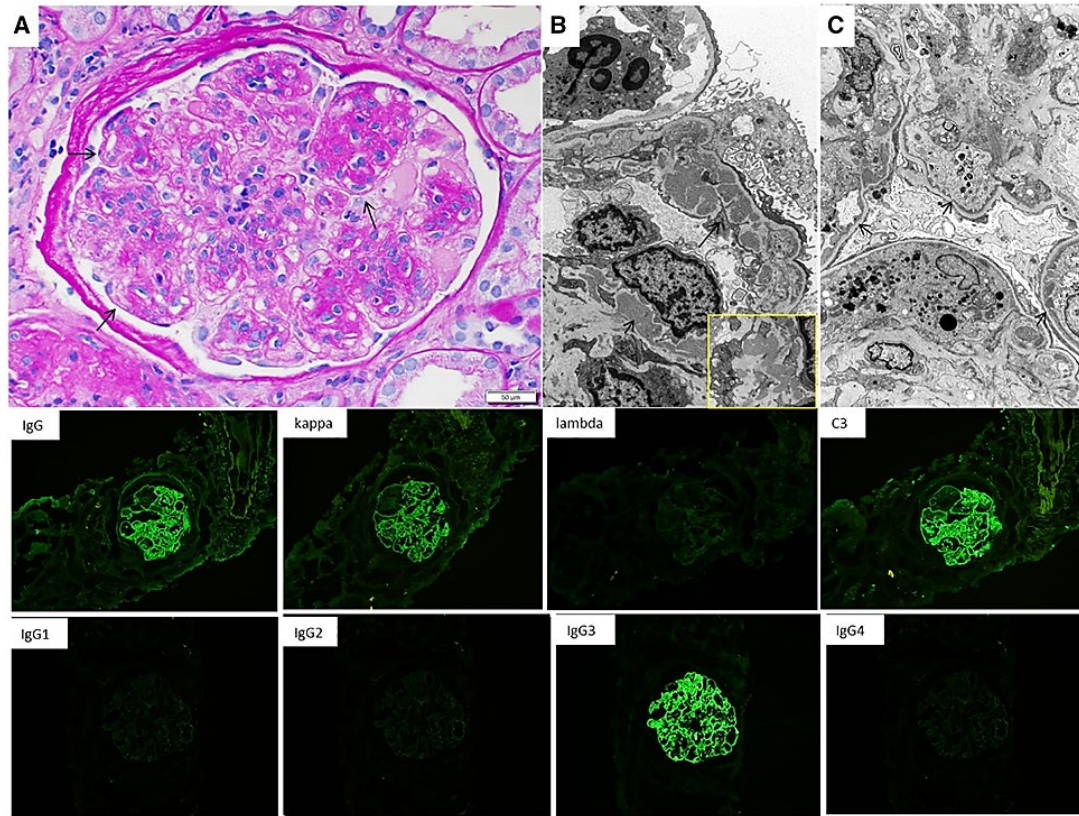
Heavy chain deposition (++ γ 1 or γ 3) is associated with co-deposition of complement proteins and hypocomplementemia



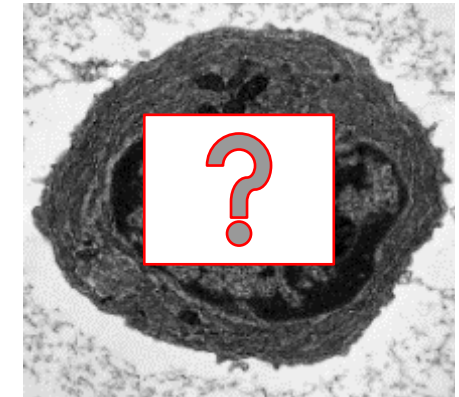
Hypocomplementemia

- ✓ Cryoglobulinemic GN
 - Type 1: 65%
 - Type 2: 80%
- ✓ HCDD ~ 60%
- ✓ Immunotactoid GN ~ 30%
- ✓ PGNMID 10-25%

GN à dépôts non-organisés non-Randall (PGNMID)

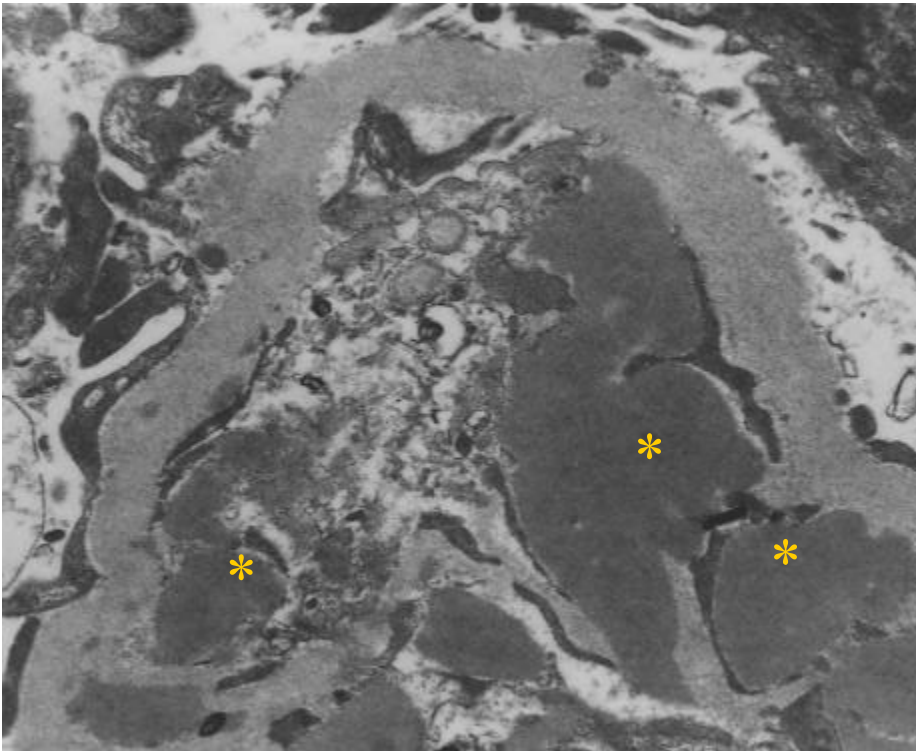


Detection of
the circulating
nephrotoxic
monoclonal Ig
~30%

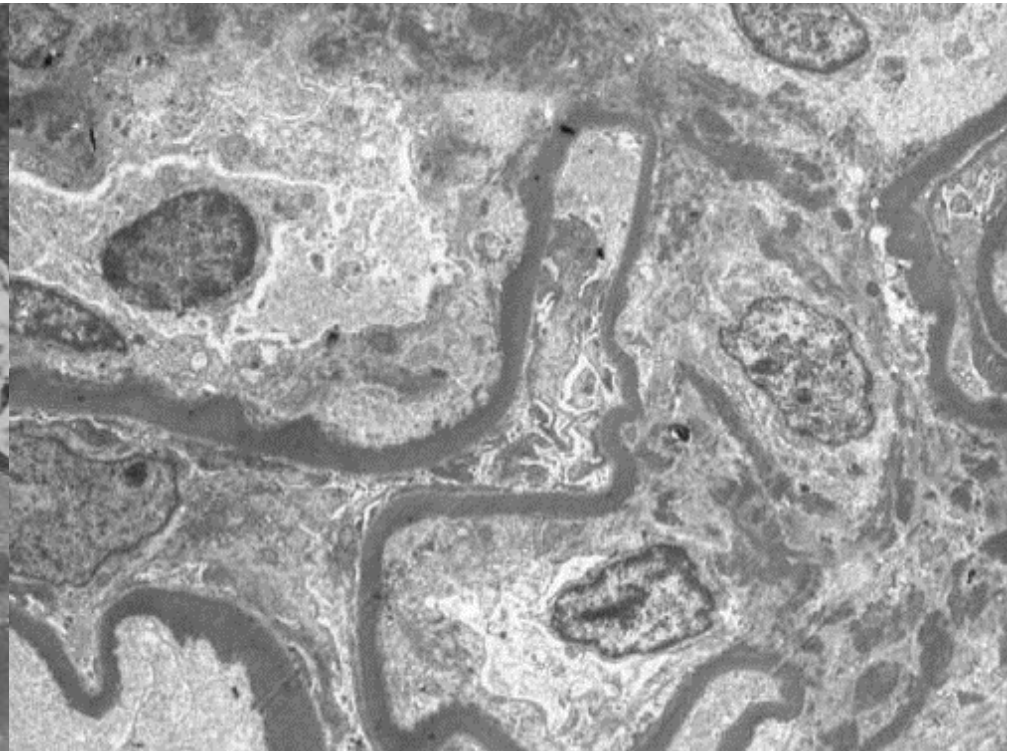


Clone detection rate ~**25%**
using bone marrow flow
cytometry and IHC analysis
(Bhutani et al. *Mayo Clin Proc.* 2015)

Granular deposits in mesangium and GCW
Monotypic Ig deposits (most commonly IgG3; rarely
IgG1, IgG2, IgA, IgM or light chain only)
No extraglomerular deposits
C3 almost always, C1q in ~2/3
Non-organized granular deposits by EM (++
subendothelial space)



PGNMID, ME x 5000



LCDD , ME x 2000

Aspect non-organisé non-Randall en microscopie électronique

Taux de détection du clone selon les sous-types de MGRS

Lesion	Proportion of lesions (%)				
	Monoclonal immunoglobulin deposits	Detectable monoclonal immunoglobulin	MM	MGRS	Other ^a
Light-chain cast nephropathy	100	100	99	0	~1
Immunoglobulin-related amyloid amyloidosis	96	99	16	80	1–4
MIDD	100	100	0–20	78–100	1–2
Light-chain proximal tubulopathy	100	97 ^b	12–33	61–80	3–8
Cryoglobulinaemic (type I) glomerulonephritis	100	90–100	6–8	47–52	24–56
Cryoglobulinaemic (type II) glomerulonephritis	100	49	0	20	7
PGNMID	100	30–32	4	96	~1
Crystal-storing histiocytosis	83	90	33	8	50
Cryocryoglobulin or cryoglobulin nephropathy	91	82	61	18	4
Immunotactoid glomerulonephritis	69–93	63–71	0–13	25–50	25–50
C3 glomerulopathy with monoclonal gammopathy ^c	0	100	0–40 ^d	40–90	6–10

Does the joint use of RACE-RepSeq for full-length sequence information and mass spectrometry analysis performed on laser micro-dissected tissue samples could be a powerful tool to confirm/exclude monoclonality in PGNMID?

- Since the clonotypic LC sequences are unique to each patient, they cannot be reliably detected by standard proteomic workflows that target the constant regions of LCs.

Proteomic Detection of Immunoglobulin Light Chain Variable Region Peptides from Amyloidosis Patient Biopsies

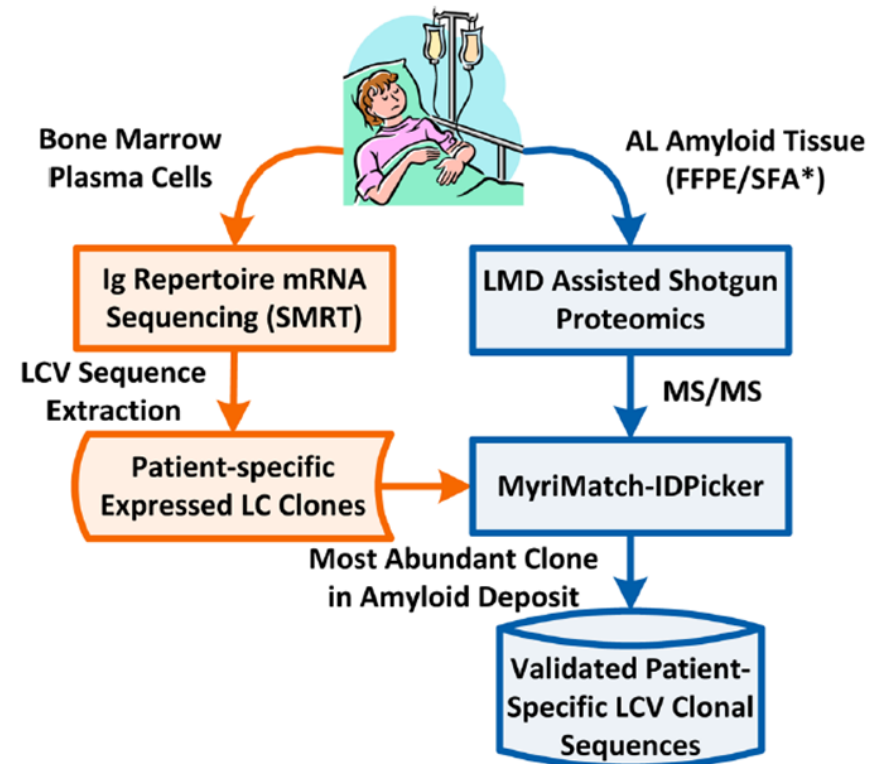
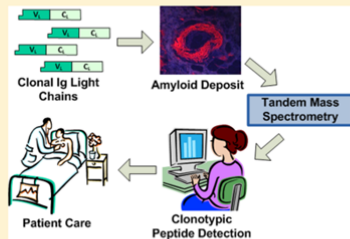
Surendra Dasari,[†] Jason D. Theis,[‡] Julie A. Vrana,[‡] Oana M. Meureta,^{‡,⊥} Patrick S. Quint,[‡] Prasuna Muppa,[‡] Roman M. Zenka,[§] Renee C. Tschumper,^{||} Diane F. Jelinek,^{||} Jaime I. Davila,[†] Vivekananda Sarangi,[†] Paul J. Kurtin,^{*,‡} and Ahmet Dogan^{‡,⊥}

[†]Department of Health Sciences Research, [‡]Department of Laboratory Medicine and Pathology, [§]Information Technology Administration, and ^{||}Department of Immunology and Division of Hematology, Mayo Clinic, 200 First Street SW, Rochester, Minnesota 55905, United States

Supporting Information

ABSTRACT: Immunoglobulin light chain (LC) amyloidosis (AL) is caused by deposition of clonal LCs produced by an underlying plasma cell neoplasm. The clonotypic LC sequences are unique to each patient, and they cannot be reliably detected by either immunoassays or standard proteomic workflows that target the constant regions of LCs. We addressed this issue by developing a novel sequence template-based workflow to detect LC variable (LCV) region peptides directly from AL amyloid deposits. The workflow was implemented in a CAP/CLIA compliant clinical laboratory dedicated to proteomic subtyping of amyloid deposits extracted from either formalin-fixed paraffin-embedded tissues or subcutaneous fat aspirates. We evaluated the performance of the workflow on a validation cohort of 30 AL patients, whose amyloidogenic clone was identified using a novel proteogenomics method, and 30 controls. The recall and negative predictive values of the workflow, when identifying the gene family of the AL clone, were 93 and 98%, respectively. Application of the workflow on a clinical cohort of 500 AL amyloidosis samples highlighted a bias in the LCV gene families used by the AL clones. We also detected similarity between AL clones deposited in multiple organs of systemic AL patients. In summary, AL proteomic data sets are rich in LCV region peptides of potential clinical significance that are recoverable with advanced bioinformatics.

KEYWORDS: amyloidosis, immunoglobulin, light chain, variable gene, variable gene family, peptides, proteomics, bioinformatics



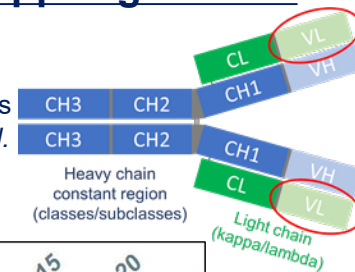
PGNMID IgG1κ with detectable clone using RACE-RepSeq

Kidney biopsy findings

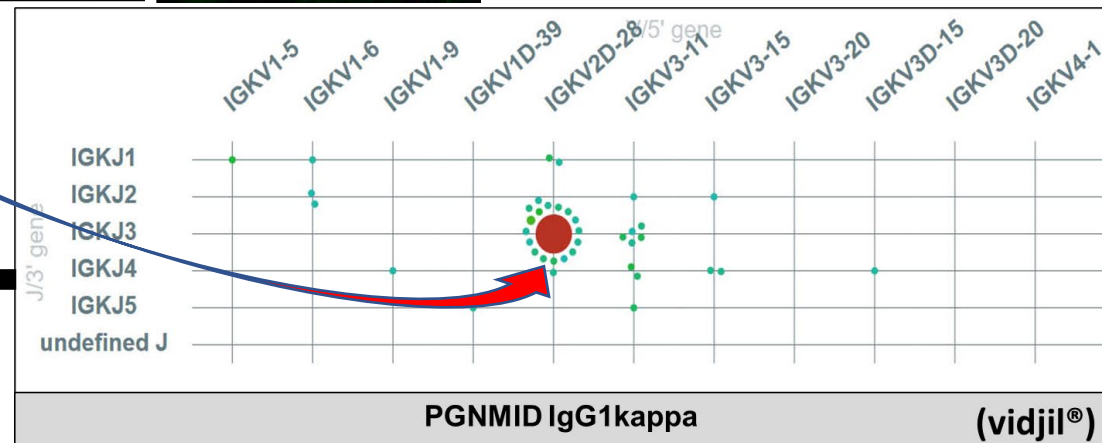
IF analysis using anti-Vκ antibodies
(variable domain specific)*

Vκ2 restricted kappa light chain

*Monoclonal anti-Vκ antibodies
(Abe M et al. *Am J Clin Pathol.*
1993)

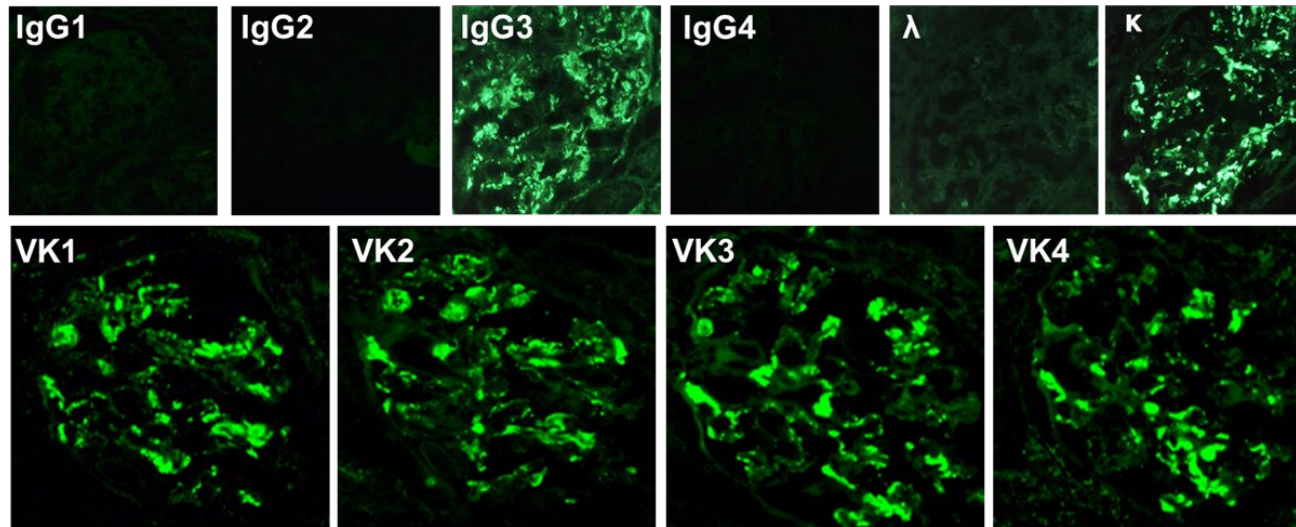


Bone marrow RACE-RepSeq analysis detected a dominant clonal light chain sequence (Vκ2 variability subgroup) matching the kidney deposits



Schematic representation (Vidjil®) showing a dominant clonotype
(Vκ2D-28) accounting for 78% of total clonotypes by RACE-
RepSeq

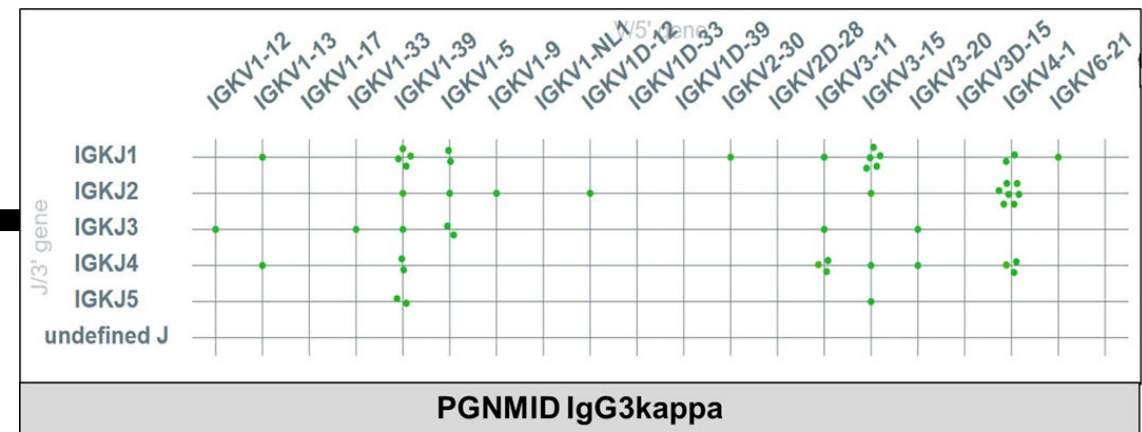
PGNMID IgG3κ without detectable clone using RACE-RepSeq



Kidney biopsy findings

No evidence of Vk restriction
(similar staining using antibodies specific for Vk1, Vk2, Vk3 or Vk4 variability subgroup)

Bone marrow and blood RACE-RepSeq analysis showed no dominant clonal light chain sequence



Schematic representation (Vidjil®) showing the absence of dominant clonal kappa sequence by RACE-RepSeq

Revisiting proliferative glomerulonephritis with monoclonal immunoglobulin deposits through immunoglobulin repertoire sequencing

kidney
INTERNATIONAL

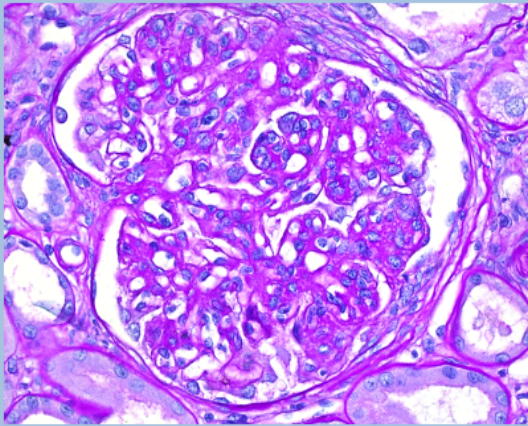


56 patients with kidney biopsy-proven PGNMID and Ig repertoire sequencing analysis



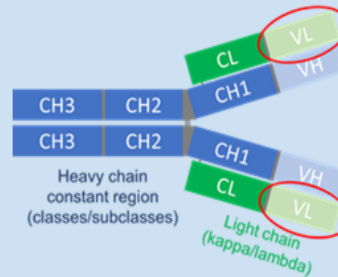
13 with clone

43 without clone

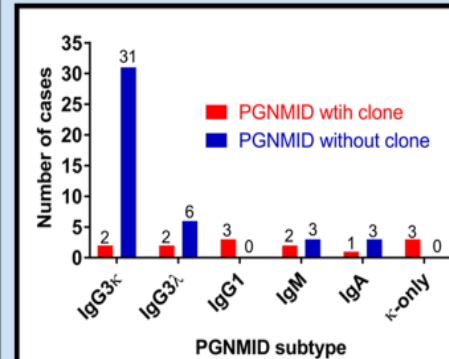


Comparison between clone-positive and clone-negative patients

- ✓ Clinical data
- ✓ Hematologic workup
- ✓ Pathologic analysis (proteomic and IF on kidney biopsies using anti-V κ antibodies)



PGNMID subtypes according to clone detection using RACE-RepSeq analysis

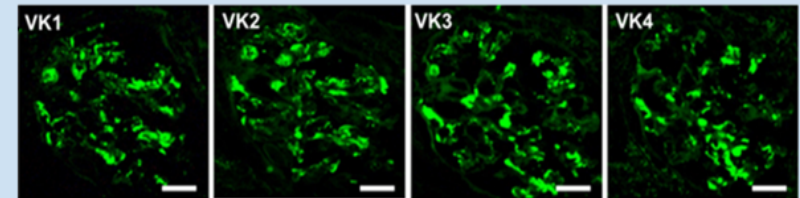


Clone-positive patients :

- ✓ Older age
- ✓ Lower proteinuria
- ✓ More frequent hypocomplementemia

Clone-negative patients :

- ✓ More frequent PGNMID-IgG3 subtype
- ✓ Polyclonal deposits (anti-V κ)

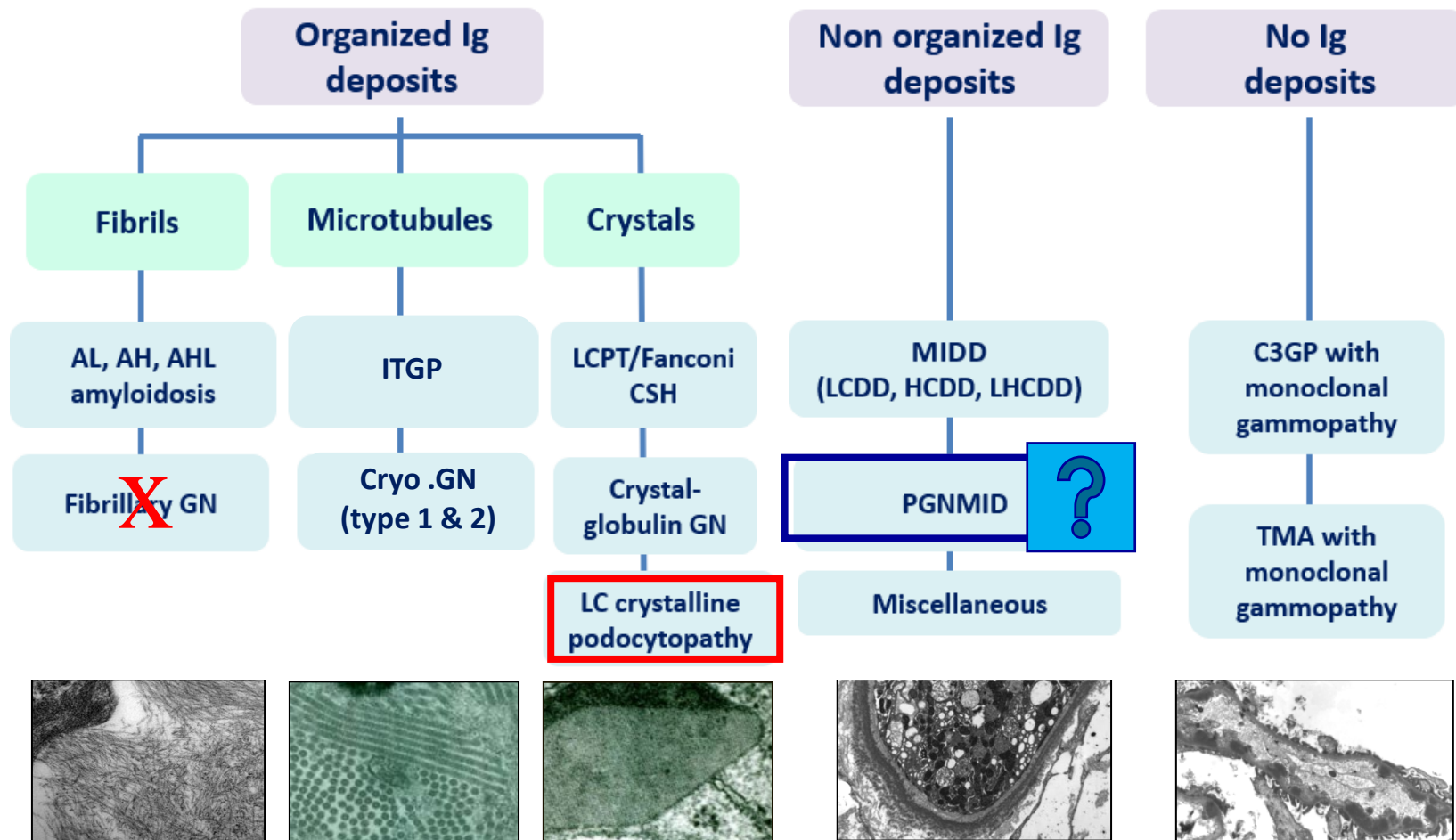


IF analysis (anti-V κ antibodies) in a PGNMID-IgG3 κ case without clone

Javaugue, Pascal et al, 2025

CONCLUSION: PGNMID cases with undetectable clones after thorough hematologic workup, including most IgG3 subtypes, are associated with oligoclonal deposition and should be excluded from the MGRS spectrum

Revised IKMG classification of MGRS-related renal diseases

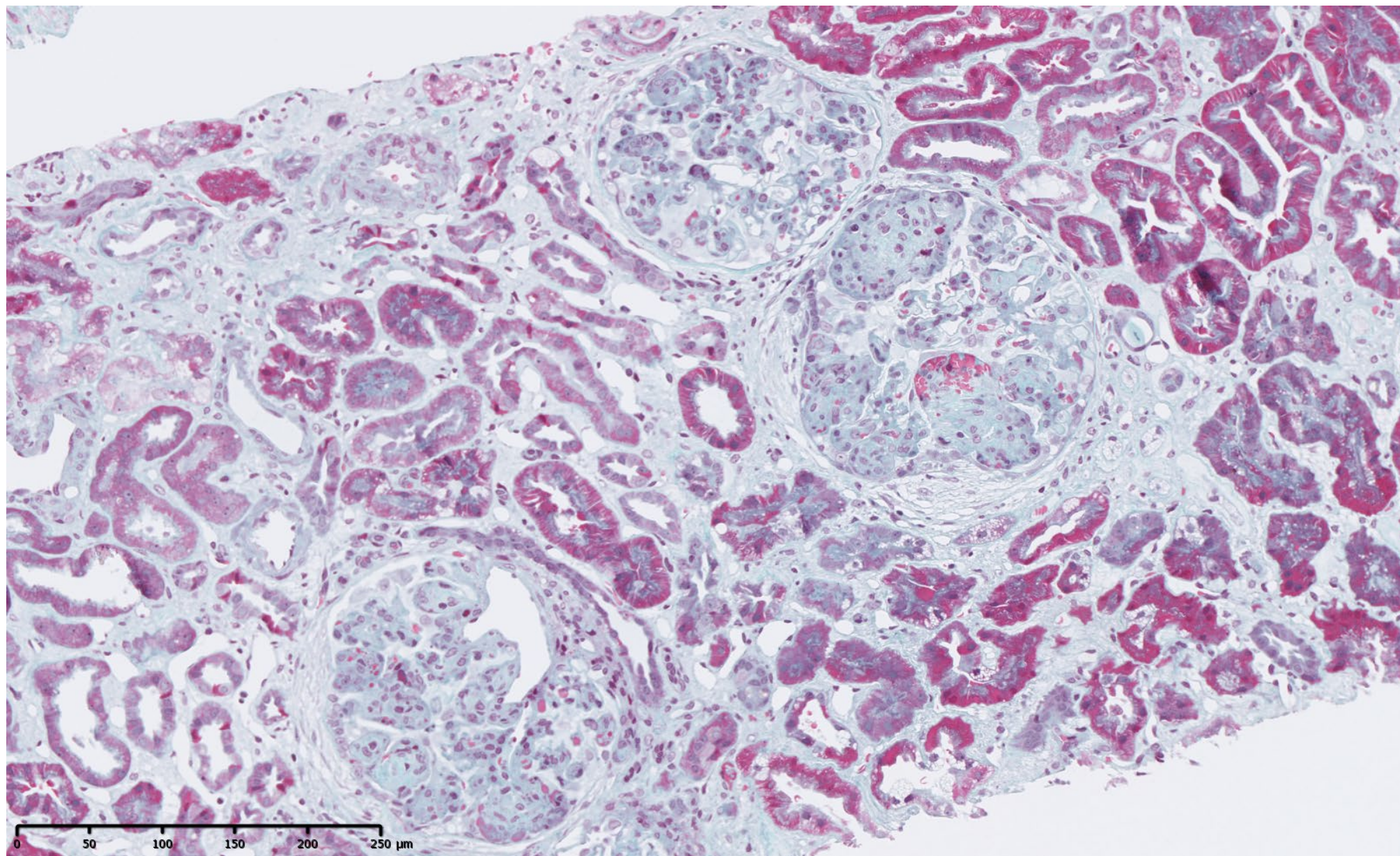


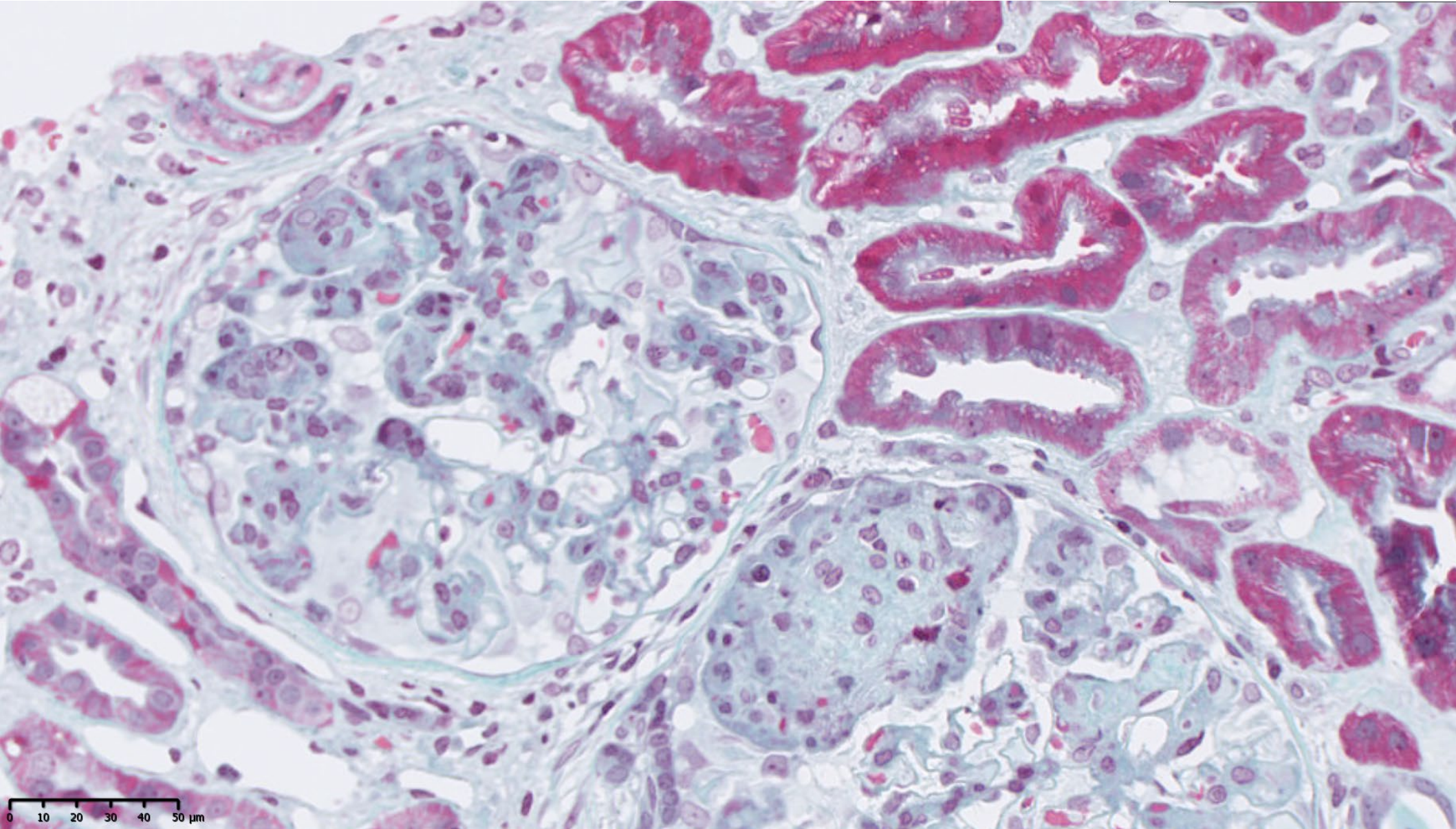
Leung N, Bridoux F, Nasr SH. N Engl J Med 2021



Pour finir, une MGRS exceptionnelle de Marseille...

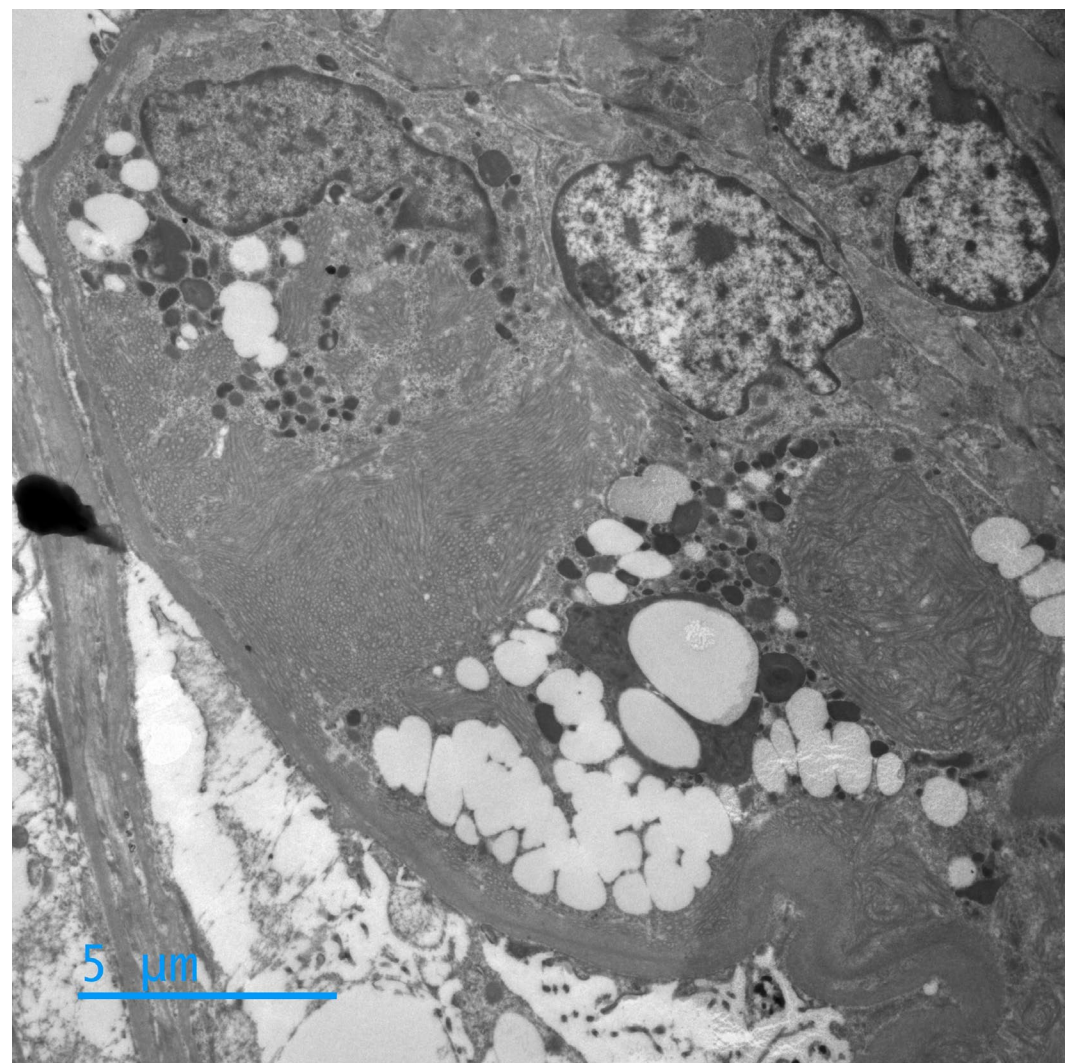
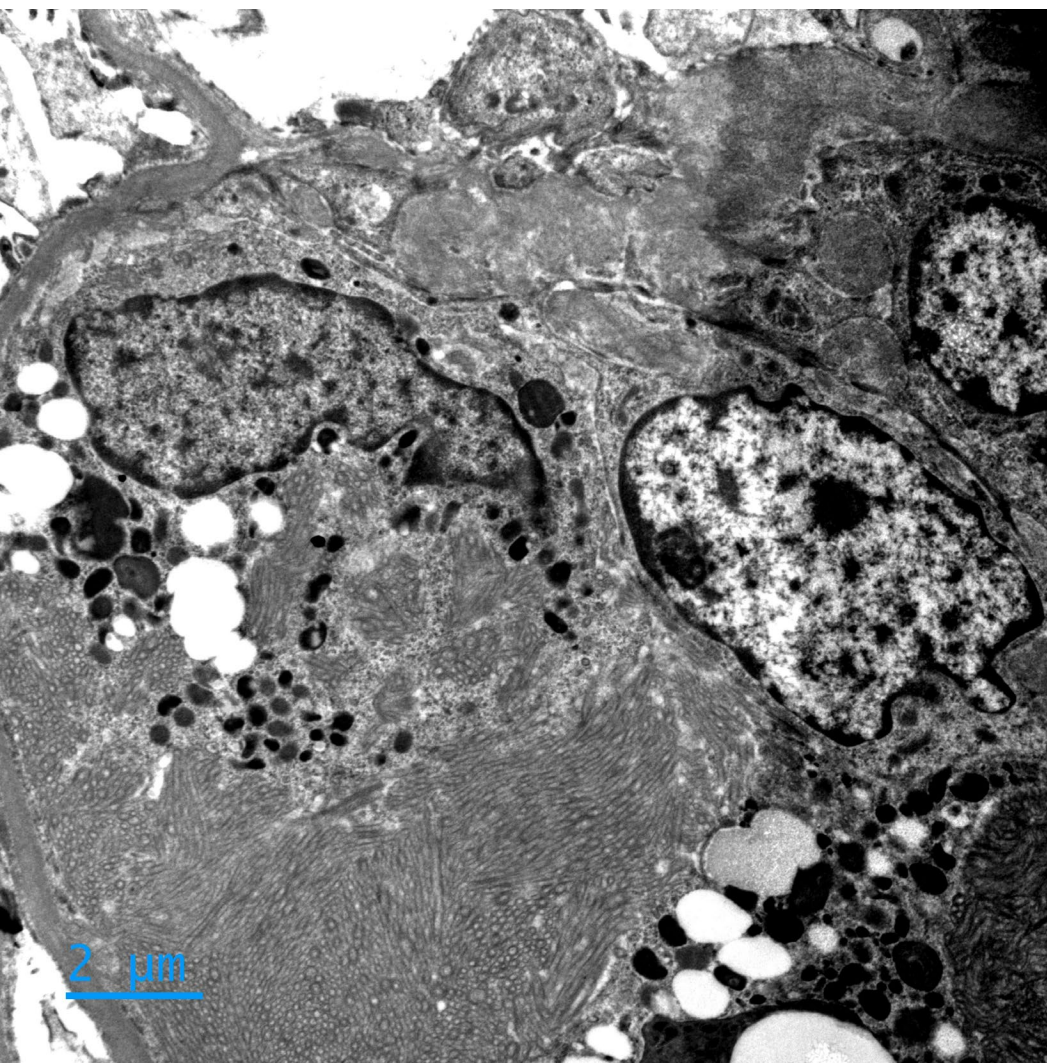
- Patient âgé de 71 ans. Antécédent de GN à dépôts de C3 et de mono-neuropathie multiple (vascularite des petits vaisseaux sur biopsie nerveuse sans dépôts en IF) traitée par Eculizumab et corticoïdes depuis 4 ans
- Amélioration initiale de la fonction rénale et de la neuropathie mais devant l'échec de l'espacement de l'Eculizumab, décision de réaliser une nouvelle biopsie rénale
- Au moment de la seconde biopsie rénale
 - Créatininémie à 149 $\mu\text{mol/l}$ (DFG = 39 ml/min/1,73m²)
 - Albuminémie = 27 g/l et rapport protéinurie/créatininurie = 332 mg/mmol
 - Hématurie microscopique (66/mm³).
 - Mise en évidence d'une IgG kappa monoclonale (pic connu et stable à 5 g/l depuis 4 ans)
 - C3 bas, C4 normal, CH50 indosable (sous Eculizumab)
 - Sérologies VIH, VHB, VHC et syphilis négatives

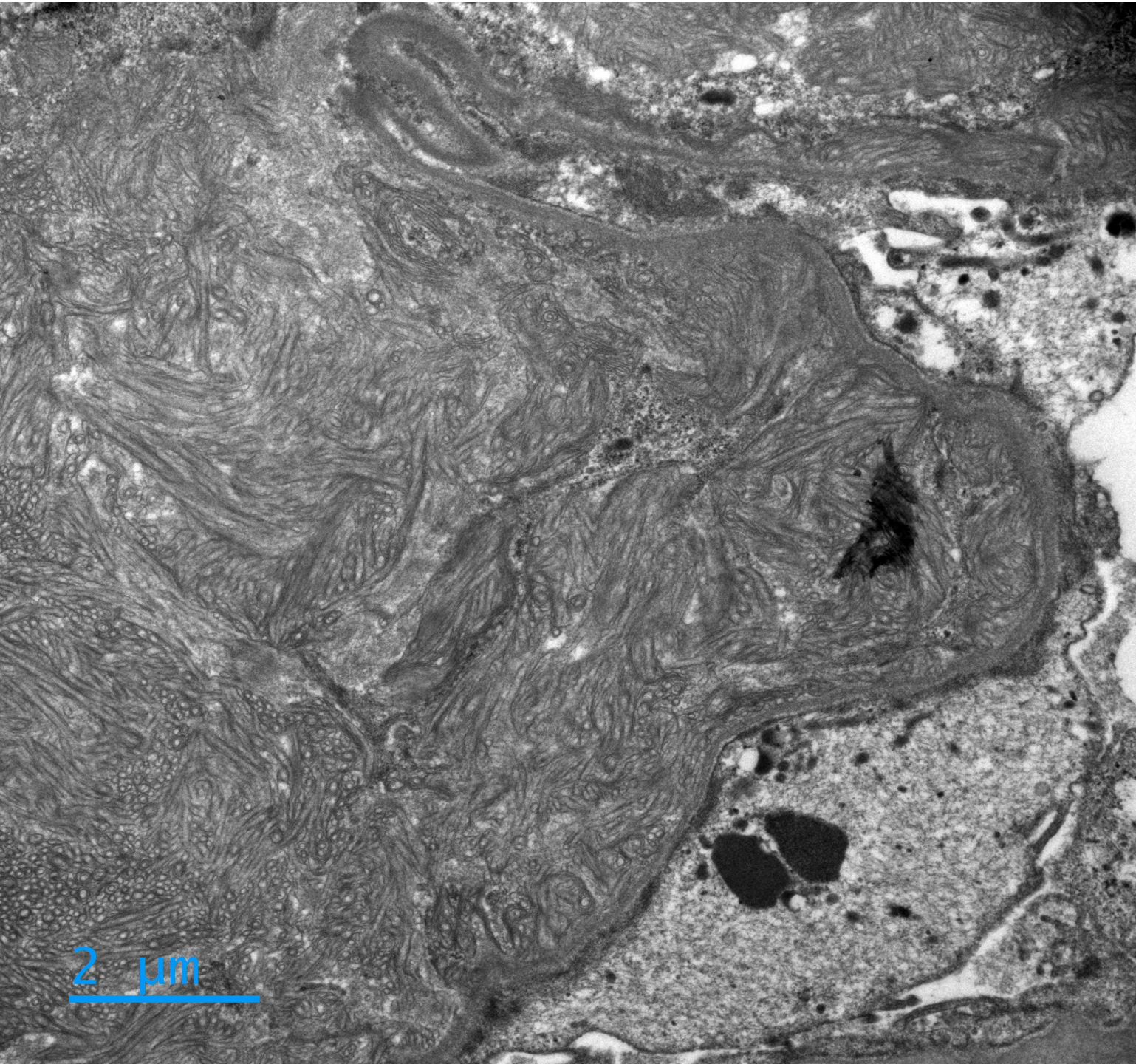




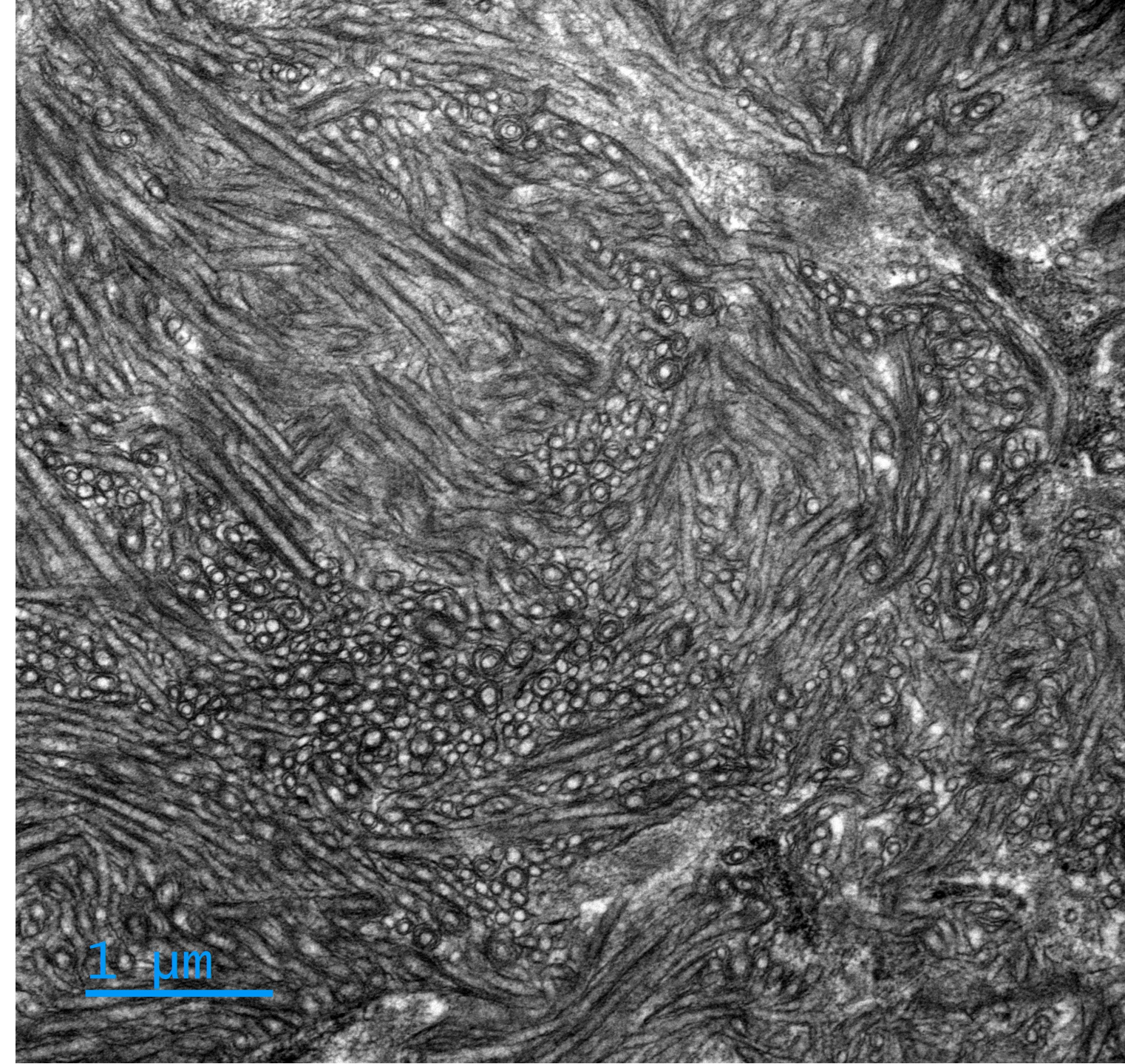
0 10 20 30 40 50 μm

**L'analyse en IF montre un marquage
glomérulaire de faible intensité (1+)
endo-membraneux globaux et diffus de
C3, segmentaire d'IgM et de C1q**

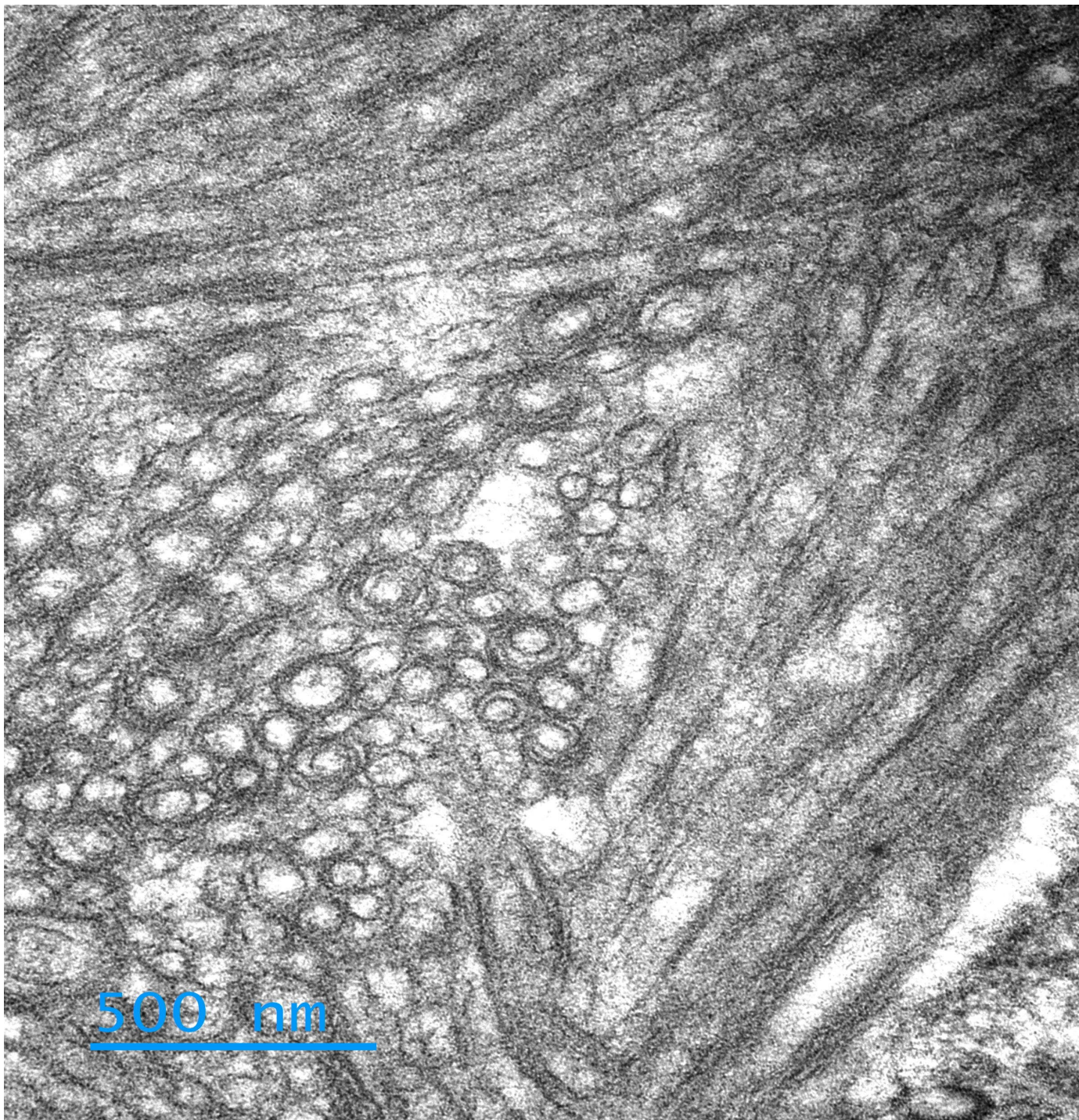




ME x 10000

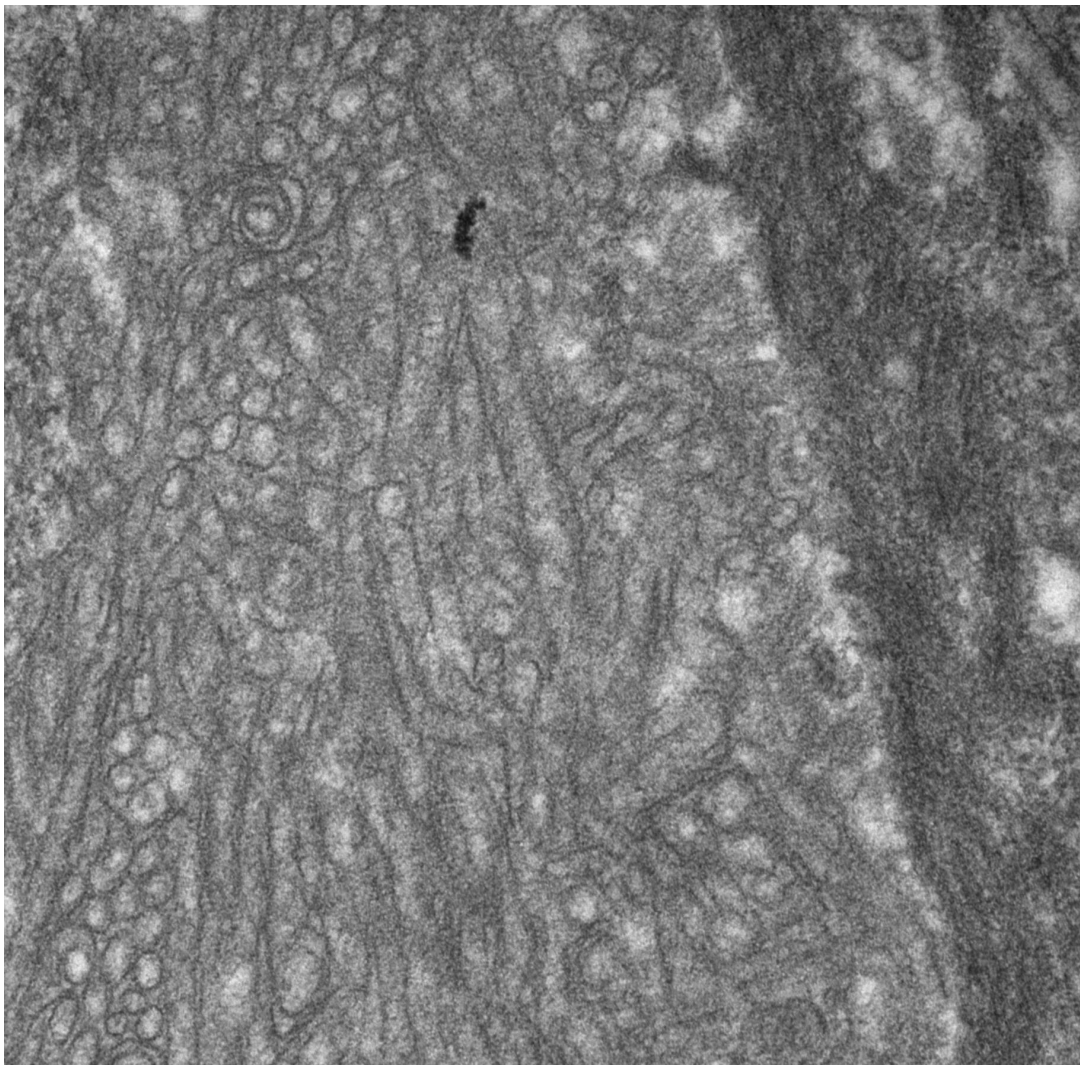


ME x 15000

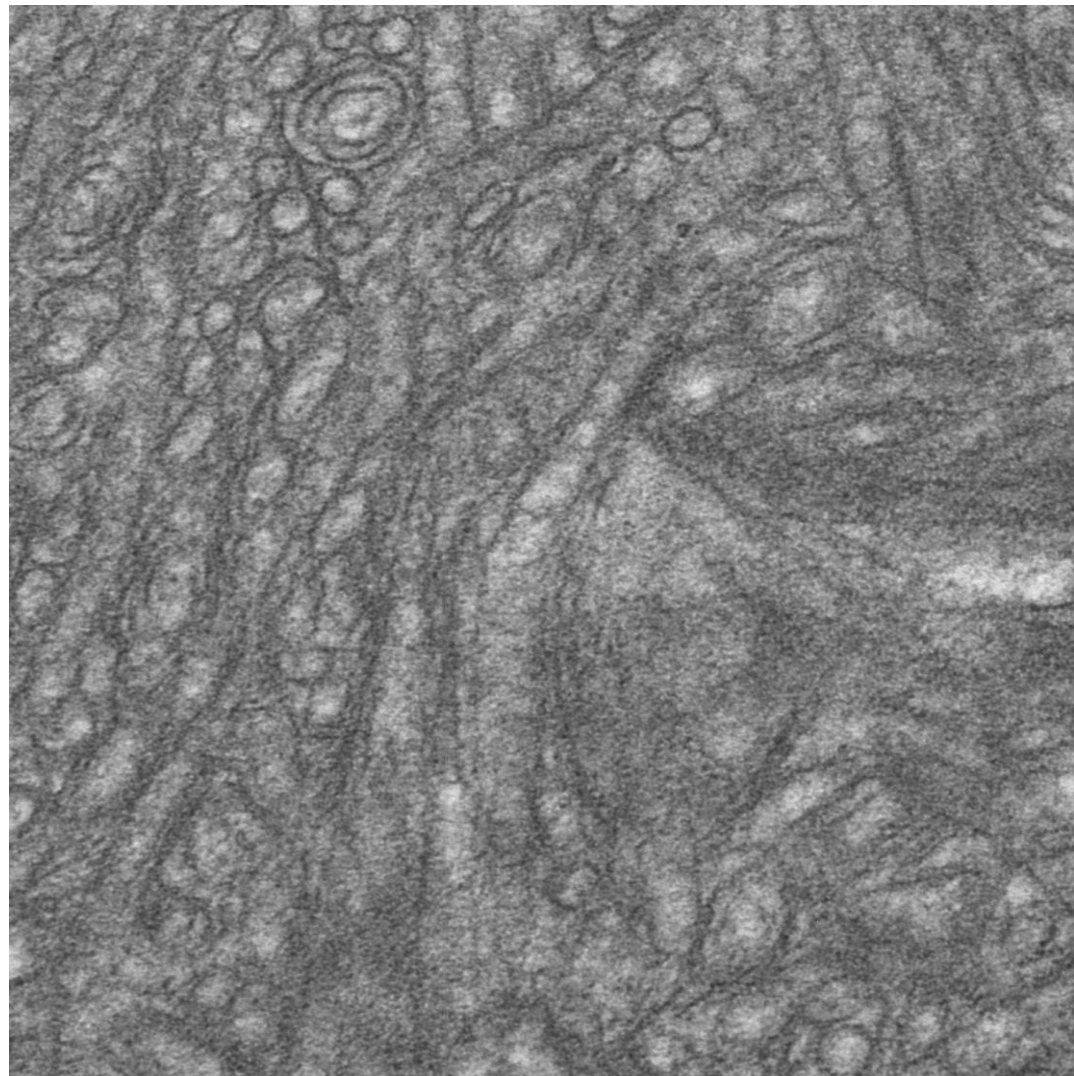


500 nm

ME x 50.000

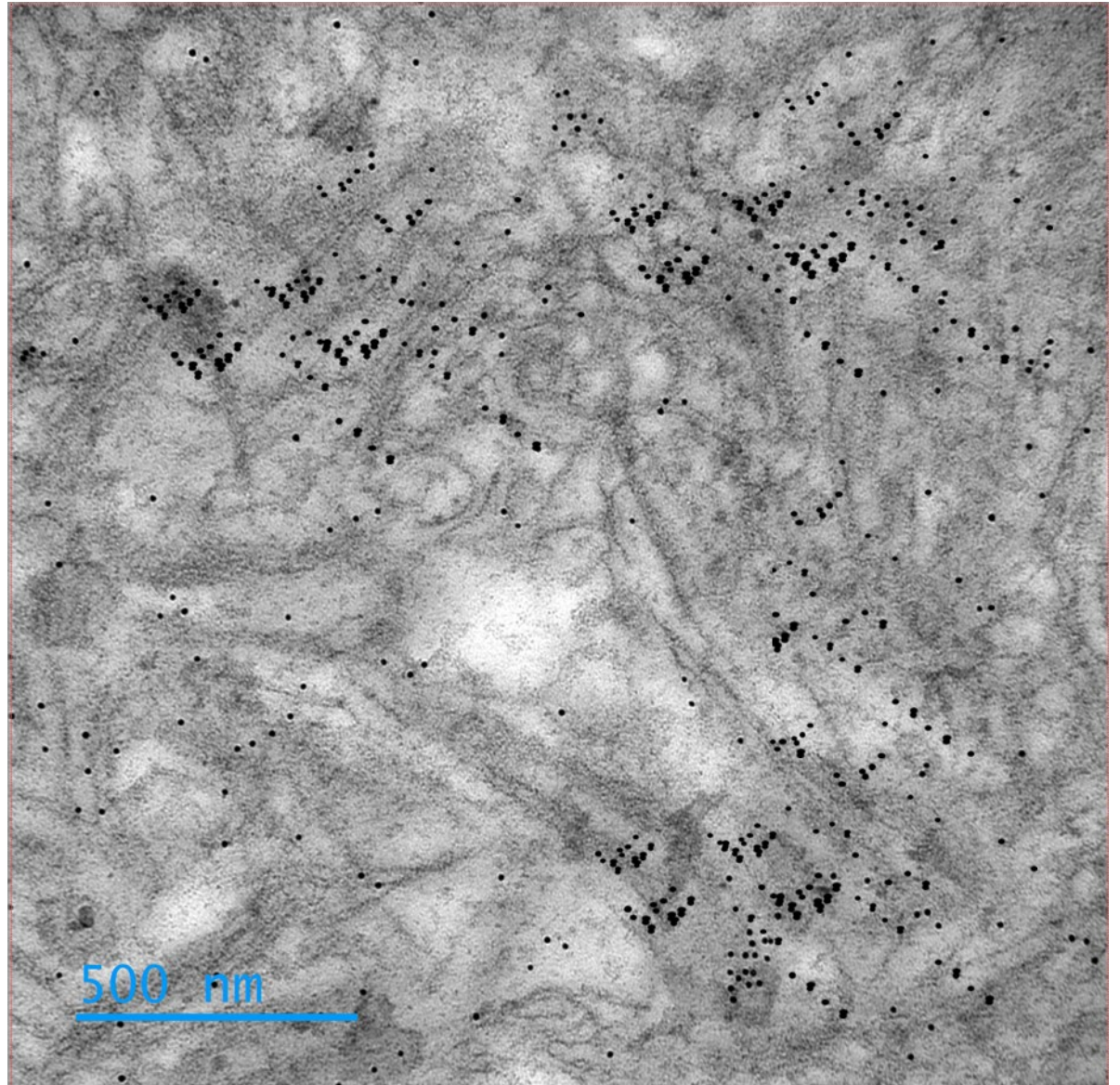


Immunogold anti-kappa

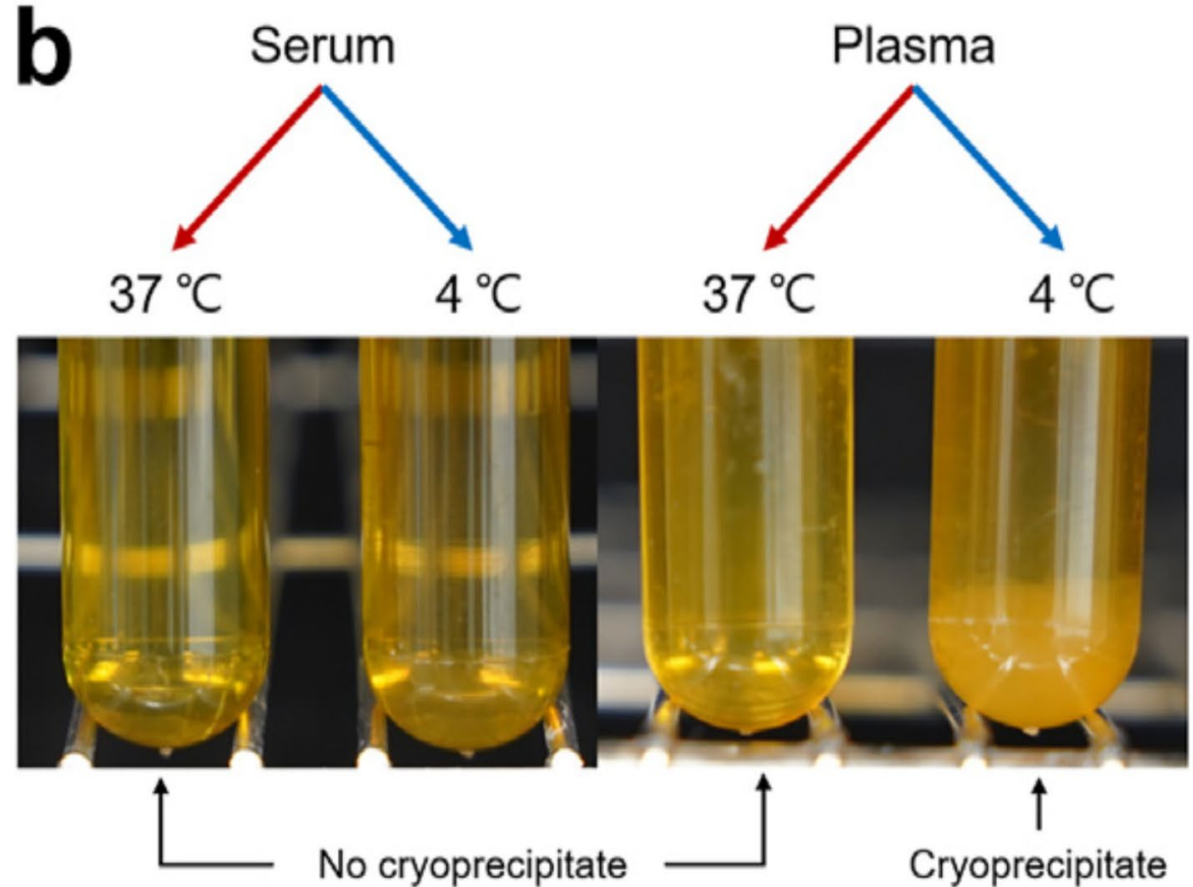
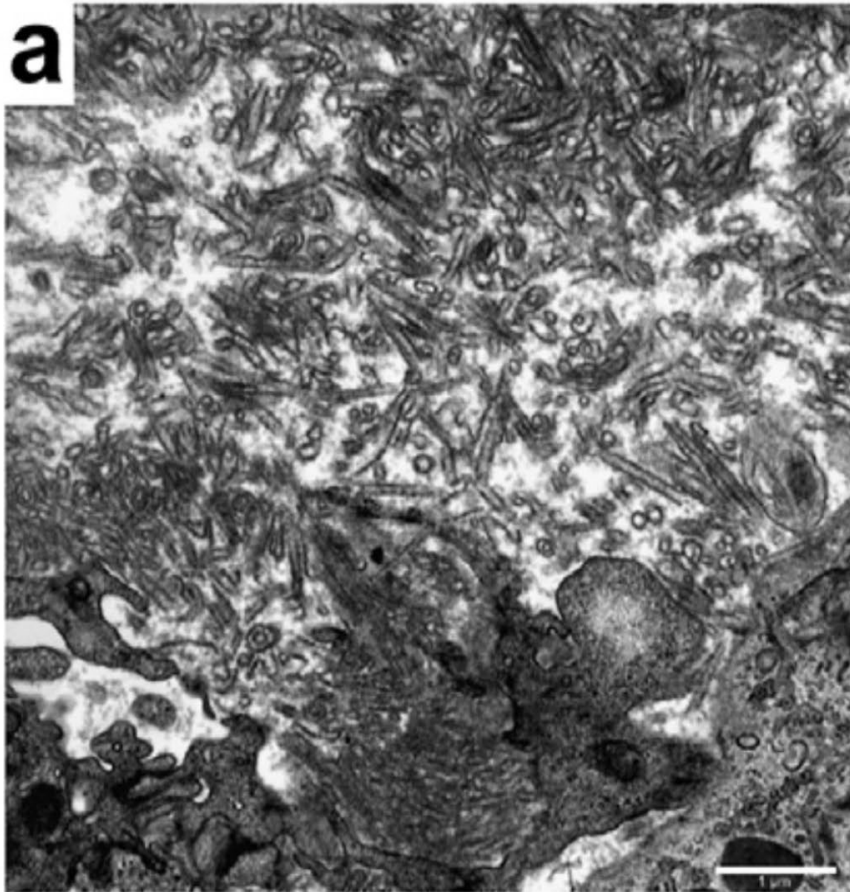


Immunogold anti-lambda

ImmunoGold
anti-
fibrinogène



Cryofibrinogen-associated membranoproliferative glomerulonephritis



Predicting the Pathogenicity of Monoclonal Immunoglobulins ?

- *Molecular analysis indicates major role of V domain characteristics*
 - **Fanconi syndrome** : Vk1 predominance, restriction IGLK1-33 et 1-39
Somatic mutations in CDRs introducing hydrophobic amino-acids
→ lysosomal dysfunction and resistance to proteolysis in PTC
 - **LCDD** : Vk4 predominance (IGLK4-1). Cationic CDRs favoring deposition to anionic basement membranes, abnormal N-glycosylation sites
 - **HCDD** : CH1 deletion, cationic entire variable domain → deposition if secreted
 - **Renal AL amyloidosis**: Vλ6 predominance, somatic mutations, hydrophobicity, post-translational modifications (glycosylation), aggregation features may predict amyloidogenicity ?



Merci !

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