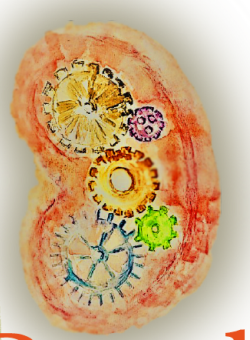
Anno VII

Cadernos de Patologia Renal



COMPLEMENTO E DOENÇAS RENAIS.

08 a 10 de maio, 2023
Hospital do Rim,
São Paulo, SP

Proteomica de Complemento:
um caminho para Medicina de Precisão

Dra. Lilian Monteiro P. Palma

Nefrologista Pediátrica – UNICAMP e LUME Nefrologia e Dialise

Realização: hrim FUNDAÇÃO OSWALDO RAMOS NÚCLEO DE ENSINO E PESQUISA

Apoio: Sociedade Brasileira de Nefrologia

SONESP Sociedade de Nefrologia do Estado de São Paulo

Conflitos de interesse

Anno VII
Cadernos de Patologia Renal
COMPLEMENTO E DOENÇAS RENAIS.

- Palestrante Síndrome Hemolítico Urêmica atípica para Alexion
- Consultora científica Glomerulopatia C3 para Apellis
- Esta não é uma aula patrocinada

Clinical Case

- Female, 16 years-old
- Dry weight 55 kg Height 160 cm
- Fever&illness
- Edema (3 kg), arterial hypertension (BP 140x90 mmHg)
- Creatinine 1.6 mg/dL (eGFR: 41 mL/min/1.73m^2)
- Hematuria (15 RBC/field) with codocytes
- Proteinuria 2 g/24 h
- Albumine 3.9 mg/dL and EFP normal
- Secondary causes negative
- C3 ↓, C4 normal
- US: normal sized kidneys, mild increase in echogenicity

Glomerulonephritis
Acute Kidney Disease
Chronic Kidney Disease?

Acute Kidney Disease Glomerulonephritis

Histology

1. Location of injury
2. Type of cells
3. Glomerular response

Patterns

- Mesangial proliferative
- Diffuse proliferative
- MPGN
- Crescentic
- Necrotizing

Pathophysiology

1. Proliferation of cells
   a. Leukocytes
   b. Endogenous
2. Synthesis of material
   a. Mesangial matrix
   b. Basement membrane
   c. Fibrin

What leads to glomerular injury?

Etiology of proliferative GN

Immune complex ± C3

Polyclonal IgG
Infection-related GN

Polyclonal IgM
Chronic infection (Hepatites)
Autoimmune

Polyclonal Igs
Lupus GN
Autoimmune Fibrillary

IgA
IgA Infectious

Anti-GBM

Renal limited
Goodpasture

Monoclonal Ig

IgG κ/λ
IgM κ/λ

Pauci-immune

ANCA+
ANCA –
Necrotizing/crescentic GN

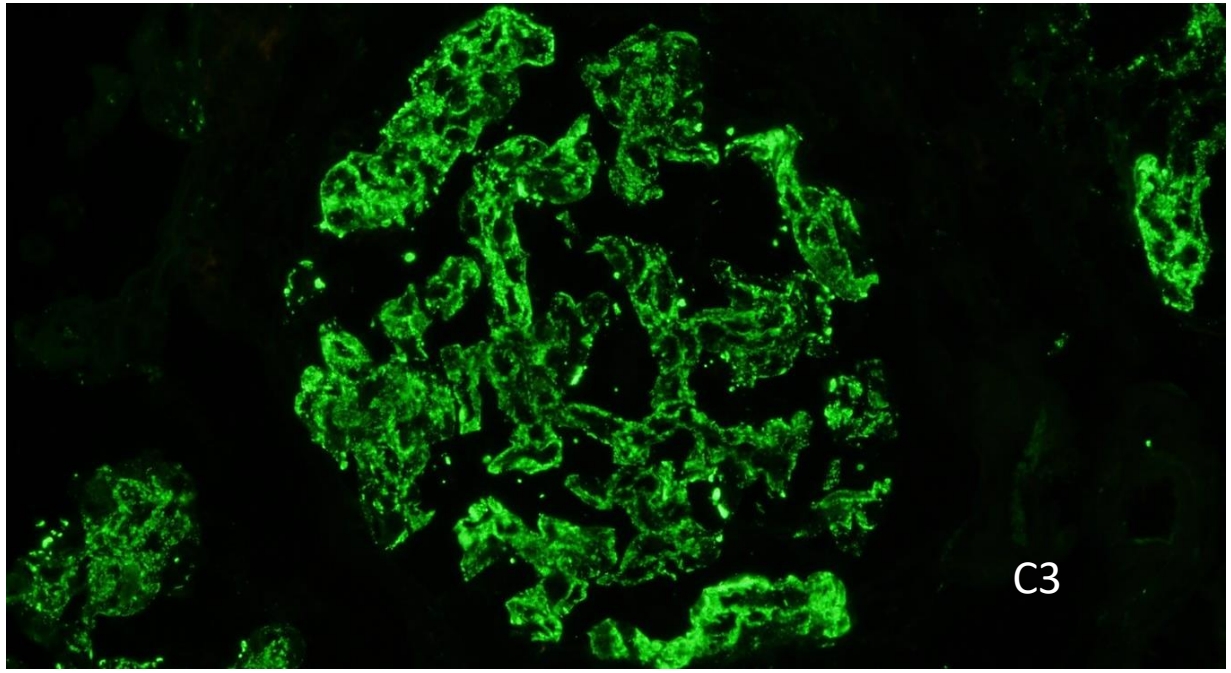
Complement±Ig

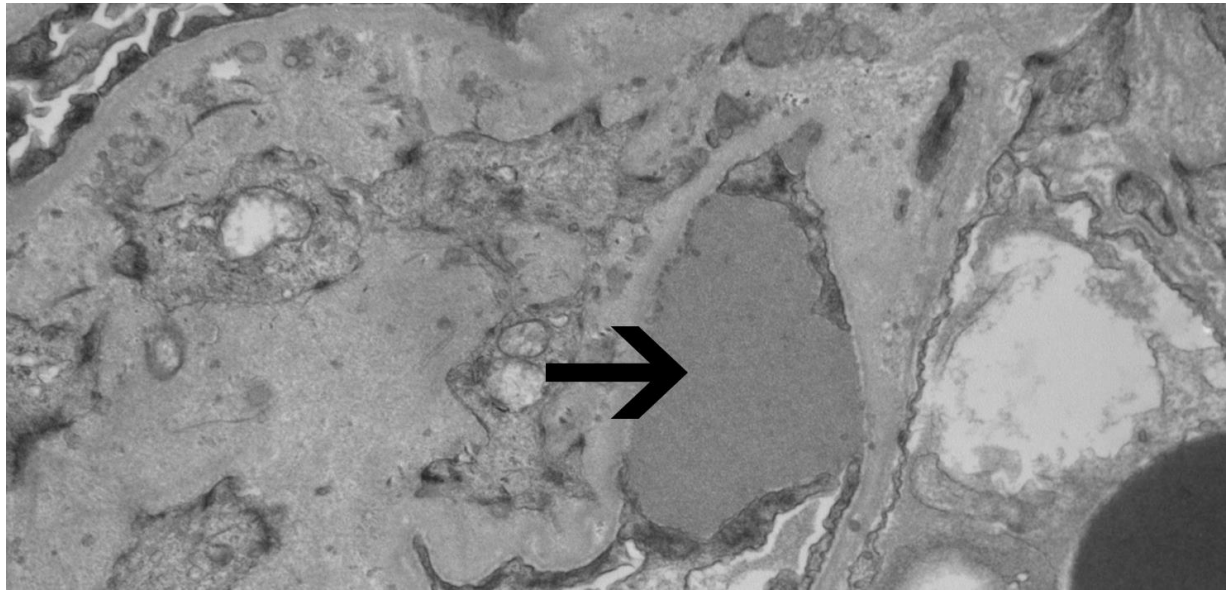
C3 dominant

C3G
C3GN
DDD

Patterns → Type of Deposits (IF)

Adapted from Sethi S *et al*. *Am J Kidney Dis* (2014), 63 (4): 561 and Sethi S et al. J Am Soc Nephrol (2016), 27(5): 1278

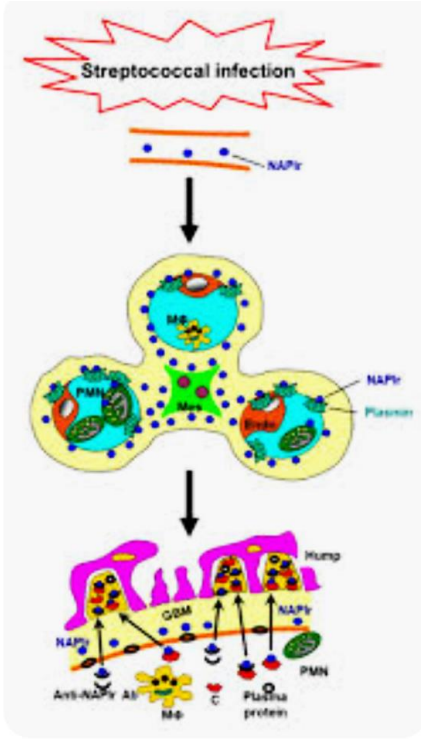




Subepithelial deposit

Post-infectious GN

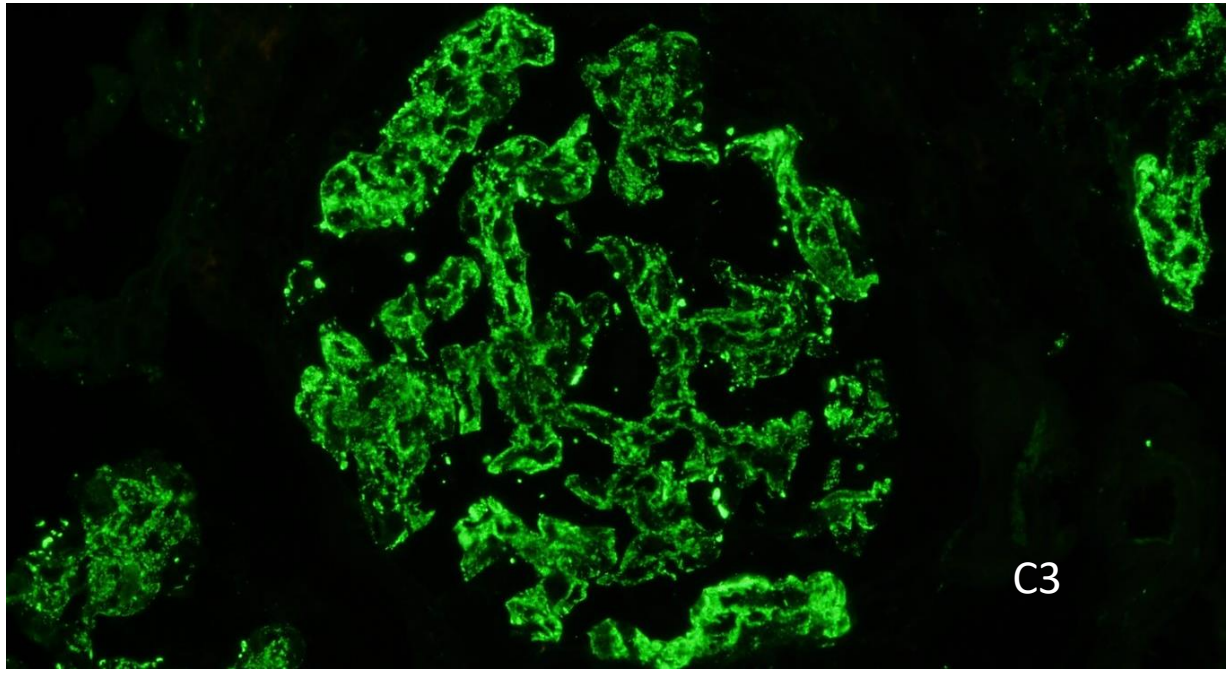
Initiation: immunecomplex and **alternative/lectine**
Proliferation: leukocyte influx and mesangial matrix degradation
2ary Complement activation
Repair: restitutio ad integrum

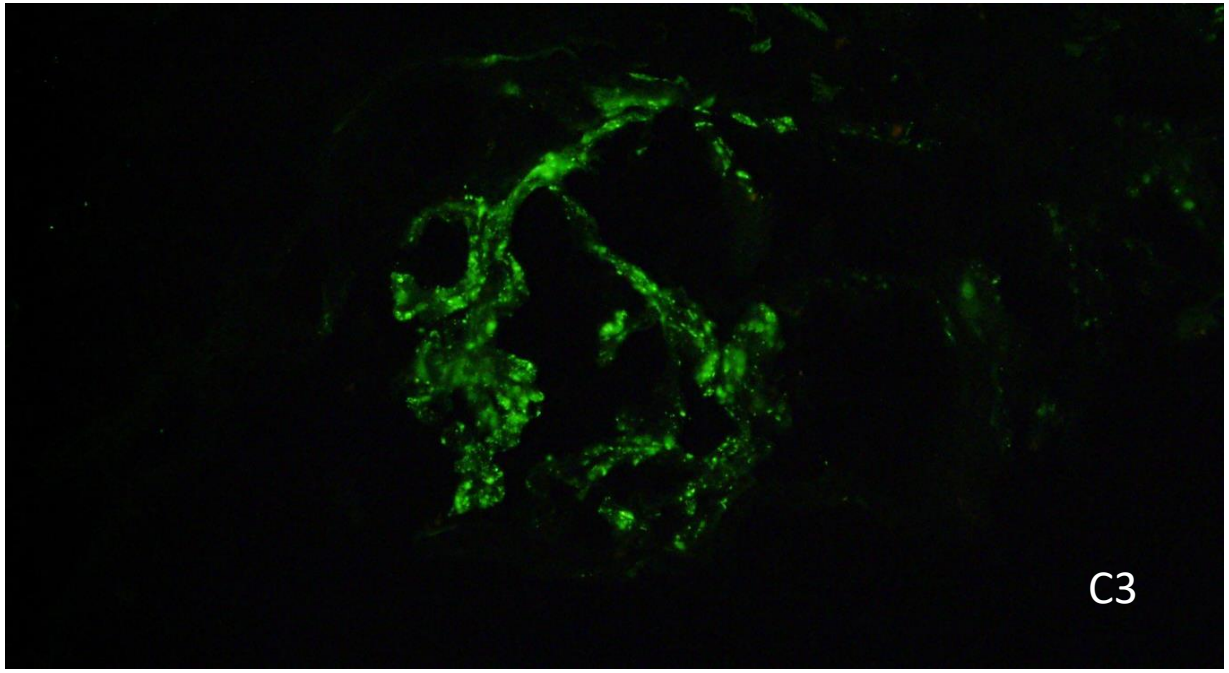


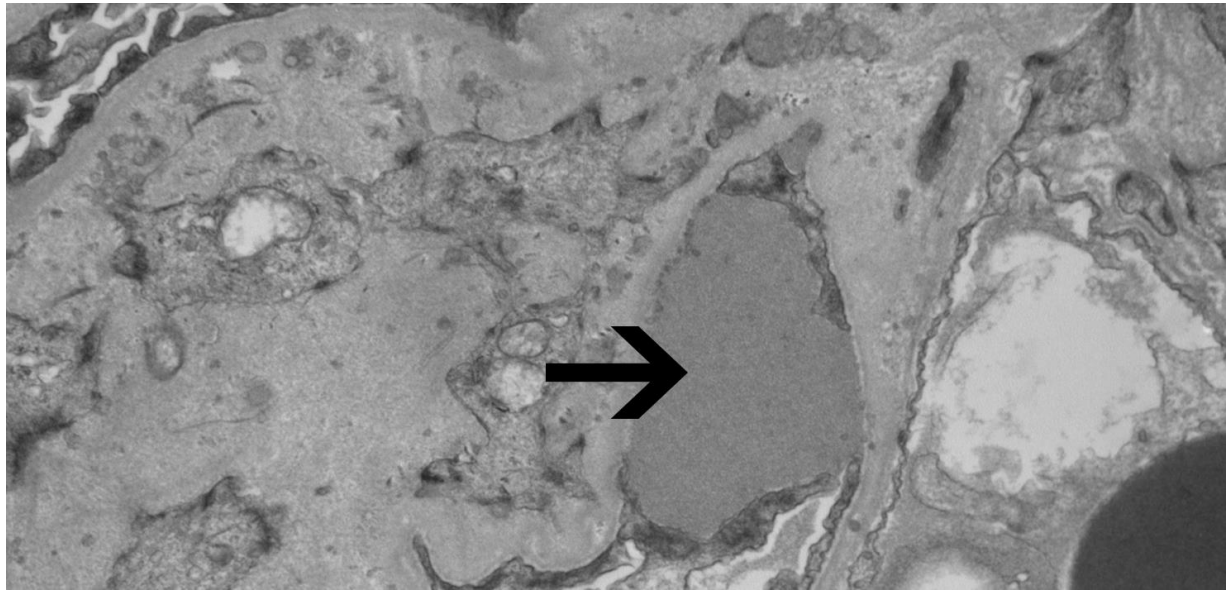
Pathophysiology

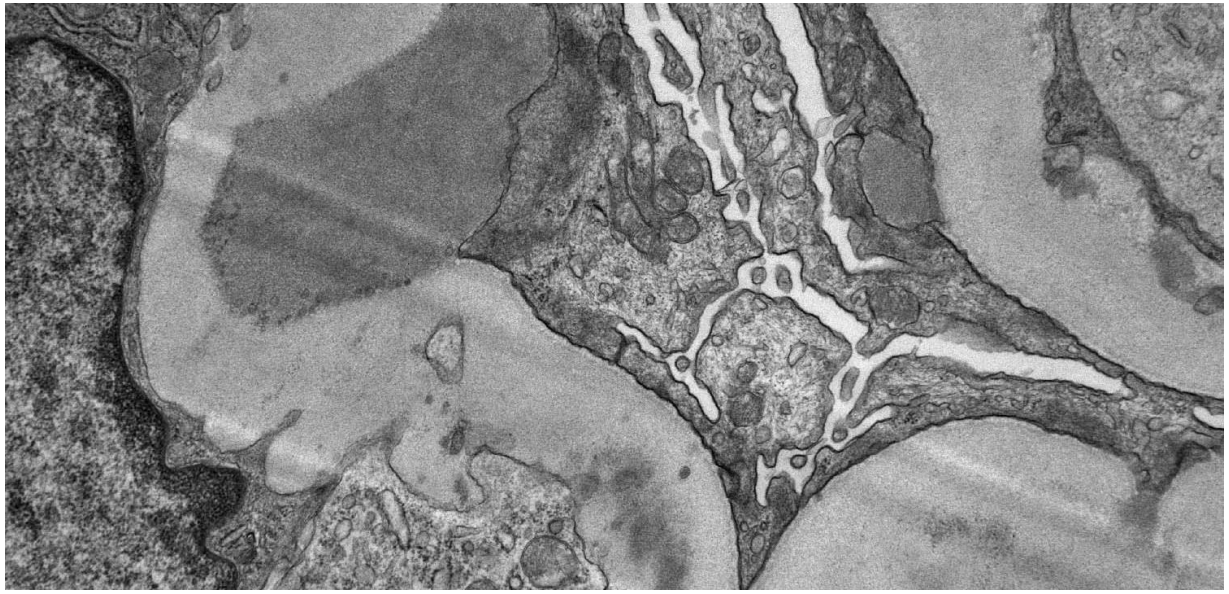
Antigens:
NAPlr: nephritis associated plasmin receptor
SPeB: Streptococcus pyrogenic exotoxin B

Balasubramanian & Marks. *Ped Int Child Health* (2017)









Subepithelial deposit

Subepithelial deposit

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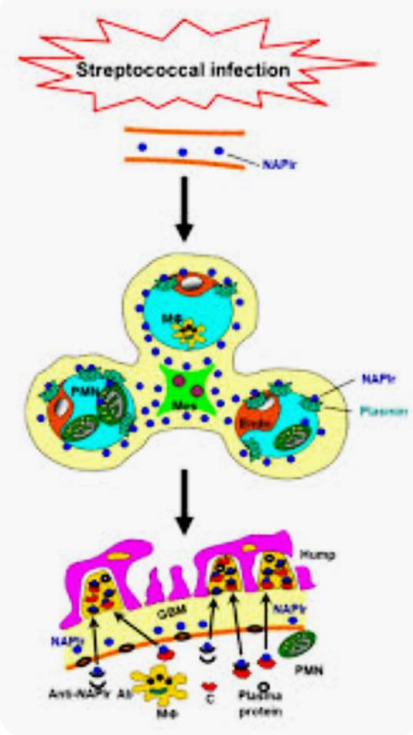


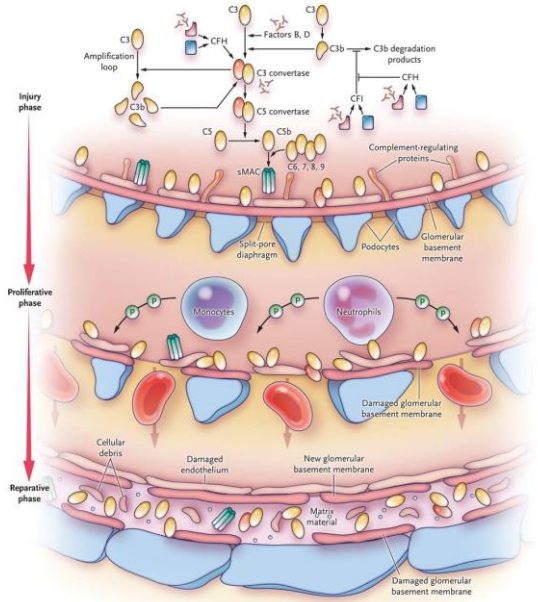
Antigens:
NAPlr: nephritis associated plasmin receptor
SPeB: Streptococcus pyrogenic exotoxin B

Balasubramanian & Marks. *Ped Int Child Health* (2017)

Pathophysiology

C3GN

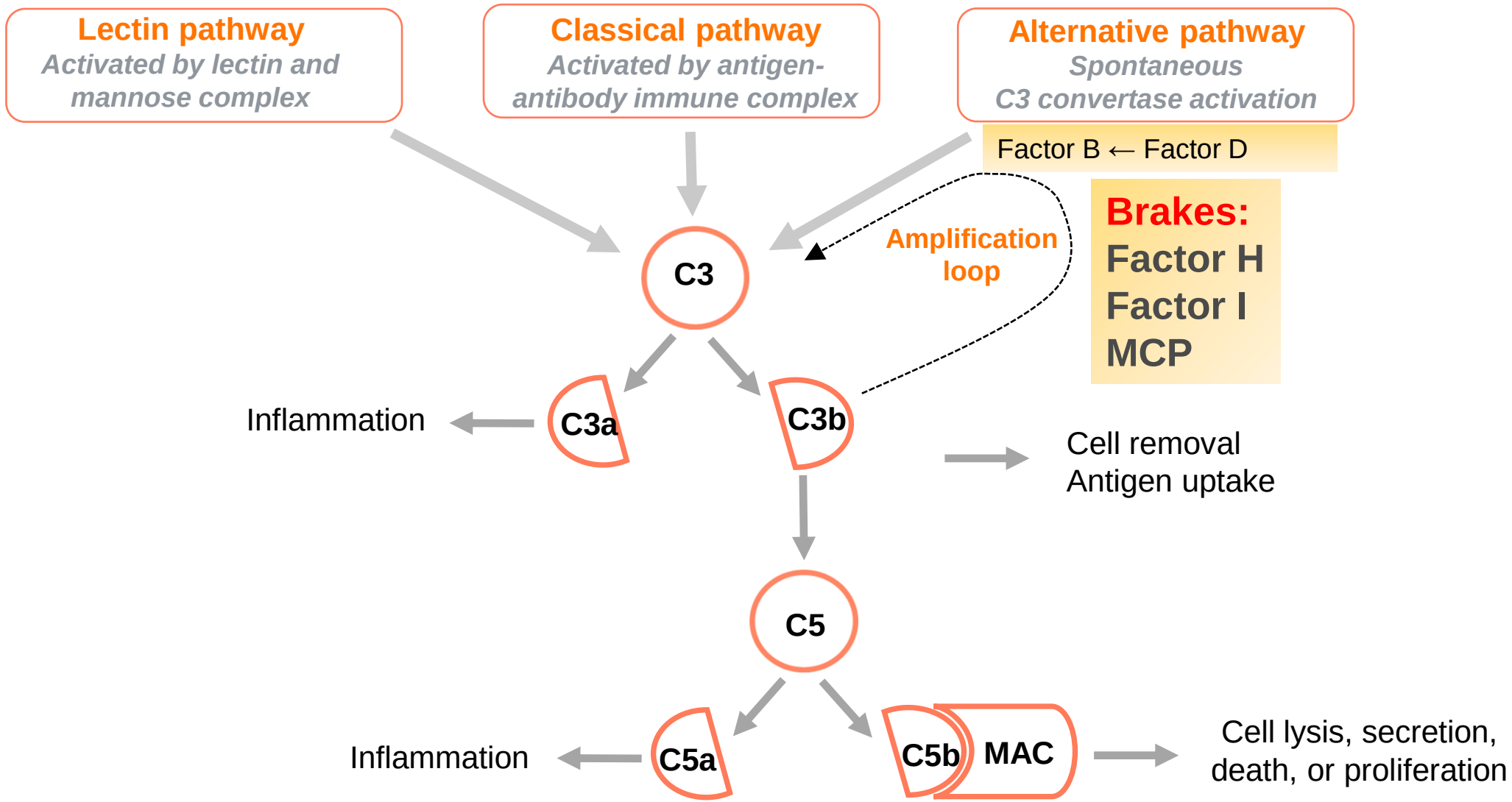
Initiation: deposition of **alternative and terminal pathway complement factors**
Proliferation: leukocyte influx
1ary complemente activation
Repair: formation of new basal membrane resulting in double contours of capillary walls



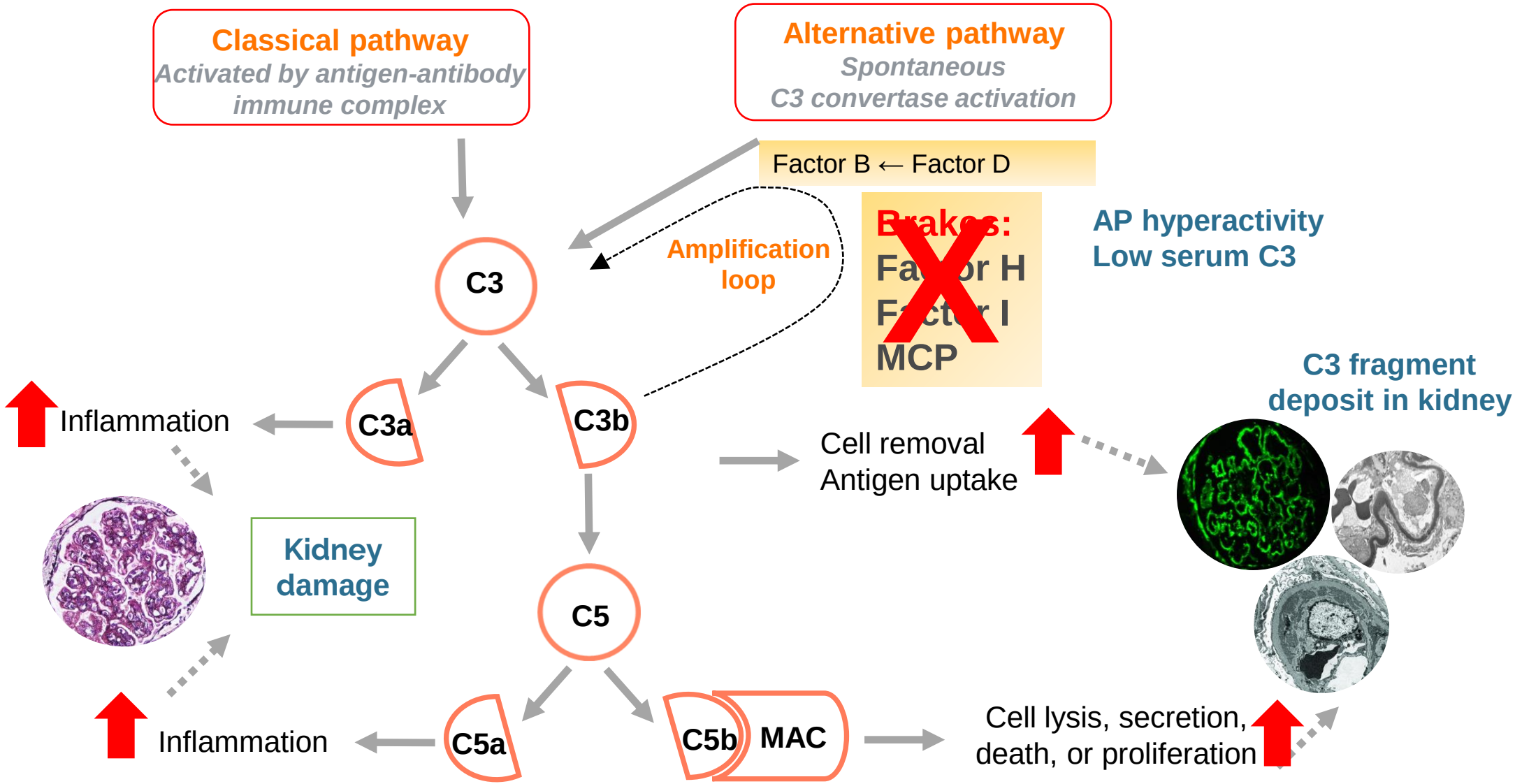
IF:
C3 > 2x immunoglobuline
(C4d negative in glomerular capillaries)

Sethi S & Fervenza F. *N Engl J Med* (2012): 366

The Complement System



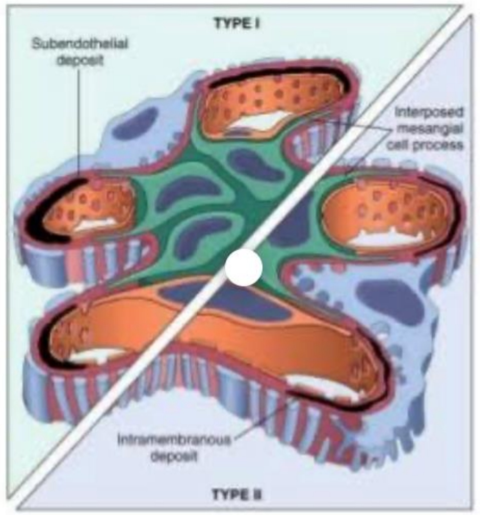
The Complement System in C3G



MPGN Type I
Subendothelial deposits
West et al, J Pediatr 1965

MPGN Type II / DDD
Intramembranous deposits
Galle, Thesis 1962; Habib et al, Kidney Int 1975

MPGN Type III
Subendothelial and subepithelial deposits
Burkholder et al, Am J Pathol 1969
Anders et al, Virchows Arch A Pathol Anat Histol 1997
Strife et al, Clin Nephrol 1984



B

Change in classification of MPGN on 2012 based on IF* and not on EM**

N Engl J Med 2012;366:1119-31.
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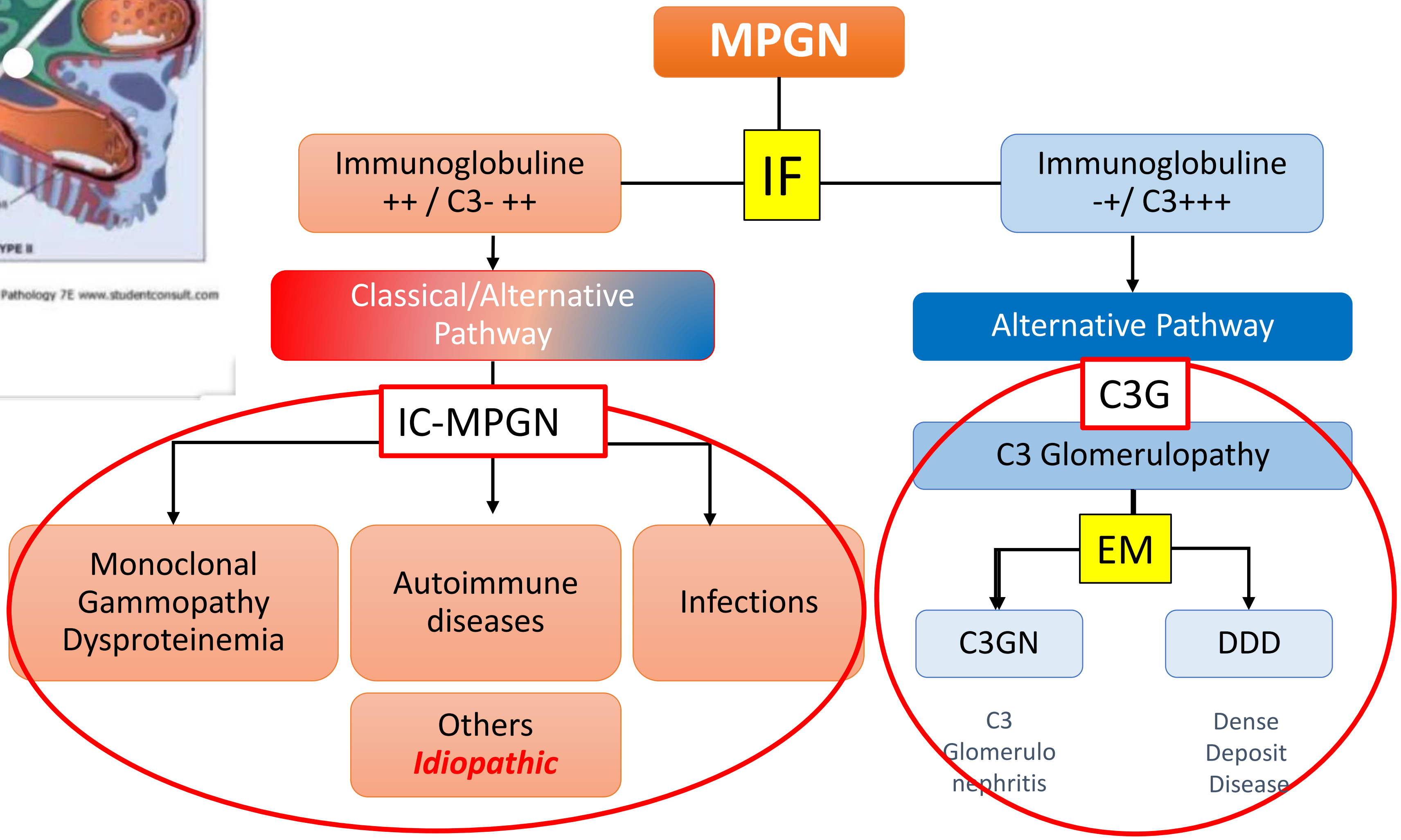
Membranoproliferative Glomerulonephritis — A New Look at an Old Entity

Sanjeev Sethi, M.D., Ph.D., and Fernando C. Fervenza, M.D., Ph.D.



**IF: immunofluorescence*
***EM: electronic microscopy*

Adapted from Sethi S *et al. Kidney Int* (2012) 81, 434-441

Proteinuria, haematuria, other markers of glomerulonephritis, ± renal insufficiency (hypertension ± nephrotic syndrome)

Investigations

- Laboratory tests: basic metabolic panel, CBC, C3, C4, ANA, ANCA, UPC, ± hepatitis screen and other tests if indicated
- Biopsy if persistent proteinuria >500mg in 24h, reduced GFR and/or unexplained hypertension with haematuria

Biopsy-confirmed C3 dominant glomerulonephritis

Diagnosis of C3 glomerulopathy

Assess complement levels and activity

- C3, C3c, Bb, C4, nephritic factors (including stabilizing capacity), soluble C5b-9, properdin, factor H, CH50 and APH50
- A complete biomarker profile including genotyping (recommended before transplantation) and additional complement biomarkers and autoantibodies

Treat according to disease activity

- **Normal renal function and proteinuria <0.5g /24h**: supportive care
- **Proteinuria 0.5–2g/24h, moderate inflammation on biopsy, or recent rise in SCr**: MMF and prednisone (tapered)
- **Proteinuria >2g in 24h, severe inflammation on biopsy, or progressive renal insufficiency on MMF**: add pulse methylprednisolone and consider anti-complement treatment

Rule out secondary forms of C3 deposition

- **Classic PIGN is ruled out by resolution of glomerulonephritis and/or normalization of C3 within 12 weeks**
- Evaluate patients aged >50 years for paraproteinassociated disease: urine and blood protein electrophoresis, urine and blood immunofixation ± bone marrow biopsy

Adapted from Smith R et al.*Nat Review Nephrol.* 2018

Proteomics of Complement Proteins in C3 Glomerulonephritis and Post-infectious Glomerulonephritis

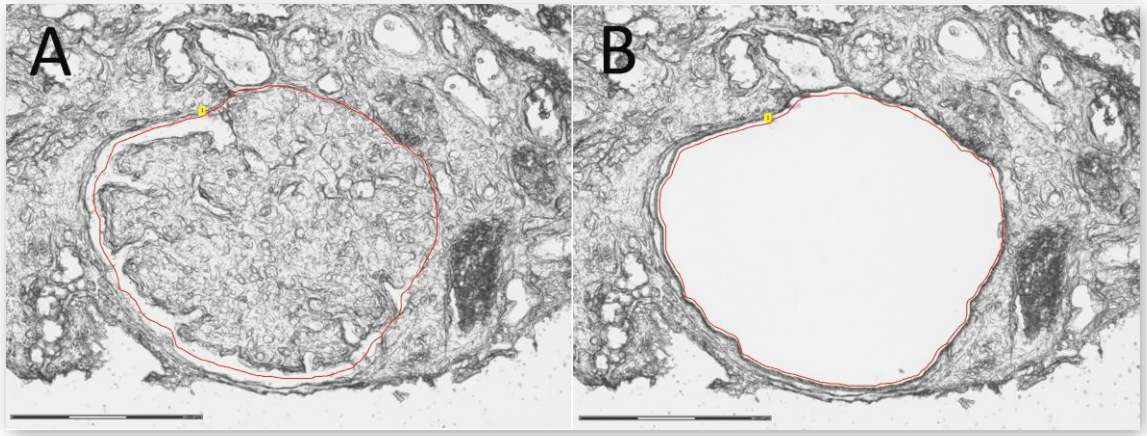
Lilian M.P. Palma[1], Jason D. Theis[2], Maria Izabel N. de Holanda[3],

Fernando C. Fervenza[2], Sanjeev Sethi[2]

[1]Universidade Estadual de Campinas (UNICAMP)
[2]Mayo Clinic Rochester, MN, USA
[3]Hospital Federal de Bonsucesso, RJ

Methods (MS/MS)

Laser Microdissection (MS) of Glomeruli



A: In the formalin-fixed paraffin sections of patients presenting different causes of Thrombotic Microangiopathy, 10 micra thick formalin-fixed paraffin sections were obtained and mounted on a special PEN membrane laser slide

B: glomeruli were microdissected using a Zeiss Palm Microbean microscope the to reach approximately 250-500,000 mM^2 per case

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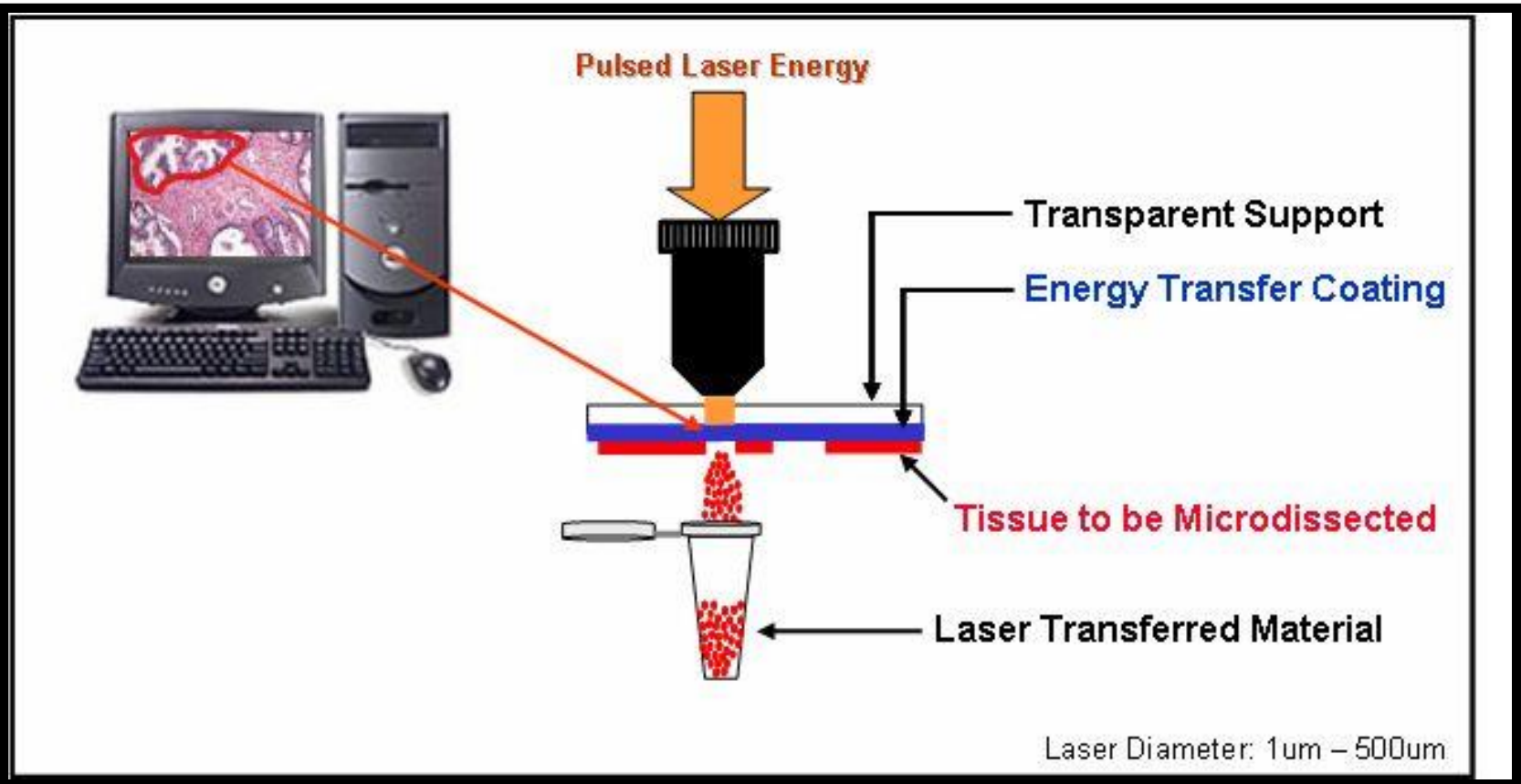
Laser micro dissection and mass spectrometry



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Microdissection

Trypsin digestion

TOT: 3-4 days

HPLC → ESI → Tandem MS/MS

High Pressure Liquid Chromatography

Electro Spray Ionization

Data analysis

- All samples were analyzed using Mascot and X! Tandem set up to search a Swissprot human database
- Scaffold (version 4.8.3, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications

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Adequate sample size

	final vol ul	lab code	date cut	# elements (gloms)	cut area square microns
1	41	TMA06	02/10/20	28	544803
2	42	TMA07	02/10/20	25	557775
3	42	TMA08	02/10/20	55	441495
4	40	TMA09	02/12/20	12	274422
5	33+9	TMA10	02/12/20	15	254066
6	40	TMA11	02/12/20	22	487985
7	40	TMA12	02/13/20	21	531776
8	41	TMA13	02/13/20	21	223899
9	40	TMA14	02/13/20	31	529639
10	42	TMA15	02/13/20	20	251106
11	41	TMA16	02/13/20	12	302852
12	41	TMA17	02/13/20	30	534455

Adequate Sample Size

10 – 30 glomeruli

250,000 – 500,000 square micra

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Total Spectral Count (TSC) – Quantitative Method

Probability Legend:

- over 95%
- 80% to 94%
- 50% to 79%
- 20% to 49%
- 0% to 19%

Peptide Prophet algorithm

#	Starred	Bio View: Identified Proteins (15/219) Including 0 Decoys	Case 10	Case 11	Case 12	Case 13	Case 14	Case 15	Case 16	Case 17	Case 18
1	☆	Complement C3	101	37	198	155	42	132	162	24	315
2	☆	Complement C4-A						64		9	
3	☆	Complement C4-B		5	6	2	2	76	1	8	5
4	☆	Complement C5	9		22	2		2	15		73
5	☆	Complement component C6	11	7	17	12	10		11		37
6	☆	Complement component C7	2		1				8		32
7	☆	Complement component C8 alpha chain					1		4		25
8	☆	Complement component C8 beta chain	14		15	4			3		25
9	☆	Complement component C8 gamma chain	3								10
10	☆	Complement component C9	22	20	29	33	15	6	27	4	67
11	☆	Complement factor H		2	22	10	6	5	32	1	50
12	☆	Complement factor H-related protein 1			20		14	7	72	5	103
13	☆	Complement factor H-related protein 2							22		47
14	☆	Complement factor H-related protein 3			12						2
15	☆	Complement factor H-related protein 5	1		13	9	11		22	8	70

Complement deposition based on TSC

- > 50: very high 4+
- 16-50: high 3+
- 6-15: moderate 2+
- 2-5: low 1+
- 0-1: baseline

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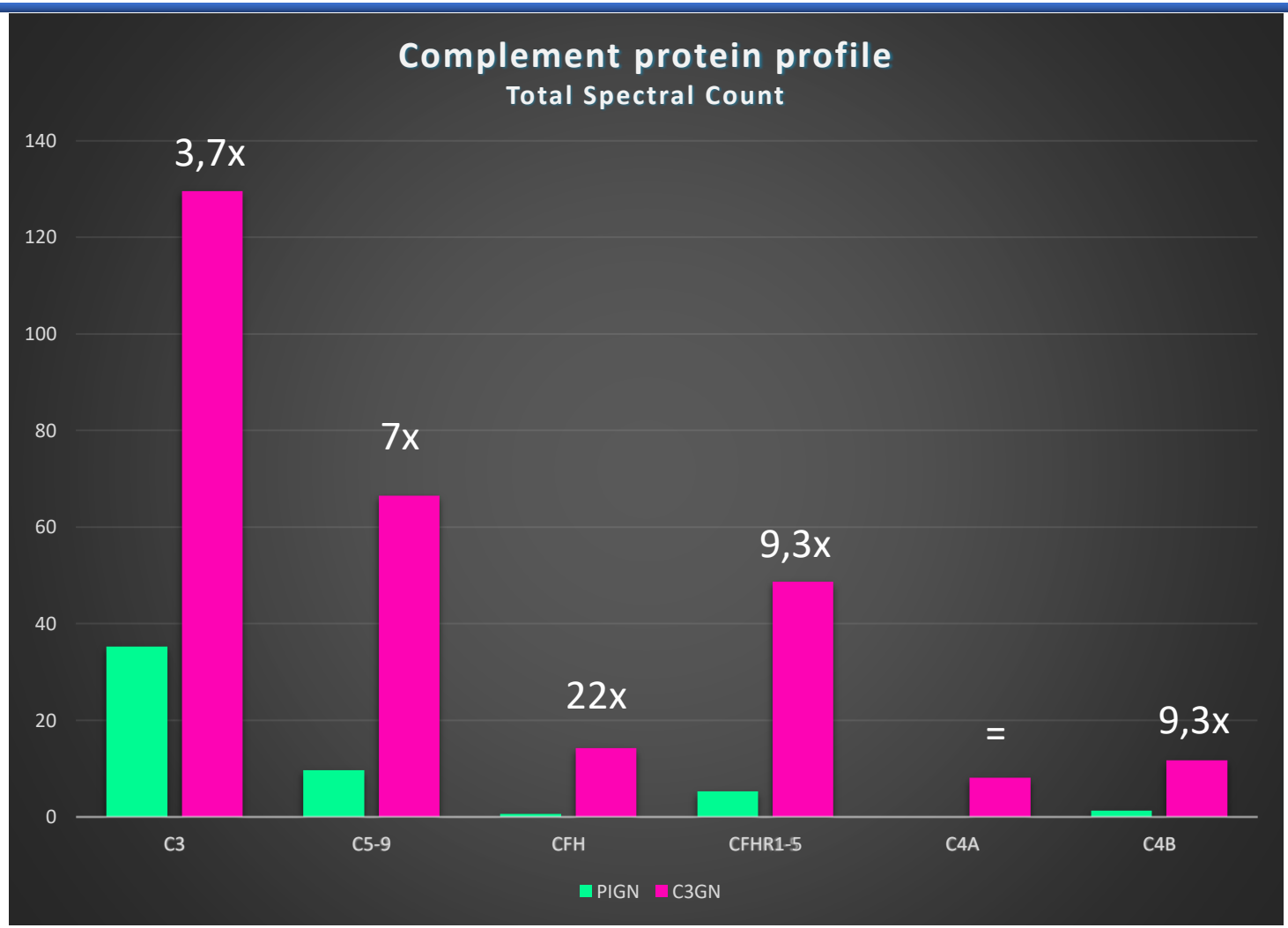
TSC of alternative and terminal complement pathway proteins are much higher in C3GN compared to PIGN



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Conclusion & D

- These studies show t lement pathway proteins is much high greater role for anti-complement therapy
- By including more ca Count (TSC) to define patients who would b e therapies
- Laser Microdissectio le and affordable provided there is a tr

Complement and Kidney

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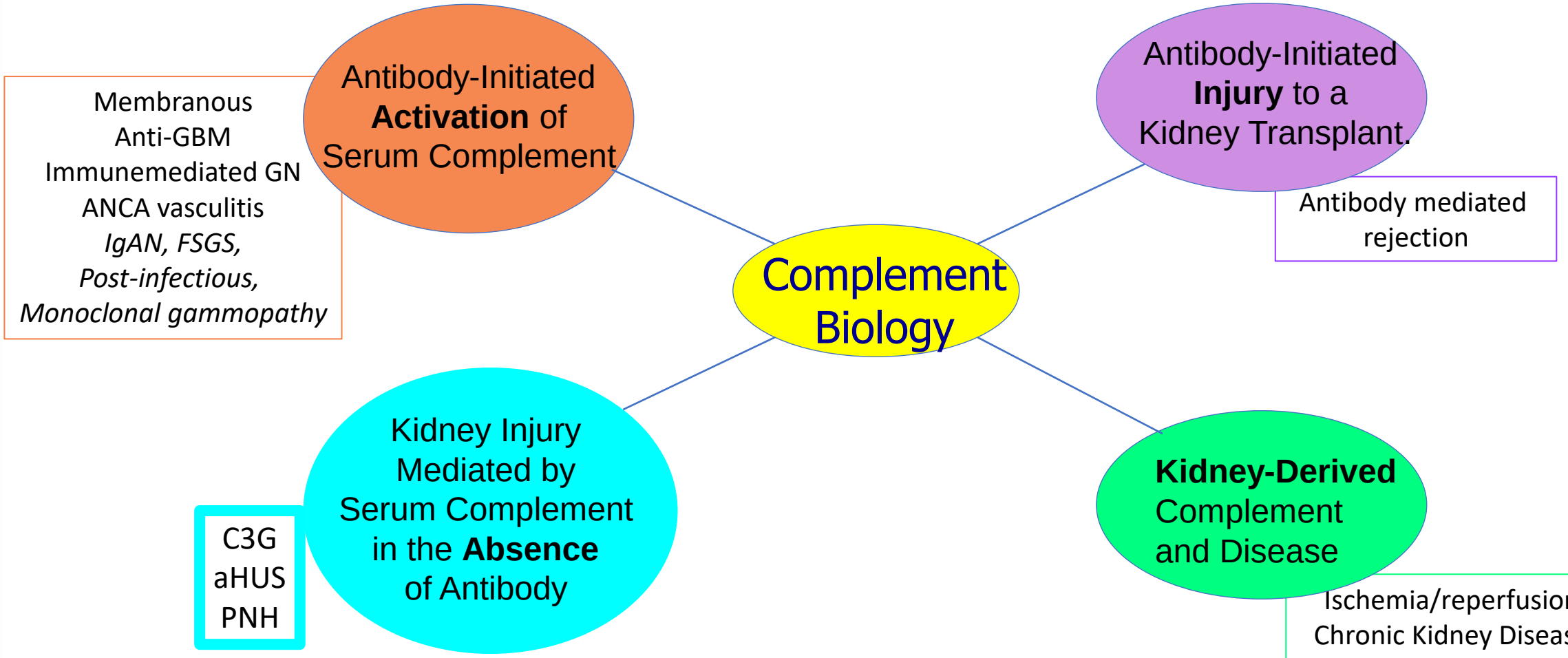


Mathern & Heeger. *Complement and Kidney. CJASN 2015, 10: 1636*

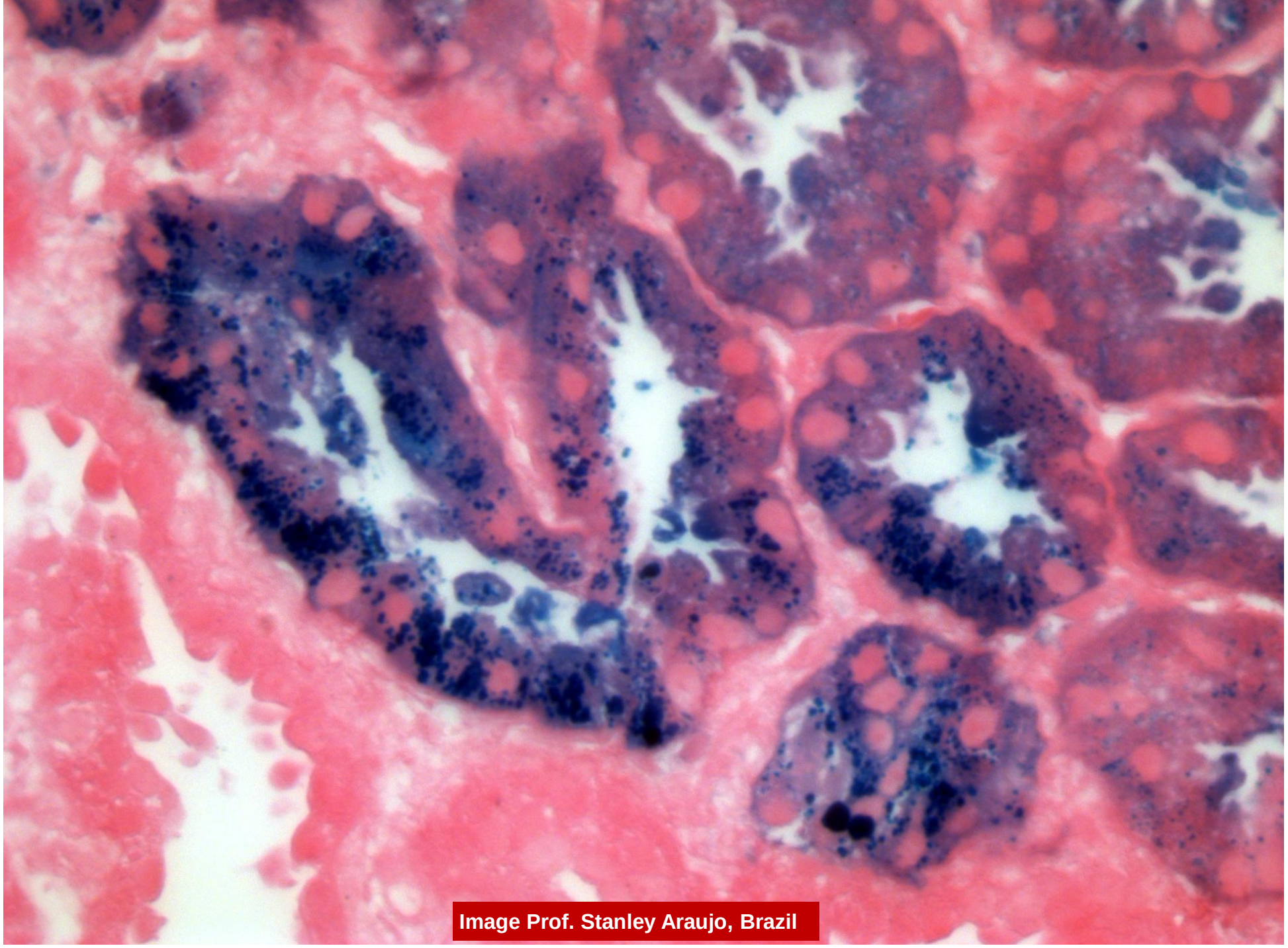
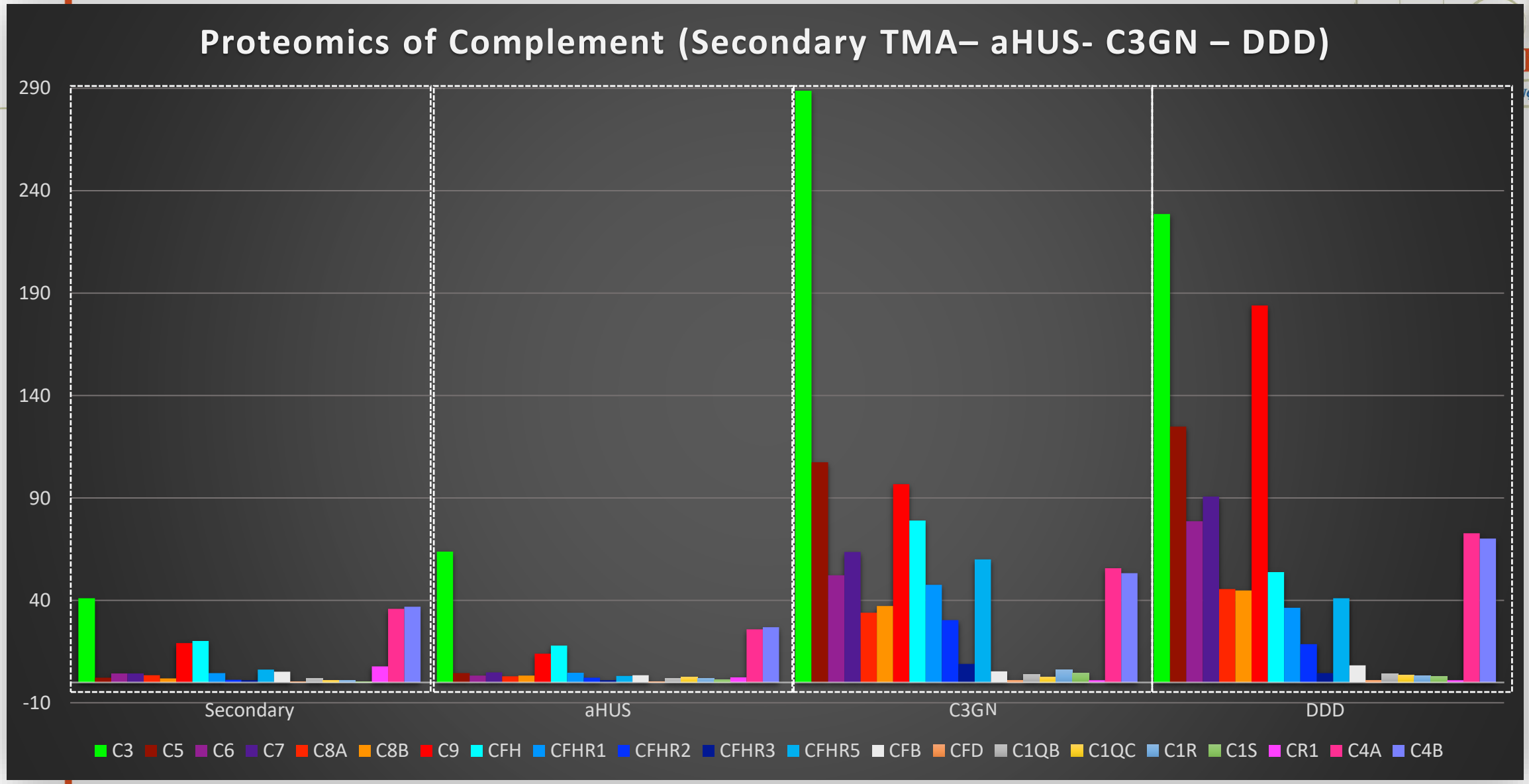


Image Prof. Stanley Araujo, Brazil



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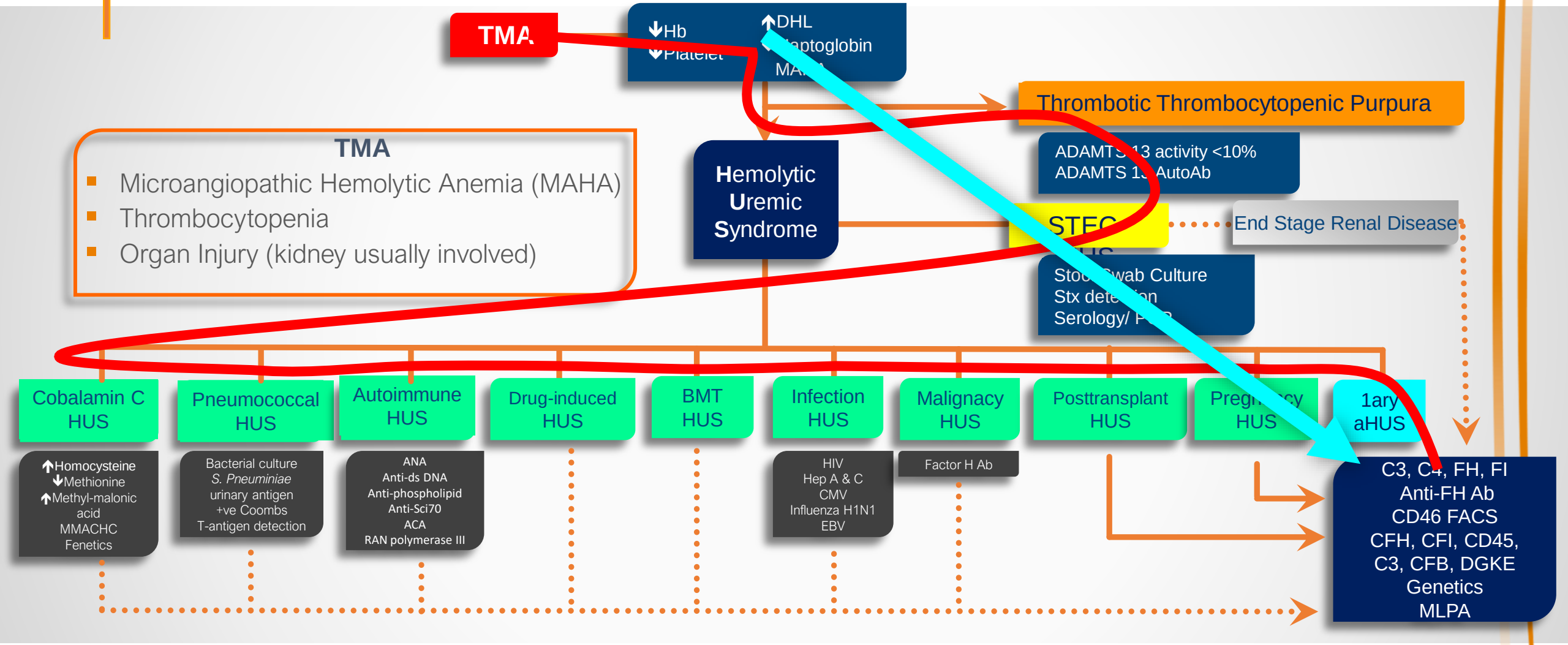
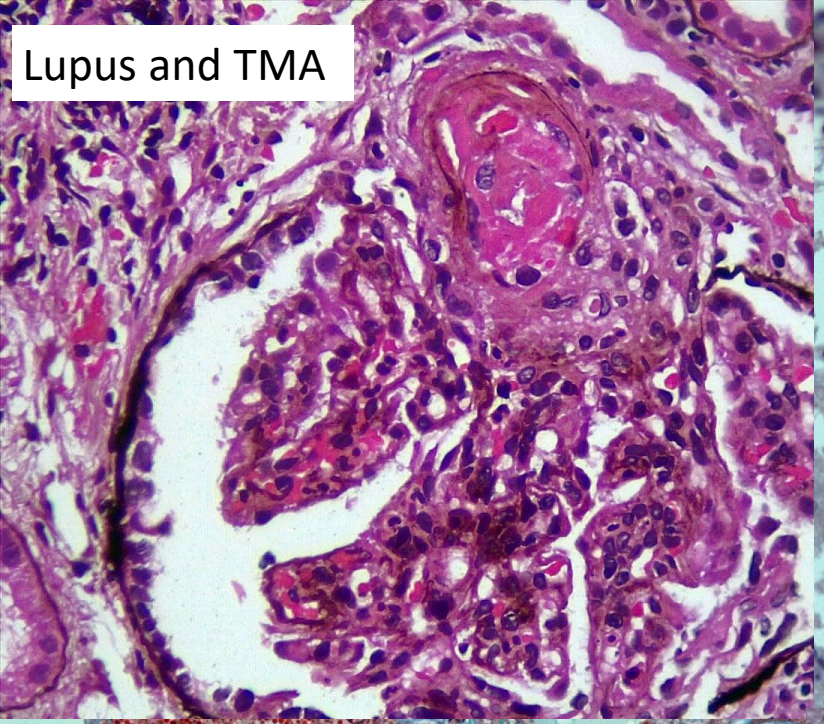
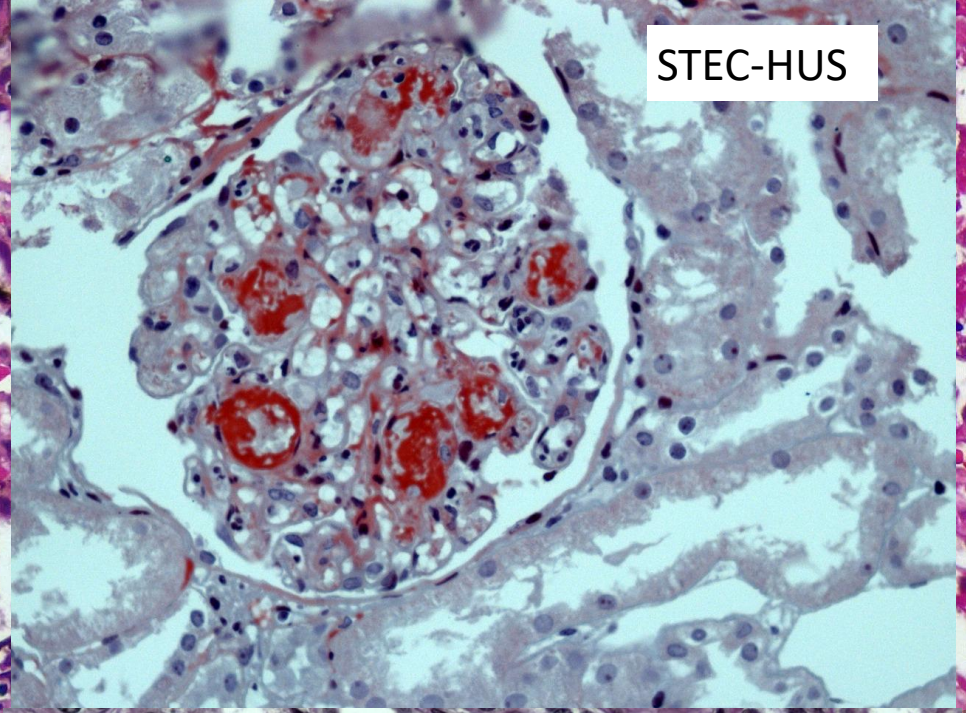
Unpublished data

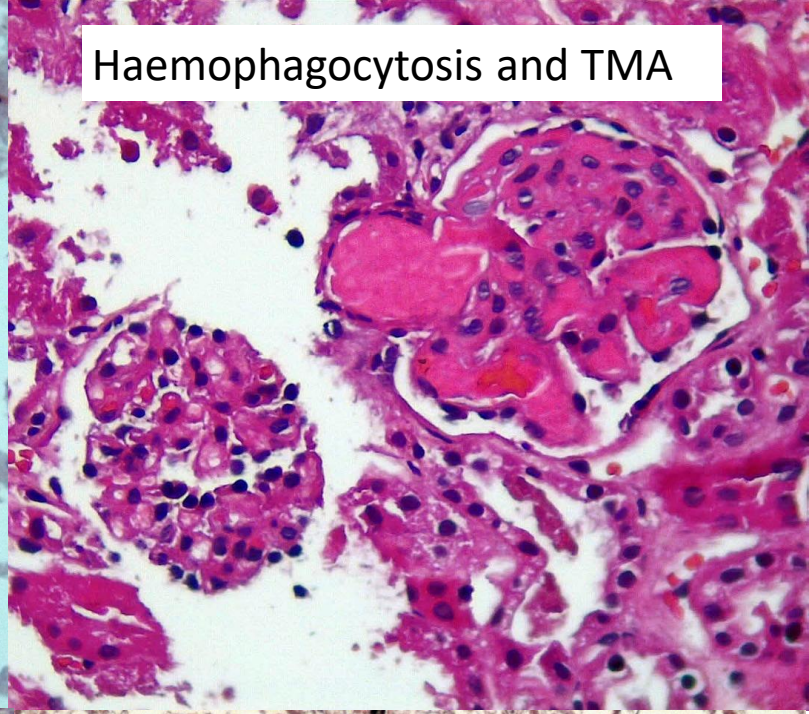
The Thrombotic Microangiopathy (TMA) Spectrum

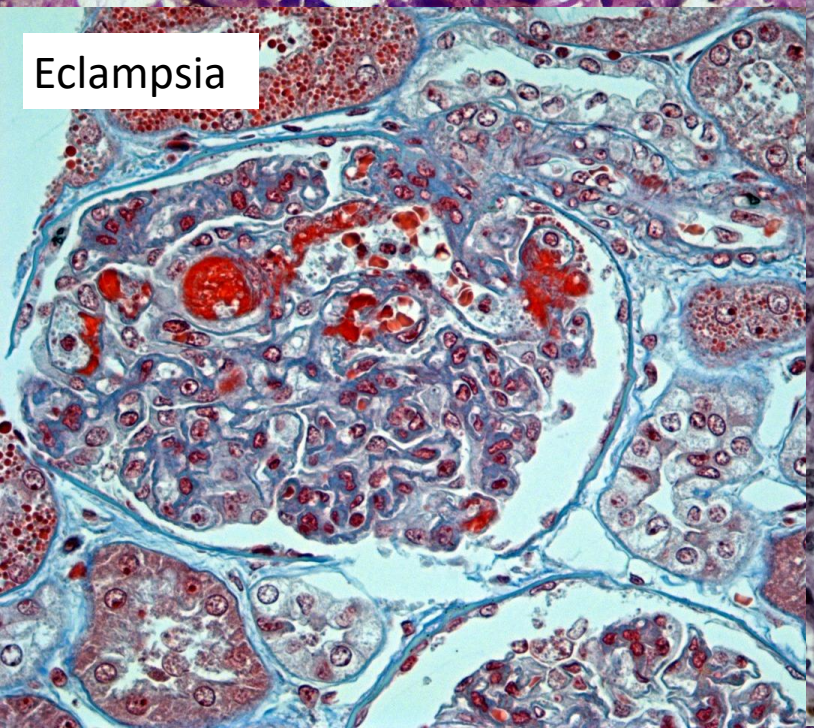


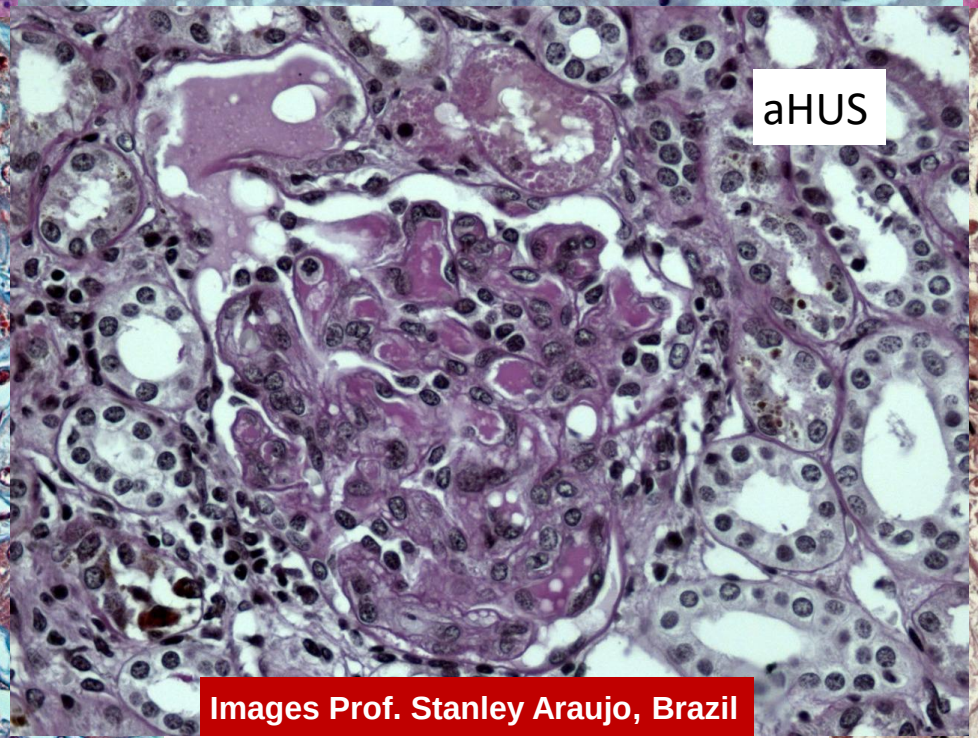
Modified from: Goodship et al, *Kidney Int,* 2017 (KDIGO)

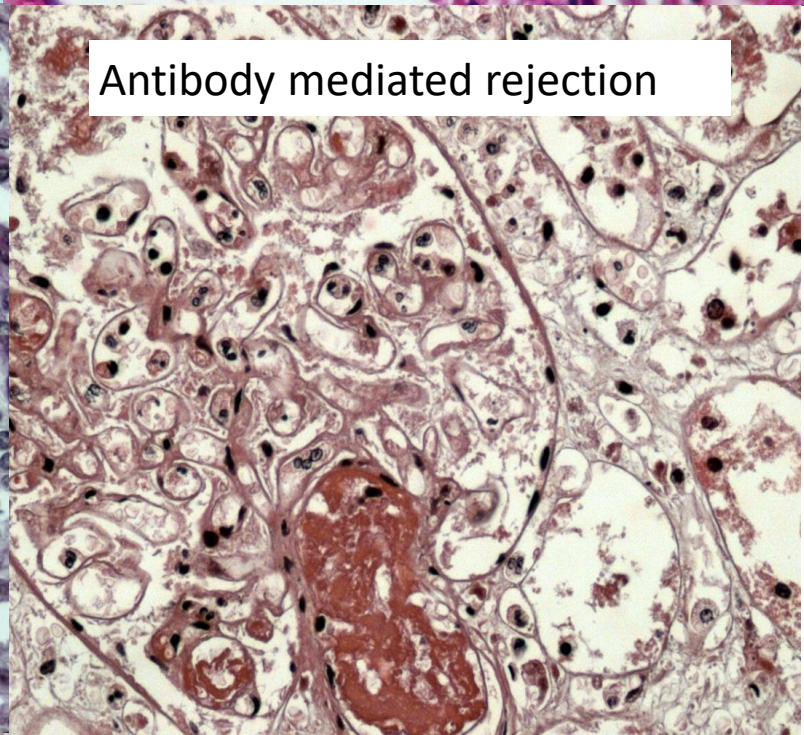

Lupus and TMA


STEC-HUS


Haemophagocytosis and TMA

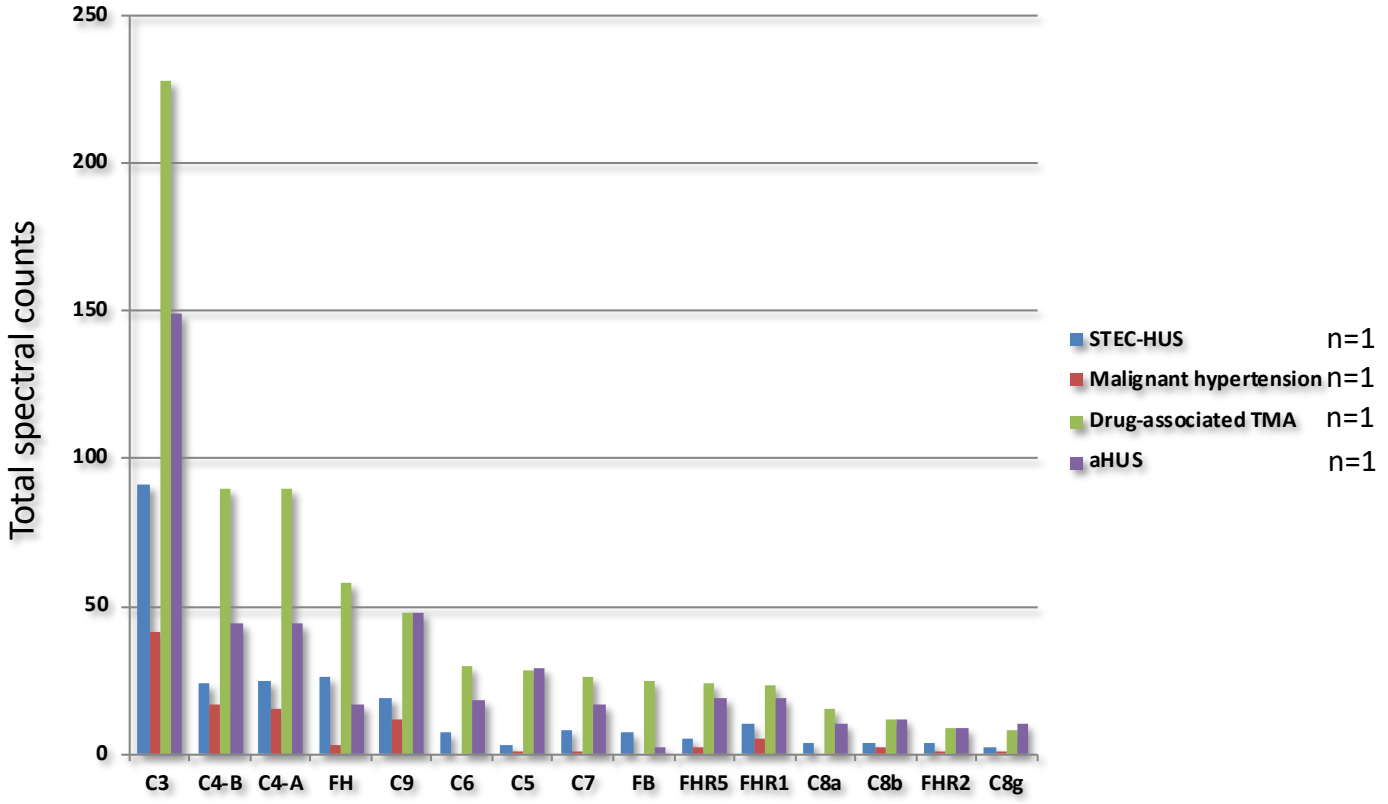

Eclampsia


aHUS
Images Prof. Stanley Araujo, Brazil


Antibody mediated rejection

Different Causes of TMA

Mass spectrometry of complement proteins in kidney biopsies according to the cause of TMA



Legend: STEC-HUS: shigatoxin related hemolytic uremic syndrome; TMA: thrombotic microangiopathy; aHUS: atypical hemolytic uremic syndrome, C: complement protein; FHR: Factor H related protein

Protein category	Proteins	aHUS (n=12)	Secondary TMA (n=12)
C3	C3	63.75	53
Terminal	C5/6/7/8A/8B/9	32.9	50.1
CRP*	CFH/CFHR1-2-3-5/CFB/CFD	32.9	48.25
Classical	C1QB/C1QC/C1R/C1S/CR1/ C4A/C4B	63	97
Total		192.55	248.35

CRP*: complement regulatory proteins

Protein category	Hypertension (n=5)	Autoimmune (n=3)	Drug (n=4)
C3	42.6	52	67
Terminal	56	46	46
CRP*	36.1	47.6	63.75
Classical	85.2	72.3	130.2
Total	219.9	217	306.9

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Proteomics of Complement in Thrombotic Microangiopathy

Lilian Monteiro P. Palma MD[1], Meera Sridharan, MD[2], Benjamin Madden BA[3], M. Cristine Charlesworth PhD[3], Sanjeev Sethi MD PhD[4]

[1]Pediatric Nephrology, State University of Campinas (UNICAMP), Brazil
[2]Hematology, Department of Internal Medicine, Mayo Clinic, Rochester, MN
[3]Medical Genome Facility, Proteomics Core, Mayo Clinic, Rochester, MNDivision of
[4]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN

Conclusions

- Complement prote … among the ~2000 proteins identified
- Terminal compleme … may likely contribute to glome
- The identification o … and/or lectin pathway
- The burden of com … ifferent according to TMA cause (higher
- Limitations: vessels

Cluster Analysis Identifies Distinct Pathogenetic Patterns in C3 Glomerulopathies/Immune Complex–Mediated Membranoproliferative GN

178 pts

- 68 C3GN
- 25 DDD
- 80 IC-MPGN

34 variables

- 7 clinical
- 17 pathology
- 10 complement and genetics

Cluster 1 | Cluster 2 | Cluster 3

Fluid phase complement activation

↓ C3
↑Genetic & acquired AP abnormalities

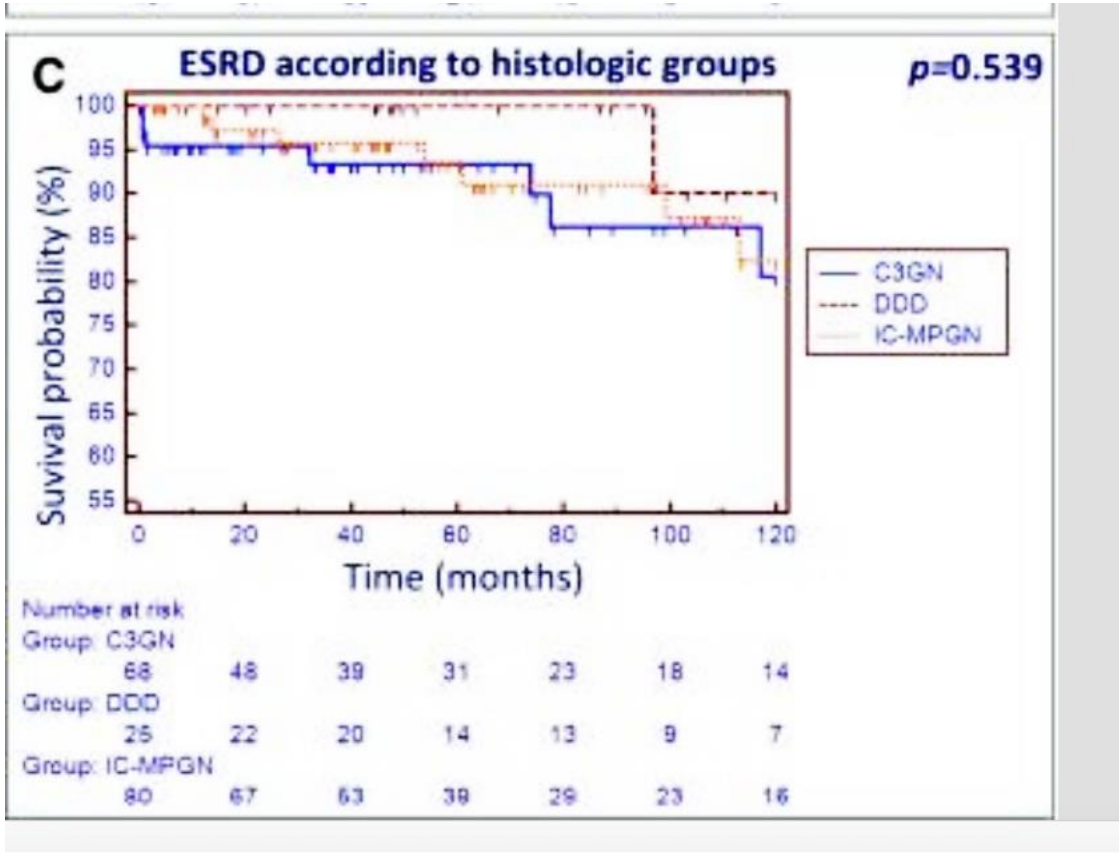
Cluster 4

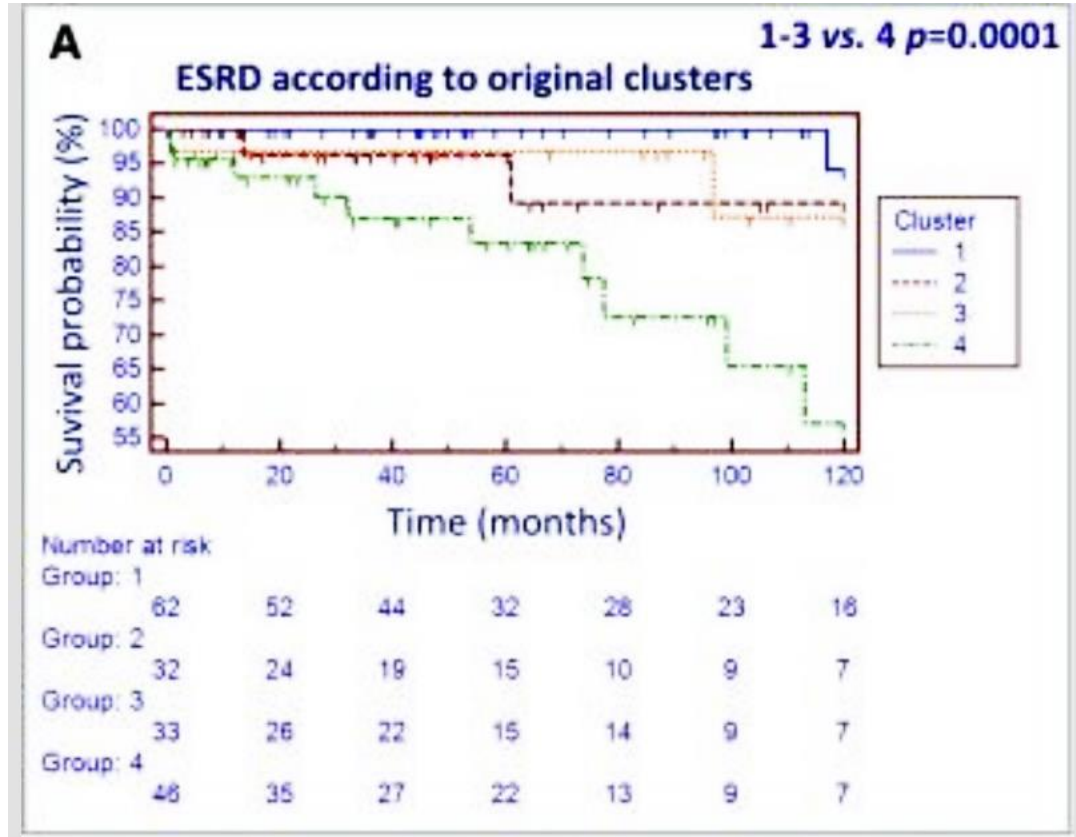
Solid phase complement activation

Normal C3
Negative AP abnormalities
More TMA

Iatropoulos P et al. *J Am Soc Nephrol*, 2018

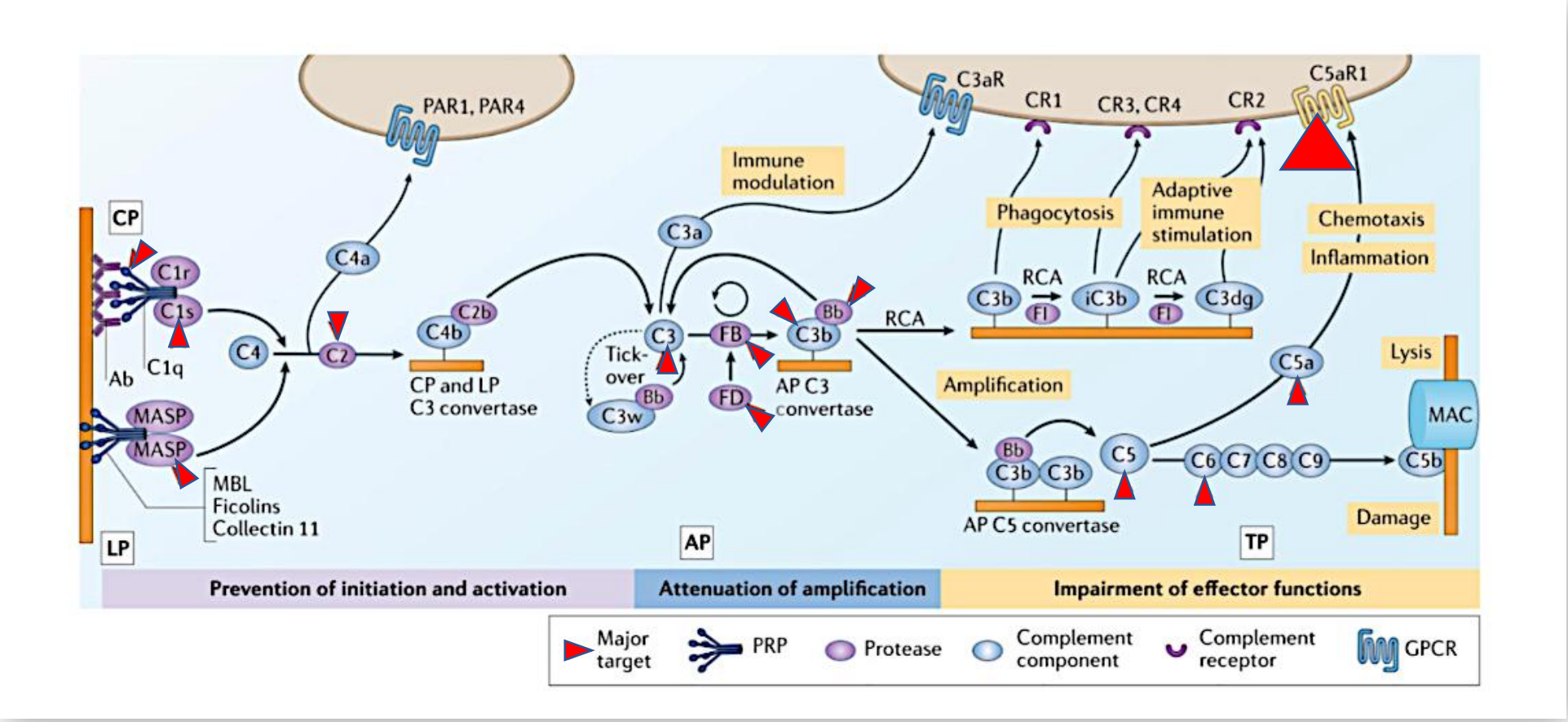
Cluster Analysis Identifies Distinct Pathogenetic Patterns in C3 Glomerulopathies/Immune Complex–Mediated Membranoproliferative GN





Iatropoulos P et al. *J Am Soc Nephrol*, 2018

The Renaissance of Complement Therapeutics



Ricklin D et al. *Nat Review Nephrol.* 2018;14 (1):26-47

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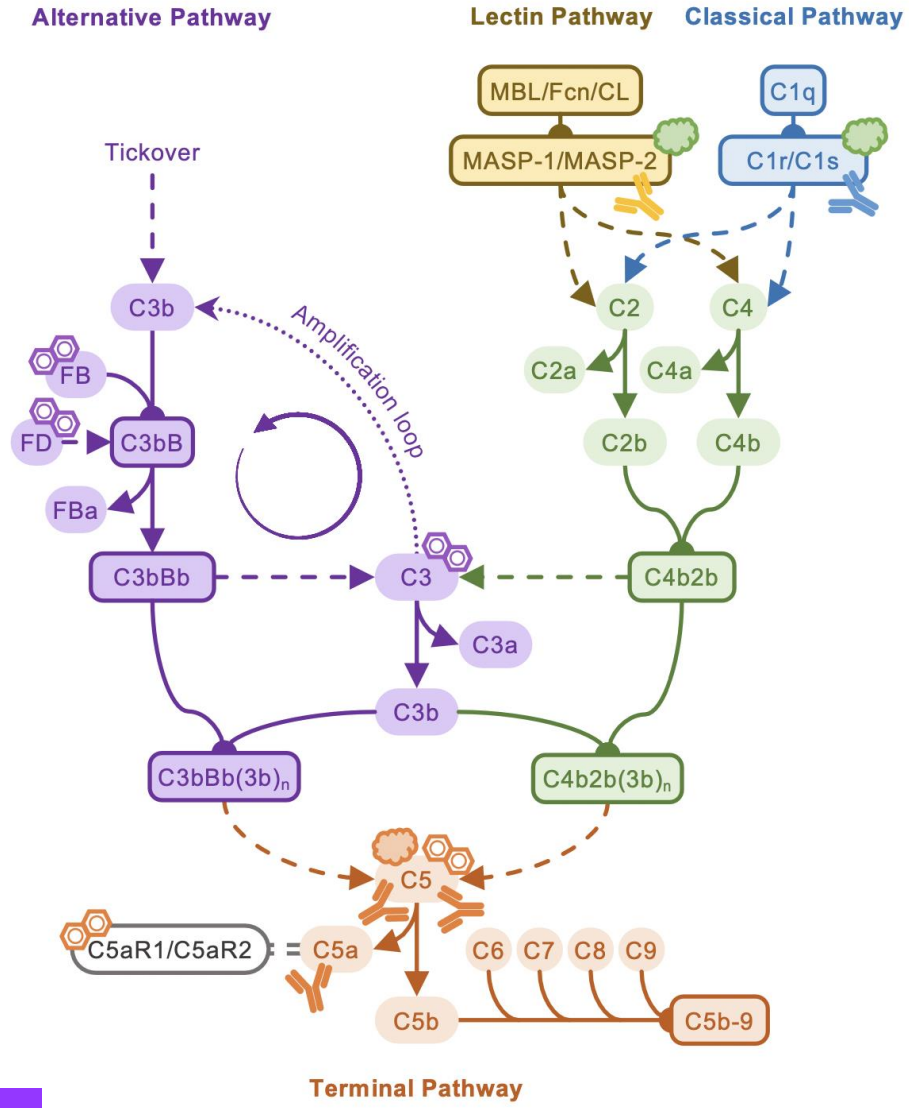


Initiation Inhibitors

- **Narsoplimab** (anti-MASP2)
- **Sutimlimab** (anti-C1s)
- **C1 esterase inhibitors**

Amplification Inhibitors

- **Pegcetacoplan** (C3 inhibitor)
- **Iptacopan** (FB inhibitor)
- **Danicopan** (FD inhibitor)

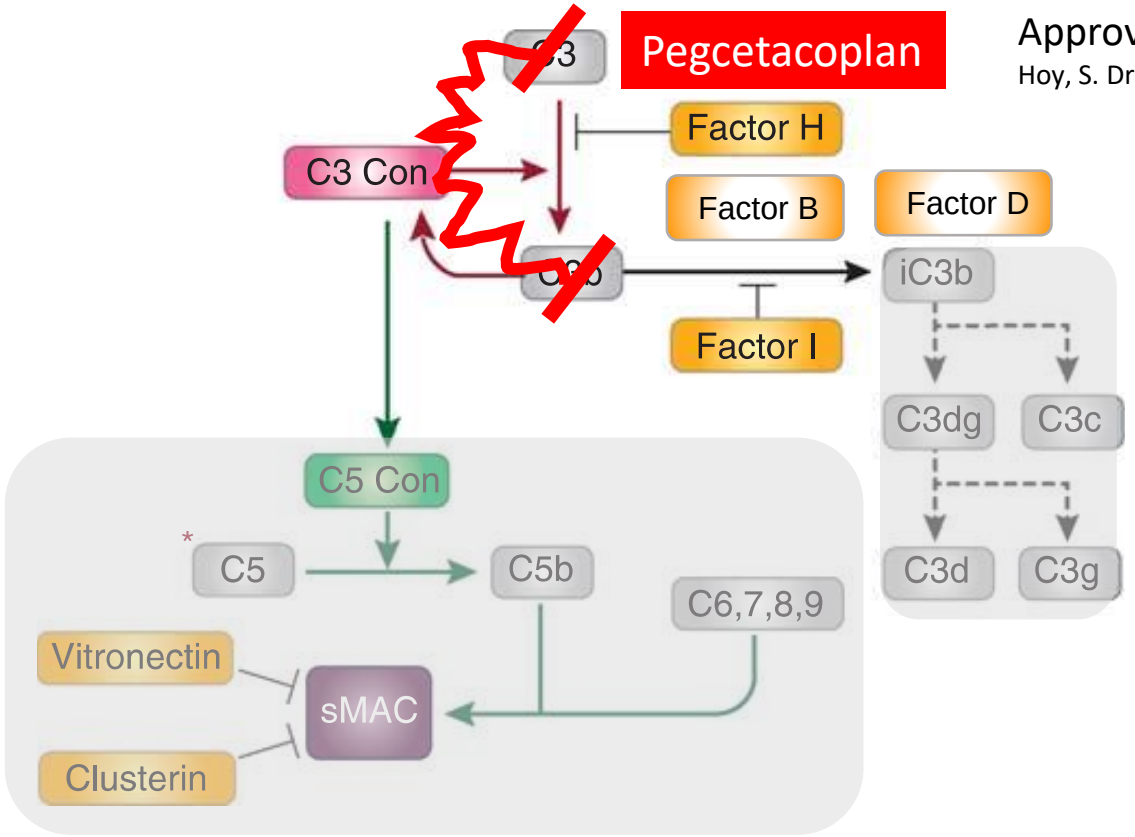
Effector Inhibitors

- **Eculizumab** (anti-C5)
- **Ravulizumab** (anti-C5)
- **Crovalimab** (anti-C5)
- **Nomacopan** (C5 inhibitor)
- **Zilucoplan** (C5 inhibitor)
- **Zimura** (C5 inhibitor)
- **Vilobelimab** (anti-C5a)
- **Avacopan** (C5aR1 antagonist)

Cortesia Dra. Silvana Miranda

ClinicalTrials.gov Search Results 09/21/2022
Richard B.P., Daniel R. Seminars in Immunopathology, 2021

C3 glomerulopathies (C3G) are caused by dysregulation of the Alternative Pathway (AP) and Terminal Complement Complex (TCC)



Approved in USA for patients with PNH

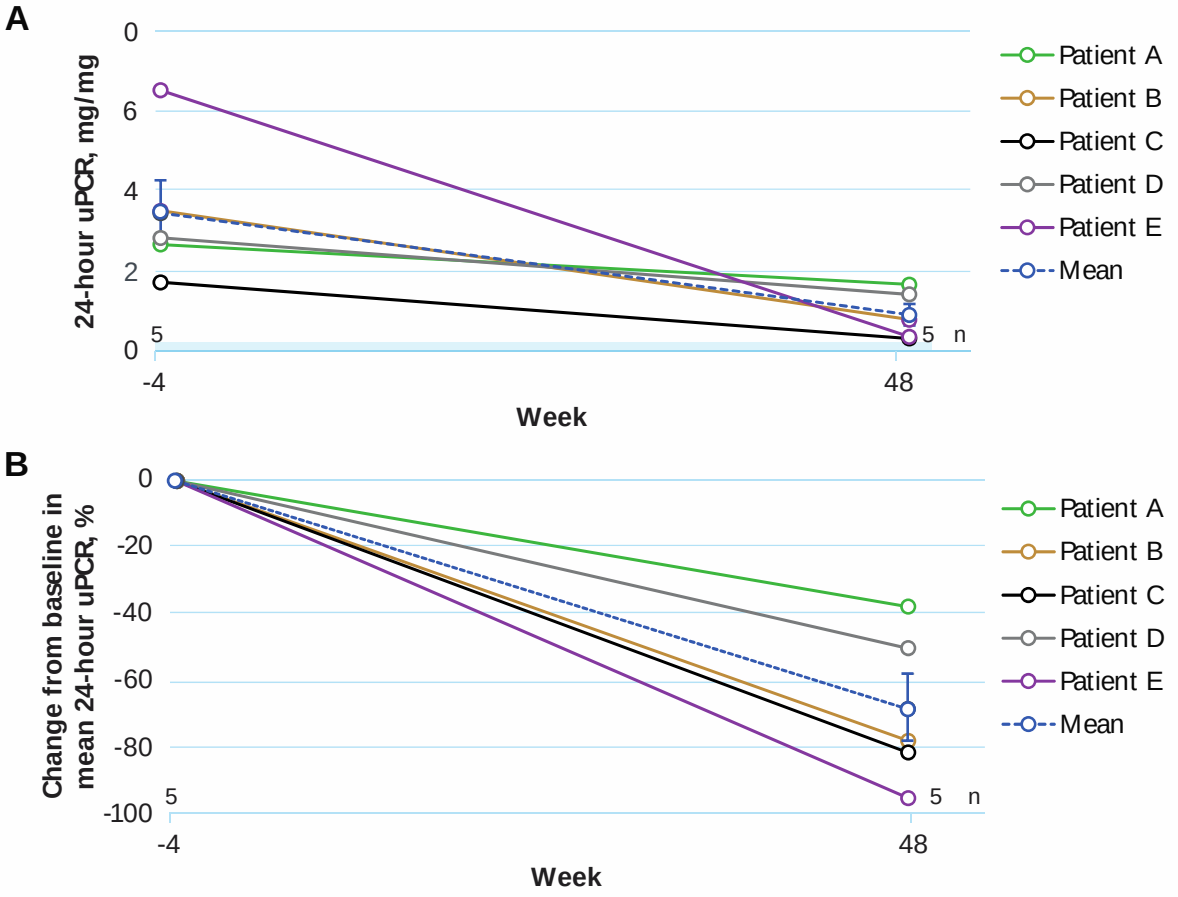
Hoy, S. Drugs. 2021 Aug;81(12):1423-1430

Adapted from Sethi S et al. *Kidney Int* (2012) 81, 434-441

C3 inhibition with pegcetacoplan targets the underlying disease process of C3 glomerulopathy.

PO1852

Figure 1. (A) Mean and individual 24-hour uPCR, **(B)** mean and individual percentage change from baseline in 24-hour uPCR, and **(C)** mean serum albumin levels



[1]University of Colorado School of Medicine, Aurora, CO, USA; [2]Emory University, Atlanta, GA, USA; [3]Tidewater Kidney Specialists, Inc., Chesapeake, VA, USA; [4]University of Tennessee Health Science Center, Memphis, TN, USA; [5]Eastern Nephrology Associates, Wilmington, NC, USA; [6]Ex-Equals, LLC, Sausalito, CA, USA; [7]Apellis Pharmaceuticals, Inc, Waltham, MA, USA

Safety and tolerability

Table 3. Complement levels through week 48

Biomarker, mean (SD), [range][a]	Baseline[b]	Week 48
Serum C3, mg/mL	61.60 (20.42) [11.00, 116.00]	252.00 (52.82) [82.00, 407.00]
Serum C4, mg/dL	19.20 (4.22) [5.00, 31.00]	17.75 (2.17) [14.00, 22.00]
CH50, U/mL	183.40 (53.17) [23.00, 298.00]	214.00 (12.52) [190.00, 248.00]
AH50, U/mL	62.00 (25.59) [0.00, 113.00]	60.75 (22.40) [0.00, 96.00]
C5b-9, ng/mL	1113.50 (675.85) [79.00, 3009.00]	385.25 (328.89) [22.00, 1371.00]

SD, standard deviation.
[a]Normal ranges for each of the biomarkers: serum C3, 0.94-1.66 mg/mL; CH50, 176-382 U/mL; AH50, 77-159 U/mL; C5b-9, 72-244 ng/mL.
[b]Baseline was the most recent result prior to the first dose.

promising therapy for C3G and support further study of pegcetacoplan in patients with C3G

- Safety outcomes, including treatment emergent adverse events (TEAEs), were monitored throughout the study
- Mean serum C3 levels increased following administration of pegcetacoplan (Figure 3; Table 3)

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KIReports.org
CLINICAL RESEARCH

Overlap of C3 Glomerulopathy and Thrombotic Microangiopathy: A Case Series

Aishwarya Ravindran[1,2], Lilian Monteiro Pereira Palma[3], Fernando C. Fervenza[4] and Sanjeev Sethi[1]

[1]Division of Anatomic Pathology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA; [2]Division of Laboratory Medicine, Department of Pathology, University of Alabama at Birmingham, Birmingham, Alabama, USA; [3]Pediatric Nephrology, State University of Campinas (UNICAMP), Campinas, Brazil; and [4]Division of Nephrology and Hypertension, Mayo Clinic, Rochester, Minnesota, USA

March, 2023

KI REPORTS
KIReports.org
LETTER TO THE EDITOR

C3 Glomerulopathy and Thrombotic Microangiopathy: A "Hybrid" Phenotype

To the Editor: C3 glomerulopathy (C3G) with morphologic features of thrombotic microangiopathy (TMA) has recently been acknowledged; the phenotype resembles either C3G or TMA.[1] Here, we studied kidney biopsies from 73 consecutive patients with TMA who had been included in the Limburg Renal Registry for morphologic features of C3G; 1 of 73 patients (1%) with TMA presented with coexisting C3G. The patient, a 73-year-old woman with no relevant medical history, presented with mild hypertension, generalized edema, acute kidney injury (creatinine, 166 μmol/l), nephrotic-range proteinuria, and glomerular hematuria. Nonhemolytic normocytic anemia was present, with platelet count at 311,000/μl. Kidney biopsy showed a diffuse endocapillary proliferative glomerulonephritis, with mesangiolysis and segmental double contours (Figure 1a), and arteriolar thrombosis. C3c deposits, but no other immunoreactants, were found along segments of the glomerular basement membrane (Figure 1b). Marked widening of the glomerular basement membrane due to electron lucent fluff was present; electron-dense deposits were found along segments of the glomerular basement membrane (Figure 1c). Therefore, C3G with morphologic features of TMA was diagnosed. The autoimmune workup, including factor H autoantibodies and C3 nephritic factor, was unremarkable; C4 and C3 were 0.08 g/l (LLN, 0.10 g/l) and 0.51 g/l (LLN, 0.90 g/l), respectively. No monoclonal protein was detected. The patient was treated with prednisolone and mycophenolate mofetil (1000 mg BID). After 1 month, she presented with altered mental status, worsening kidney function (creatinine, 348 μmol/l), Coombs negative microangiopathic hemolytic anemia, and platelet count at 91,000/μl. ADAMTS13's enzymatic activity was 61%, and secondary etiologies[2,3] were excluded. Rare variants in complement genes (i.e., *CFH, CFI, CD46, CFB, C3, CFHR1-5, C2,* and *CFP*) were not found, but the patient carried the MCPggaac at-risk haplotype. Mycophenolate mofetil was switched to cyclophosphamide IV (500 mg fortnightly) and add-on eculizumab for 3 months, followed by azathioprine (150 mg OD). A hematologic remission and partial kidney remission were achieved within 1 and 4 weeks, respectively. The patient's kidney function improved (creatinine, 124 μmol/l; estimated glomerular filtration rate, 37.0 ml/min per 1.73 m^2), with proteinuria <1 g/d, during the 6-month follow-up period. Altogether, we demonstrate the

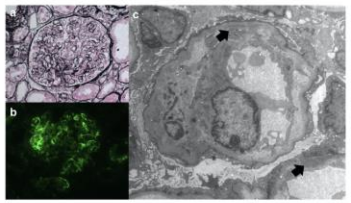


Figure 1. Light (a; Jones methenamine silver, ×400), immunofluorescence (b; C3c, ×400), and electron microscopic (c; ×4800) findings on kidney biopsy. The arrows point to electron-dense deposits.

March, 2023

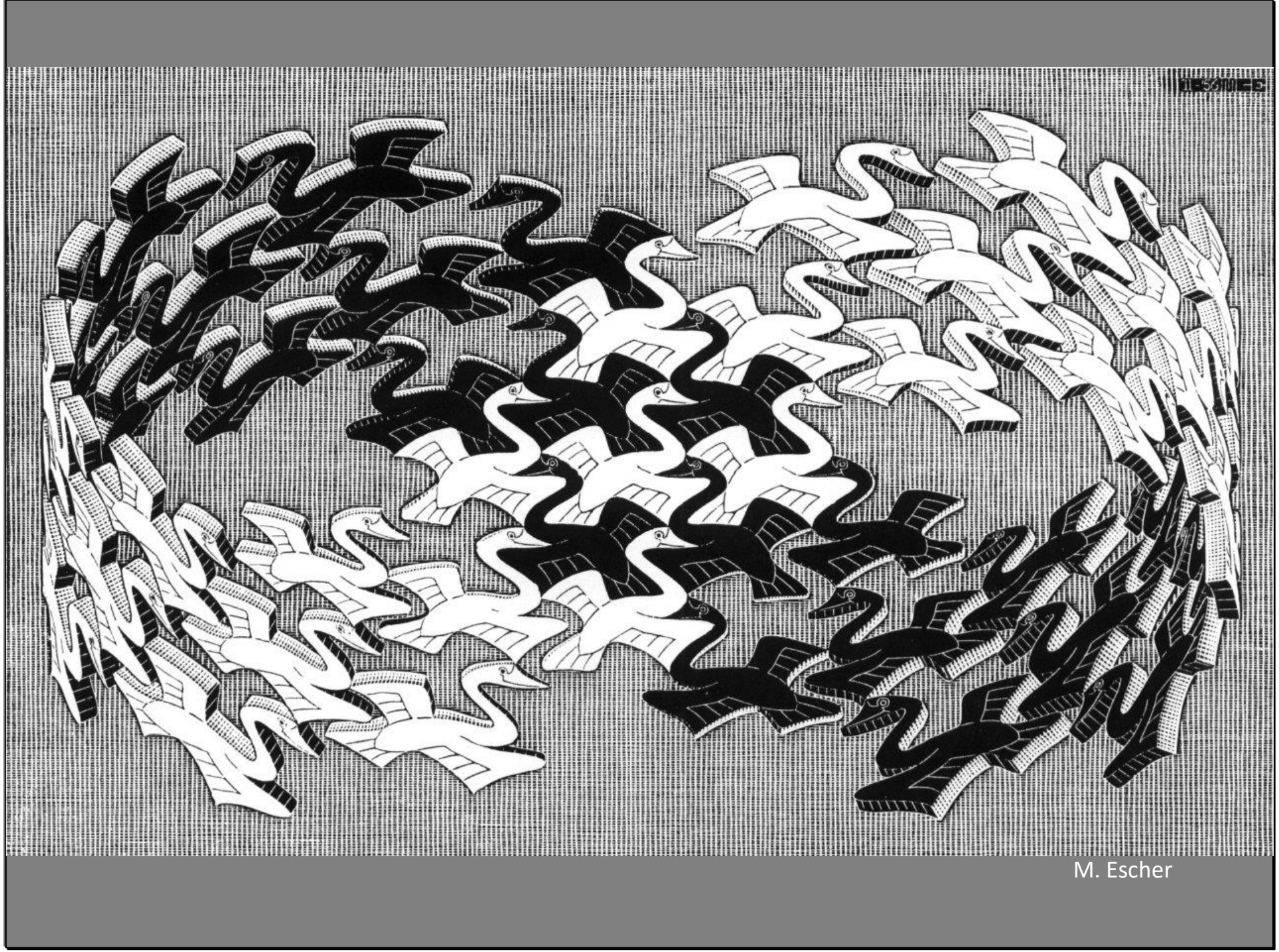
American Journal of Kidney Diseases
Available online 14 April 2023
In Press, Journal Pre-proof ? What's this? ↗

Original Investigations

C3 Glomerulopathy With Concurrent Thrombotic Microangiopathy: Clinical and Immunological Features

Melchior Chabannes MD [1],
Marion Rabant MD; PhD [2 4], Carine El Sissy MD [3],
Marie-Agnès Dragon-Durey MD PhD [3 4 5],
Paula Vieira Martins MDE [3],
Marie Sophie Meuleman MD [4 5],
Alexandre Karras MD; PhD [6 4], David Buob MD [7],
Frank Bridoux MD; PhD [8], Eric Daugas MD; PhD [9 4],
Vincent Audard MD; PhD [10 11],
Sophie Caillard MD, PhD [12], Jérôme Olagne MD [13],
Christine Kandel MD [14], Sophie Ferlicot MD, PhD [15],
Carole Philipponnet MD [16],
Thomas Crepin MD, PhD [1], Eric Thervet M.D; PhD [5],
Didier Ducloux M.D; PhD [1],
Véronique Frémeaux-Bacchi M.D; [3]

April, 2023



M. Escher

Complement and Kidney

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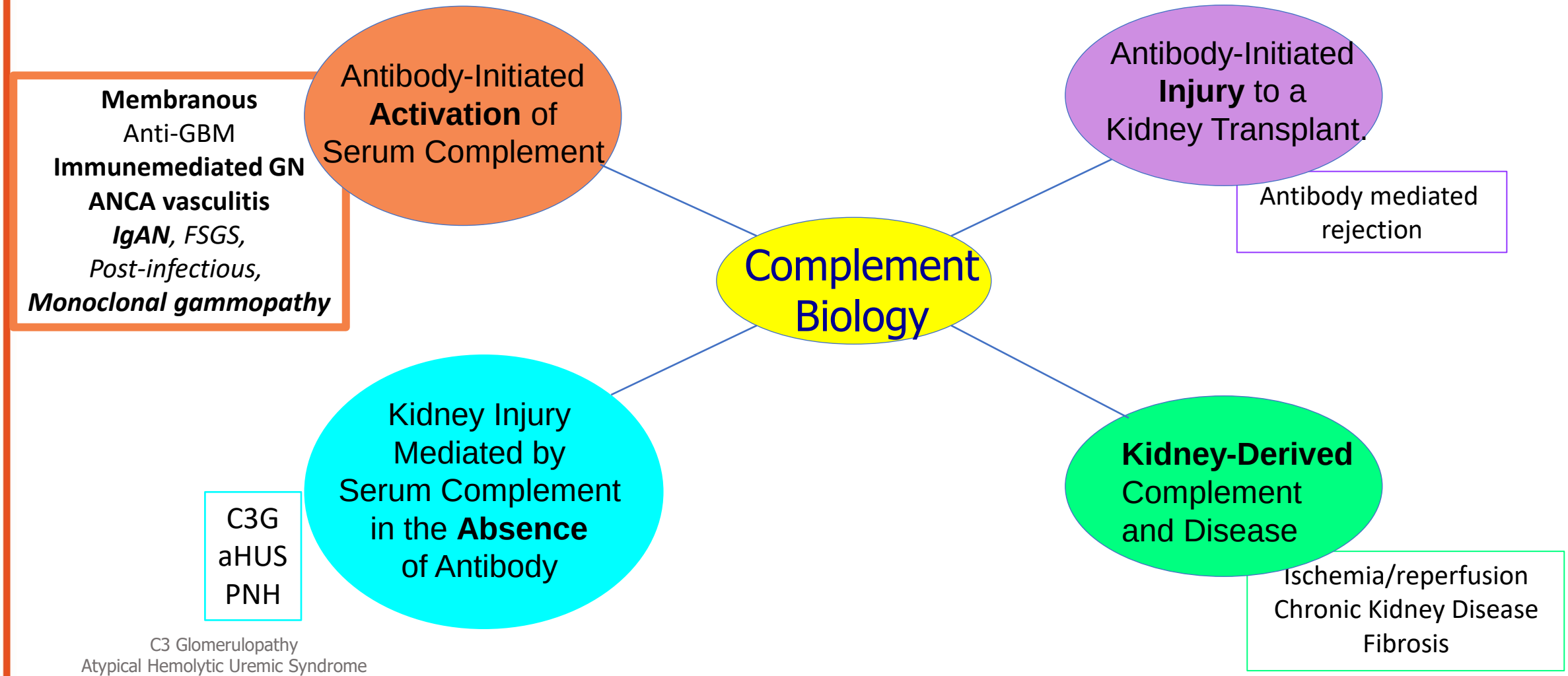
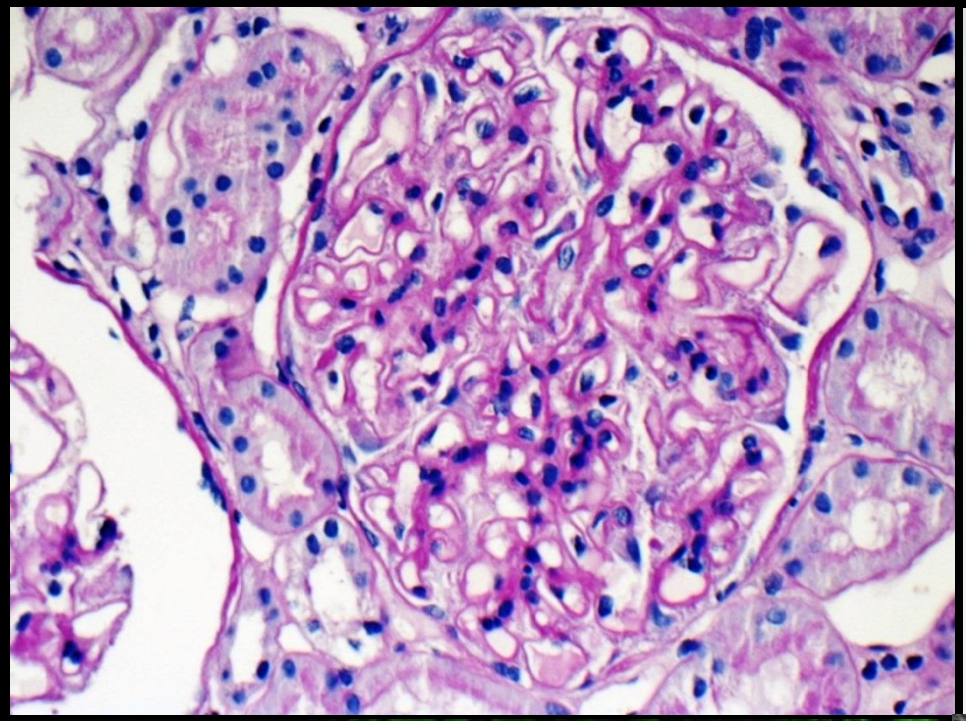
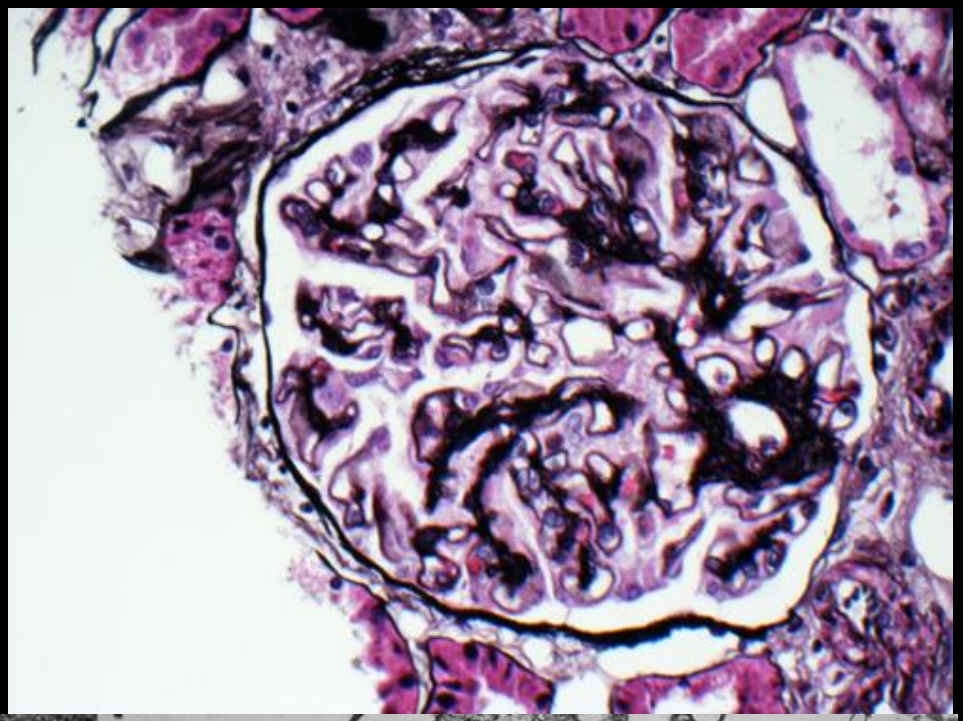
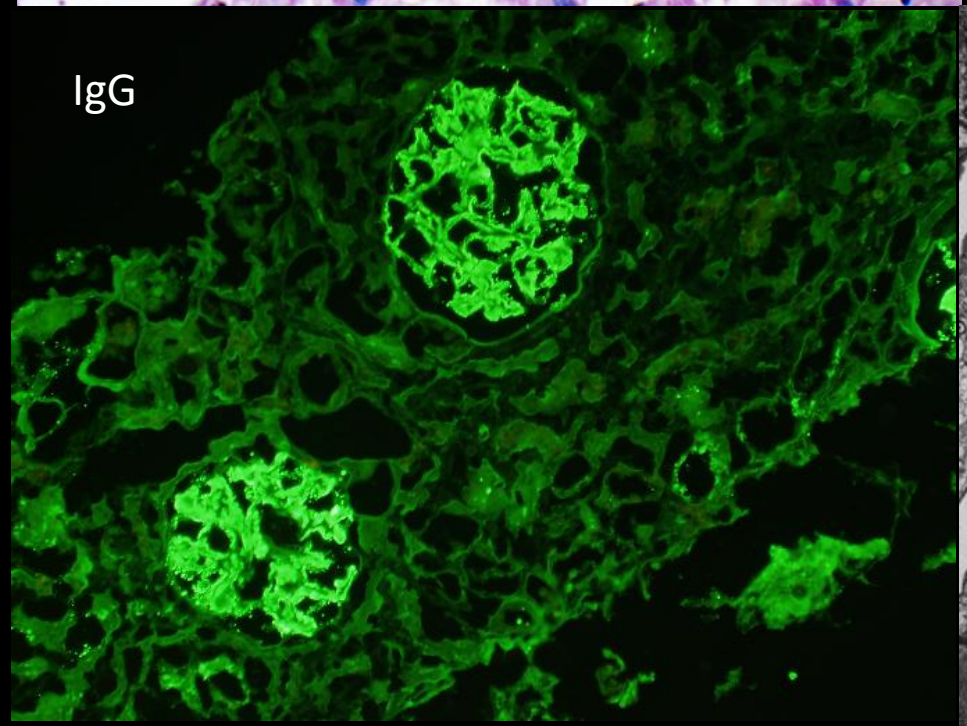


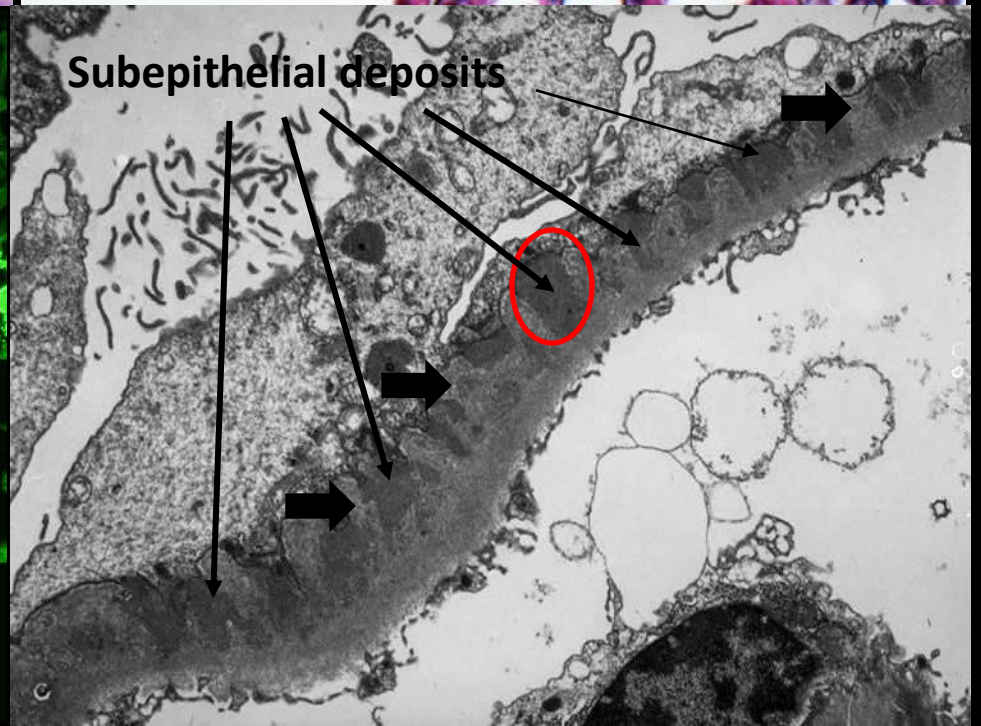
C3 Glomerulopathy
Atypical Hemolytic Uremic Syndrome
Paroxysmal Nocturnal Hemoglobinuria

Mathern & Heeger. *Complement and Kidney. CJASN 2015, 10: 1636*









Membranous Nephropathy

- Most common cause of nephrotic syndrome in adult white patients, the incidence increases in older patients
- Mean age 50-60, with 2:1 male predominance, rare in children
- Whites---Asians---Blacks---Hispanics
- 1/3 rule:
  - 1/3 spontaneous remission
  - 1/3 response to immunosuppression
  - 1/3 progression to Chronic Kidney Disease

A huge step in Membranous Nephropathy

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2009

The NEW ENGLAND JOURNAL of MEDICINE
ESTABLISHED IN 1812 JULY 2, 2009 VOL. 361 NO. 1

M-Type Phospholipase A_2 Receptor as Target Antigen in Idiopathic Membranous Nephropathy

Laurence H. Beck, Jr., M.D., Ph.D., Ramon G.B. Bonegio, M.D., Gérard Lambeau, Ph.D., David M. Beck, B.A., David W. Powell, Ph.D., Timothy D. Cummins, M.S., Jon B. Klein, M.D., Ph.D., and David J. Salant, M.D.

2014

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Thrombospondin Type-1 Domain-Containing 7A in Idiopathic Membranous Nephropathy

Nicola M. Tomas, M.D., Laurence H. Beck, Jr., M.D., Ph.D., Catherine Meyer-Schwesinger, M.D., Barbara Seitz-Polski, M.D., Hong Ma, Ph.D., Gunther Zahner, Ph.D., Guillaume Dolla, M.S., Elion Hoxha, M.D., Udo Helmchen, M.D., Anne-Sophie Dabert-Gay, Ph.D., Delphine Debayle, Ph.D., Michael Merchant, Ph.D., Jon Klein, M.D., Ph.D., David J. Salant, M.D., Rolf A.K. Stahl, M.D., and Gérard Lambeau, Ph.D.

Anti-PLA2r

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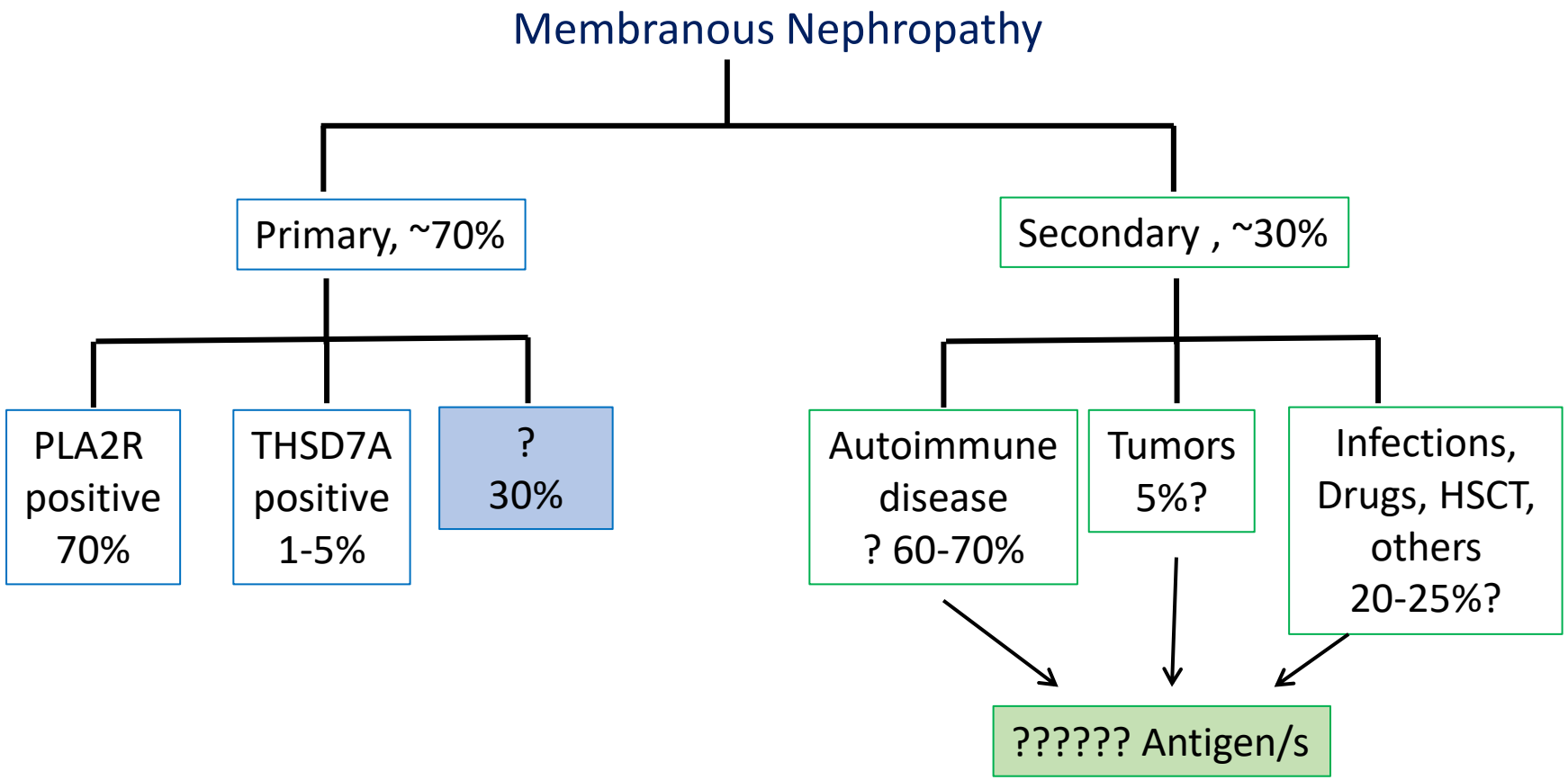


Mapping antigens of membranous nephropathy: almost there

Sanjeev Sethi[1] and Benjamin Madden[2]

Membranous nephropathy (MN) is characterized by subepithelial accumulation of immune complexes along the glomerular basement membranes. The immune complexes compromise IgG and the corresponding target antigen. Recent advances have led to the discovery of novel target MN antigens. In this study, by Caza *et al.*, 7 novel "putative" antigens are proposed. Target antigens can now be identified in approximately 90% of cases of MN. In addition to describing another 10 novel putative antigens, we propose a working algorithm for evaluating the target antigens in MN.

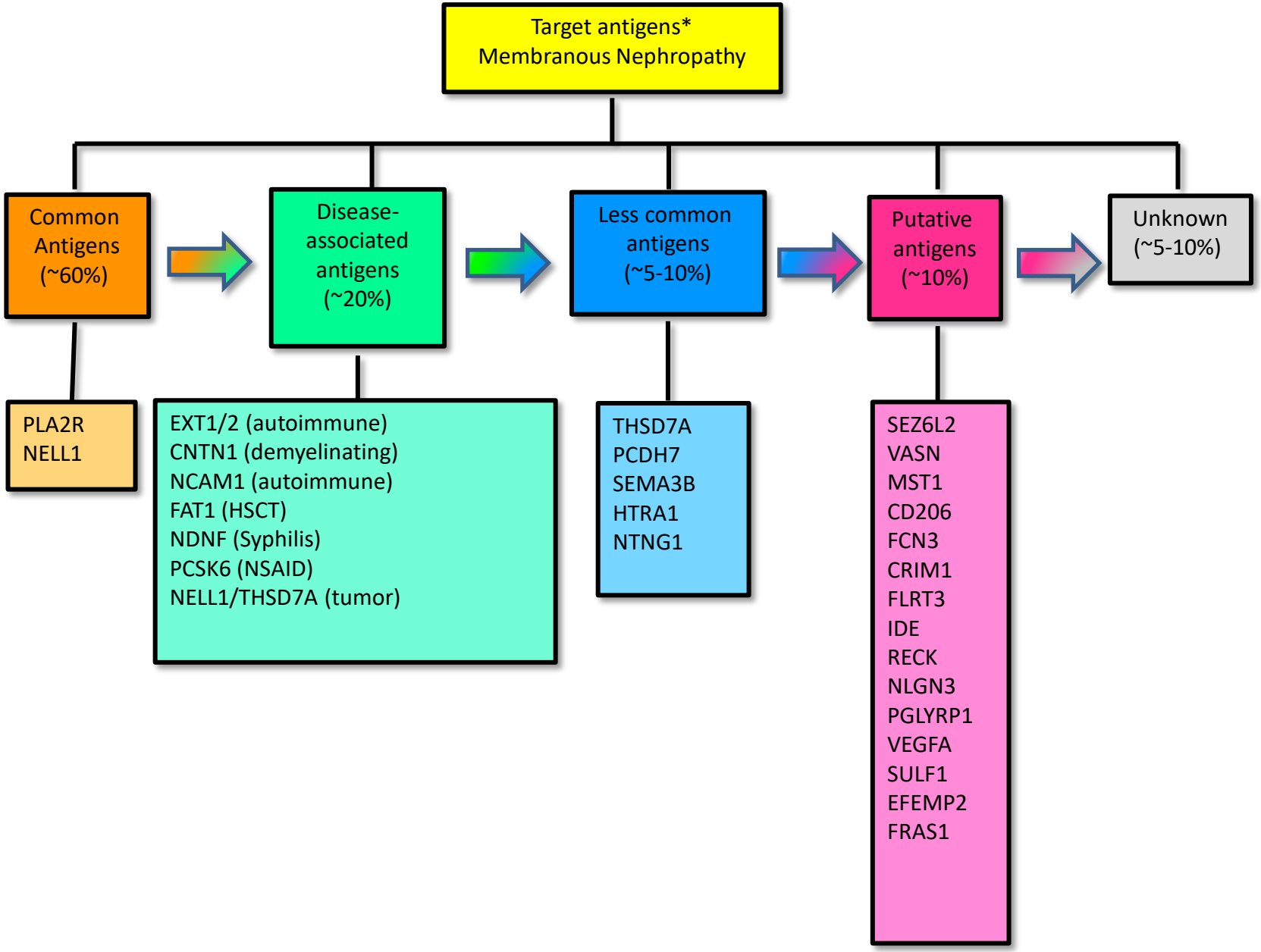
Kidney International (2023) **103,** 469–472; https://doi.org/10.1016/j.kint.2023.01.003

see clinical investigation on page 593

searchers did not find the remaining antigens.

Discovery of newer target antigens in MN

Second, discovery of newer antigens used laser microdissection of PLA2R-negative MN glomeruli from formalin-fixed, paraffin-embedded tissue followed by MS to study the proteomic profile and identify unique glomerular protein(s). The assumption was that the unique protein (target antigen) would stand out because substantial amounts of the protein were likely present as it lined the entire glomerular capillary walls. This was followed by immunohistochemistry and confocal microscopy to localize the unique glomerular protein/antigen along the glomerular basement membrane, and finally Western blot analysis to the unique protein using IgG extracted from the frozen tissue remnant and/or serum to detect



Sethi S & Madden B. Kidney Int 103, 2023

KI REPORTS
KIReports.org
CLINICAL RESEARCH

Proteomic Analysis of Complement Proteins in Glomerular Diseases

Sanjeev Sethi[1], Lilian Monteiro P. Palma[2], Jason D. Theis[1] and Fernando C. Fervenza[3]

[1]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA; [2]Pediatric Nephrology, State University of Campinas, Brazil; and [3]Division of Nephrology and Hypertension, Mayo Clinic, Rochester, Minnesota

Introduction: Complement plays an important role in the pathogenesis of glomerulonephritis (GN). Even though the underlying etiology of GN might be different, complement activation with subsequent glomerular deposition of complement proteins result in glomerular injury and progression of the lesions. Routine immunofluorescence microscopy (IF) includes staining for only complement factors C3c and C1q. Therefore, with regard to evaluation of the complement pathways, routine kidney biopsy provides only limited information.

Methods: In this study, using laser microdissection of glomeruli followed by mass spectrometry, complement proteins and pathways involved in GN were analyzed.

Results: We found that C3 followed by C9 are the most abundant complement proteins in GN, indicating activation of classical or lectin or alternative, and terminal pathways, either exclusively or in a combination of pathways. Furthermore, depending on the type of GN, C4A and/or C4B were also present. Therefore, membranous nephropathy (MN), fibrillary GN, and infection-related GN showed C4A dominant pathways, whereas lupus nephritis (LN), proliferative GN with monoclonal Ig deposits, monoclonal Ig deposition disease (MIDD), and immunotactoid glomerulopathy showed C4B dominant pathways. Significant deposition of complement regulatory proteins, factor H-related protein-1 (FHR-1) and factor H-related protein-5 (FHR-5), were also detected in most GN.

Conclusions: This study shows accumulation of specific complement proteins in GN. The complement pathways, complement proteins, and the amount of complement protein deposition are variable in different types of GN. Selective targeting of complement pathways may be a novel option in the treatment of GN.

Kidney Int Rep (2023) **8,** 827–836; https://doi.org/10.1016/j.ekir.2023.01.030
KEYWORDS: complement; glomerulonephritis; kidney; laser microdissection; mass spectrometry

Complement Burden in Glomerular Diseases

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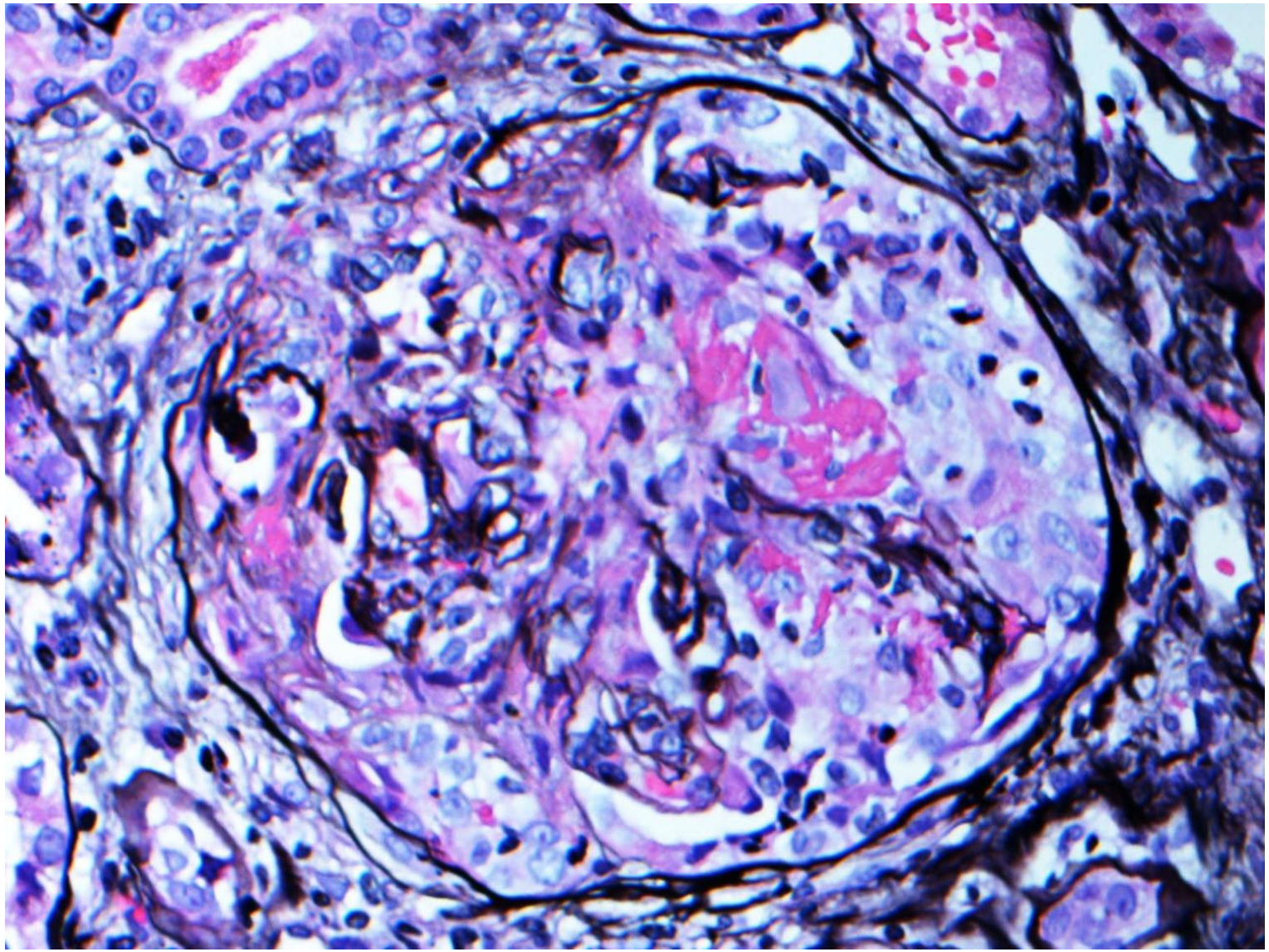
Table 1. Complement proteins in glomerulonephritis

Complement proteins	IRG	LN	IgAN	FG	ANCA + GN	ANCA - GN	C3GN	DDD	PGNMID	ITG	MIDD	MN
C3	+++	+++	+++	++++	+	+++	++++	++++	++++	++++	++++	++++
C4A	+	–	–	+++	+/–	+/–	–		++	–		+++
C4B	–	+++	–						+++	+++	++++	–
C9	++	++	++	++	–	–	+++	+++	++	++	+++	++
FHR-1	+	++	+++	+++	–	–	+++	+++	++	+++	++	++
FHR-5	+	++	+/–	+++	–	–	+++	++	++	++	++	+

ANCA, anti-neutrophil cytoplasmic antibodies; DDD, dense deposit disease; FG, fibrillary glomerulonephritis; GN, glomerulonephritis; IRG, infection-related GN; IgAN, IgA nephropathy; ITG, immunotactoid glomerulopathy; LN, lupus nephritis; MIDD, monoclonal immunoglobulin deposition disease; MN, membranous nephropathy; PGNMID, proliferative GN with monoclonal immunoglobulin deposits.
+ to ++++: –negative or baseline (0–2), + (low) spectral counts between 2–5, ++ (moderate) spectral counts between 6–15, +++ (high) spectral counts between 16–50, ++++ (very high) spectral counts over 50; ANCA + and ANCA- are pauci-immune crescentic GN with positive and negative ANCA titers, respectively.

Sethi S et al. Kidney Int Rep 8, 2023

Vasculitis with crescents and necrosis



Proteomic analysis of glomeruli in MPO-glomerulonephritis

Amit Sethi, Joseph Grande, Ulrich Specks, Fernando C. Fervenza

Mayo Clinic, Rochester, MN, USA*

BACKGROUND

Myeloperoxidase (MPO)-associated vasculitis often involves the kidney resulting a severe necrotizing and crescentic glomerulonephritis (GN). Kidney biopsy shows varying percentages of glomeruli involved by necrotizing and crescentic lesions. Yet, a proportion of the glomeruli appear normal and uninvolved by the necrotizing and crescentic lesions. In this study, we compared the proteomic profile of involved and uninvolved glomeruli in MPO-GN.

METHODS

We performed laser microdissection of glomeruli involved be crescents/fibrinoid necrosis (CF), and glomeruli that appeared normal (N) on light microscopy in 6 cases of MPO-GN. Equal number of glomeruli were dissected in each group/case. This was followed by mass spectrometry (MS/MS) to analyze the proteomic profile in the 2 groups. Sclerosed glomeruli were not dissected.

RESULTS

Proteomic profile shows higher activation of complement pathways in CF glomeruli compared to N glomeruli with higher total spectral counts (TSC) of C3 (2-fold), C5 (7-fold), C7 (10-fold), C9 (6-fold). In addition, there is 3-7-fold increase in TSC of actinin 4, laminin subunit 2, fibrinogen a, fibrillin 1, agrin, nidogen-2, heat shock protein 90 in CF glomeruli compared to N glomeruli. On the other hand, there is 3-6-fold increase in TSC of desmoplakin, protein S100, and serpin B3 and B12, and thioredoxin in N glomeruli compared to CF glomeruli.

RESULTS

Proteomic identification of complement activation in CF versus N glomeruli

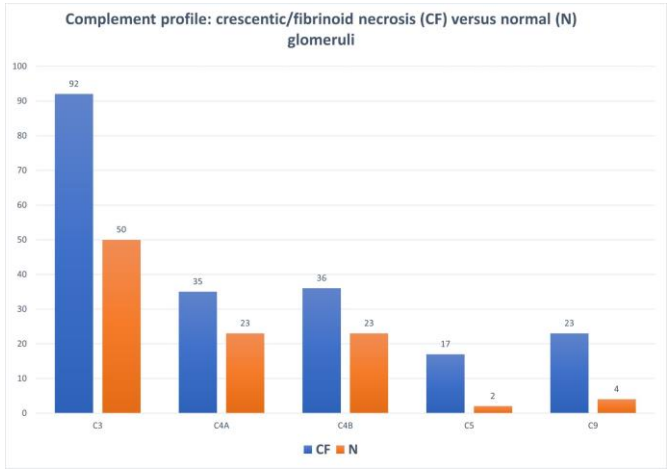


Figure 1. Proteomic profile showing increased activation of complement in glomeruli involved by crescents and fibrinoid necrosis compared to normal appearing glomeruli on the biopsy.

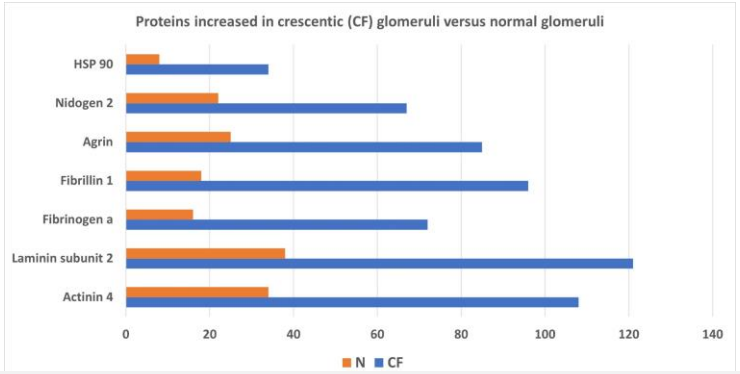


Figure 2. Proteomic profile showing increased accumulation of key protein in glomeruli involved by crescents and fibrinoid necrosis compared to normal appearing glomeruli on the biopsy.

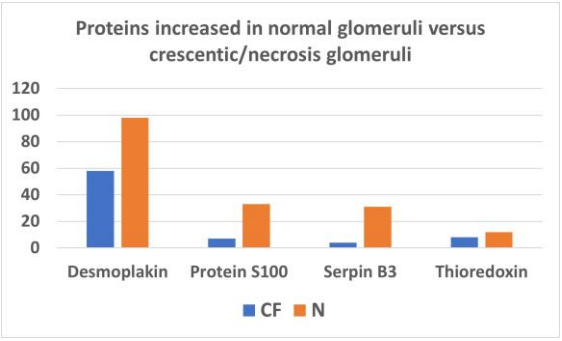


Figure 3. Proteomic profile showing increased accumulation of key protein in normal appearing glomeruli compared to glomeruli involved by crescents and fibrinoid necrosis.

CONCLUSION

- Complement activation is greater in glomeruli involved by crescents and necrosis.
- There are also differences in proteins expressed in glomeruli with crescents/necrosis compared to uninvolved glomeruli.
- Overexpression of certain proteins in normal glomeruli may protect glomeruli from developing crescents/necrosis.

The NEW ENGLAND JOURNAL of MEDICINE

RESEARCH SUMMARY

Avacopan for the Treatment of ANCA-Associated Vasculitis

Jayne DRW et al. DOI: 10.1056/NEJMoa2023386

CLINICAL PROBLEM

Patients with antineutrophil cytoplasmic antibody (ANCA)–associated vasculitis may have serious complications, decreased quality of life, and side effects from medications (e.g., glucocorticoids) used to treat the condition. Avacopan is an oral small-molecule C5a receptor antagonist that offers a potential treatment option for ANCA-associated vasculitis.

CLINICAL TRIAL

Design: A phase 3 international, double-blind, randomized, controlled trial compared oral avacopan with oral prednisone in patients with ANCA-associated vasculitis concurrently being treated with immunosuppressive drugs.

Intervention: 331 patients were assigned to receive either avacopan (30 mg twice daily) plus prednisone-matching placebo or prednisone (60 mg daily tapered to discontinuation by week 21) plus avacopan-matching placebo. All patients also received cyclophosphamide (followed by azathioprine) or rituximab. The two primary efficacy end points — clinical remission at week 26 and sustained remission at both week 26 and week 52 — were tested for noninferiority (noninferiority margin, 20 percentage points) and superiority.

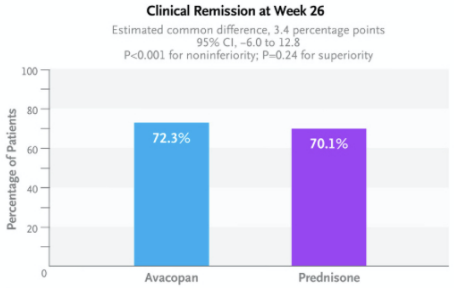
RESULTS

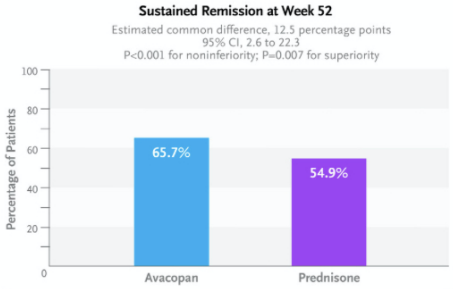
Efficacy: Avocapan was noninferior to prednisone with respect to clinical remission at week 26 and was both noninferior and superior to prednisone with respect to sustained remission at week 52.

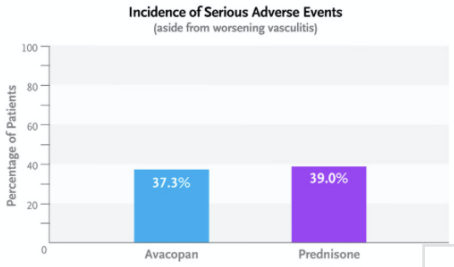
Safety: The percentage of patients who had serious adverse events (excluding worsening vasculitis) was similar in the two groups.

LIMITATIONS AND REMAINING QUESTIONS

- Patients in the avacopan group received glucocorticoids, although the mean daily dose was one third that in the prednisone group.
- The trial population was heterogeneous, including patients with newly diagnosed vasculitis and those with relapsing disease.
- The durability and safety of avacopan in patients with ANCA-associated vasculitis need to be assessed in longer-term trials.

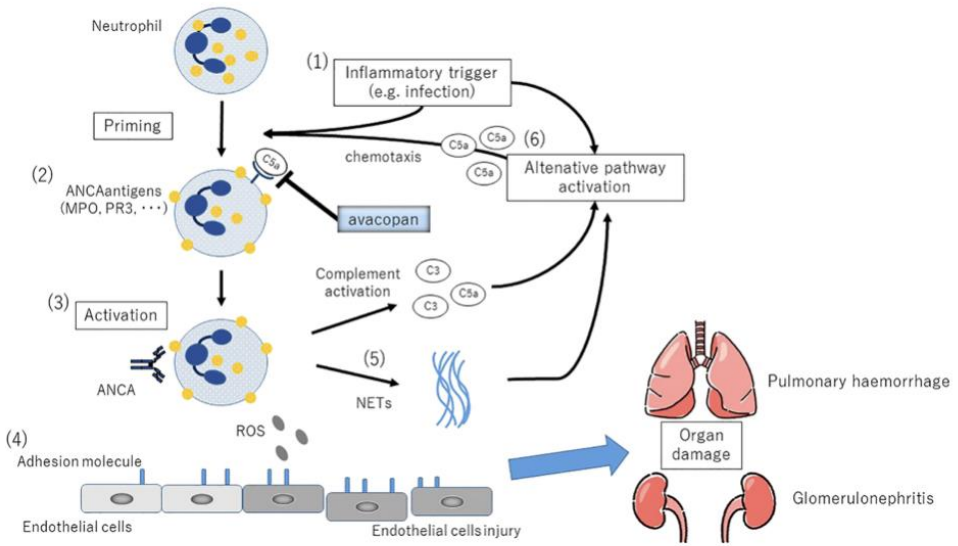






CONCLUSIONS

Among patients with ANCA-associated vasculitis, avacopa was noninferior to prednisone with respect to remission a 26 weeks and was superior with respect to sustained remission at 52 weeks.



Kimoto & Horiuchi. Fron Immunol, 2022

Proposed Complement Pathways

Anno VII
Cadernos de Patologia Renal
COMPLEMENTO E DOENÇAS RENAIS.

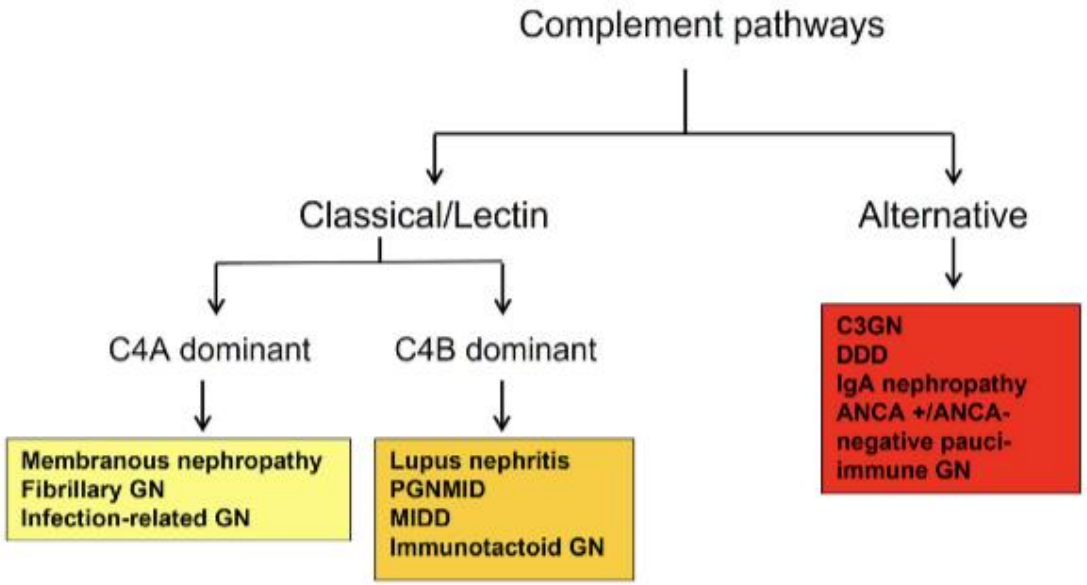


Figure 2. Proposed complement pathways in glomerulonephritis. ANCA, antineutrophil cytoplasmic antibody; DDD, dense deposit disease; MIDD, monoclonal immunoglobulin deposition disease.

Sethi S et al. Kidney Int Rep 8, 2023


É junto
dos bão
que
a gente
fica mió.
Guimarães Rosa
#A Lanterna

Obrigada