Diagnostiek en therapie op maat bij membraneuze nefropathie en de rol daarbij van ‘nieuwe antistoffen’

Anne-Els van de Logt en Jack Wetzels

Papendal 14-12-2022

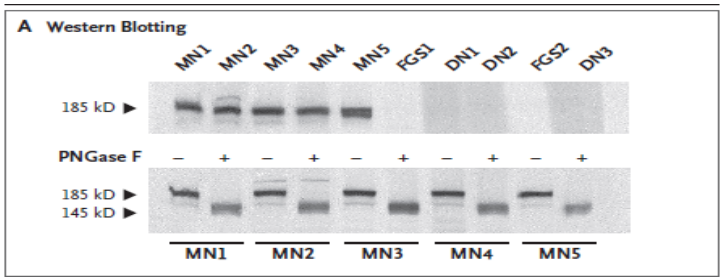
Radboudumc

Disclosure belangen spreker(s)

(potentiële) belangenverstrengeling	Zie hieronder
Voor bijeenkomst mogelijk relevante relaties met bedrijven	Novartis, Morphosys,
• Sponsoring of onderzoeksgeld • Honorarium of andere (financiële) vergoeding • Aandeelhouder • Andere relatie, namelijk …	• Klinische trial • Scientific advisory board • •

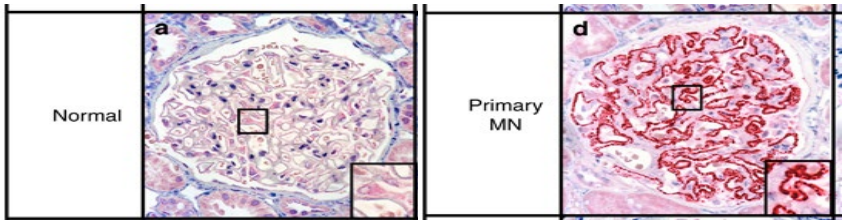
Anti-PLA2R-assays and PLA2Rag staining in biopsy

Western Blot

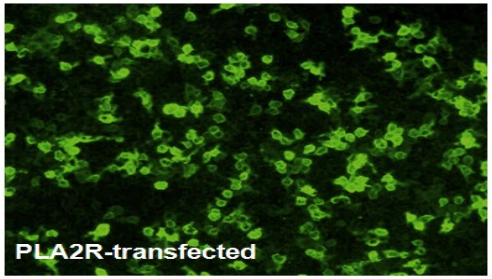


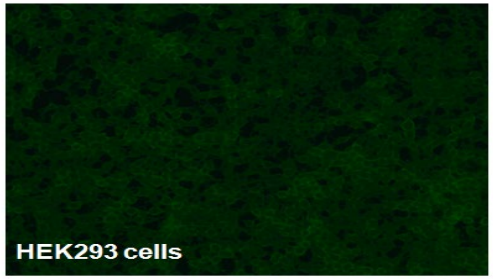
Beck et al. NEJM 2009

Biopsy staining

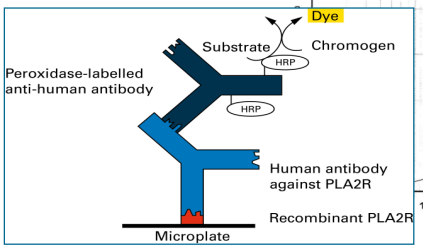


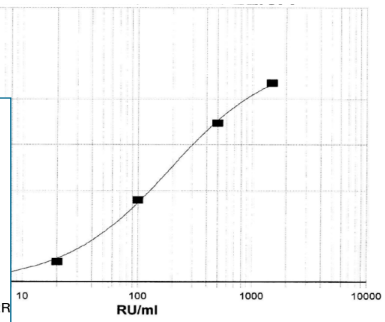
Immunofluorescence (IIFT)





ELISA





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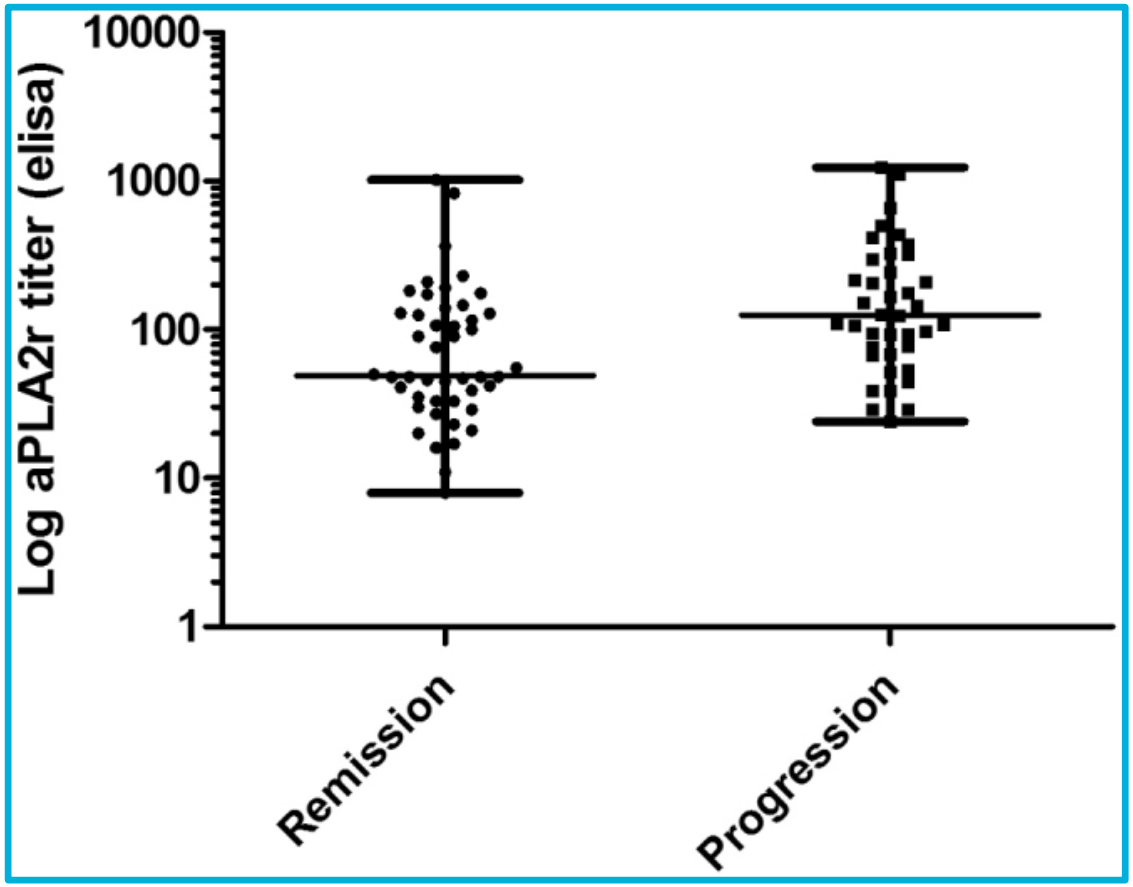
- Sensitivity: biopsy > WB > IFT > ELISA
  - *ELISA provides quantitative levels*
  - *Cut-off debated 14 vs 20 RU/ml*

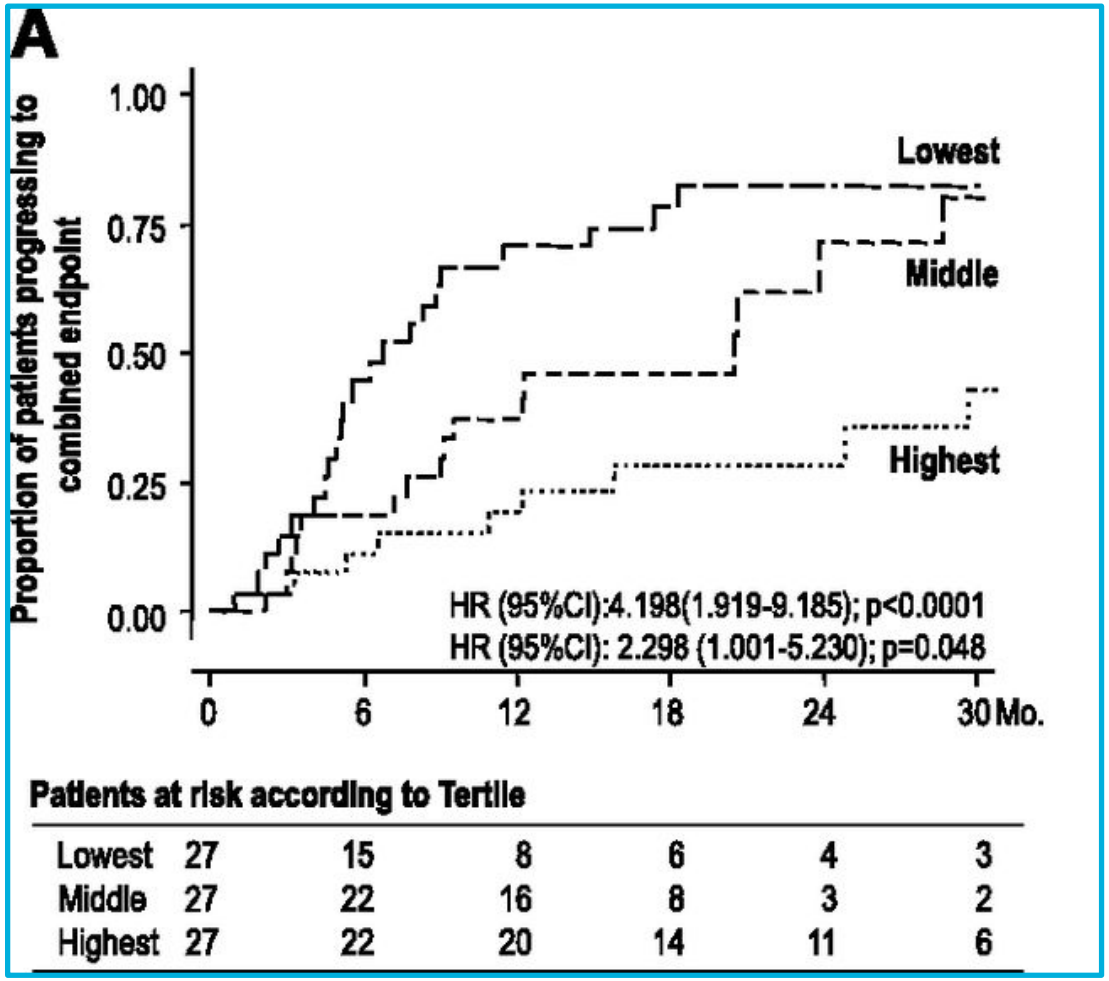
PLA2Rab levels: limited prognostic value



aPLA2R1ab levels at baseline of patients with normal serum creatinine with progression (*n* = 39) and or spontaneous remission (*n* = 46).

Logt van de AE et al. Kidney Int Rep. 2021.

PLA2R antibodies as predictive biomarker



Predictive =

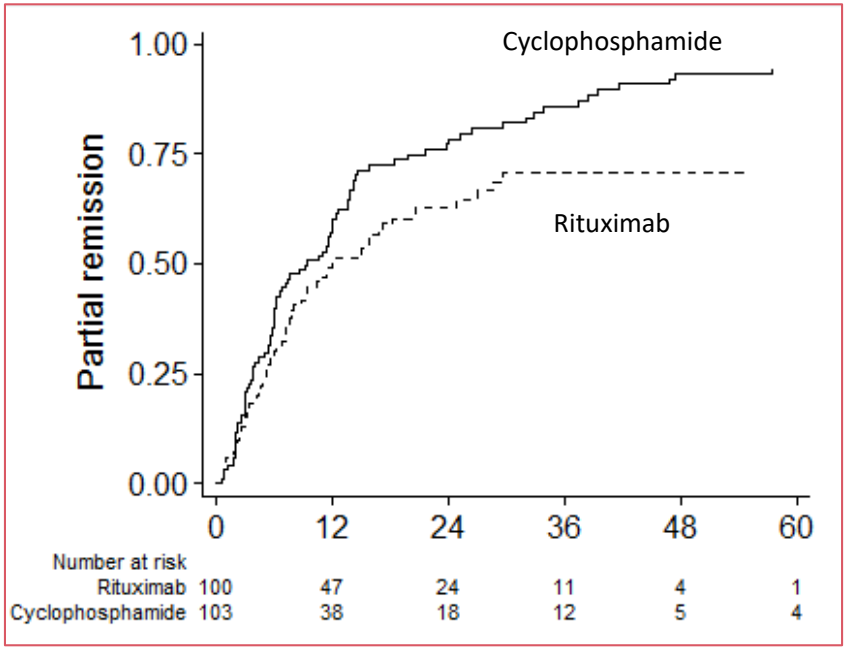
Response to therapy

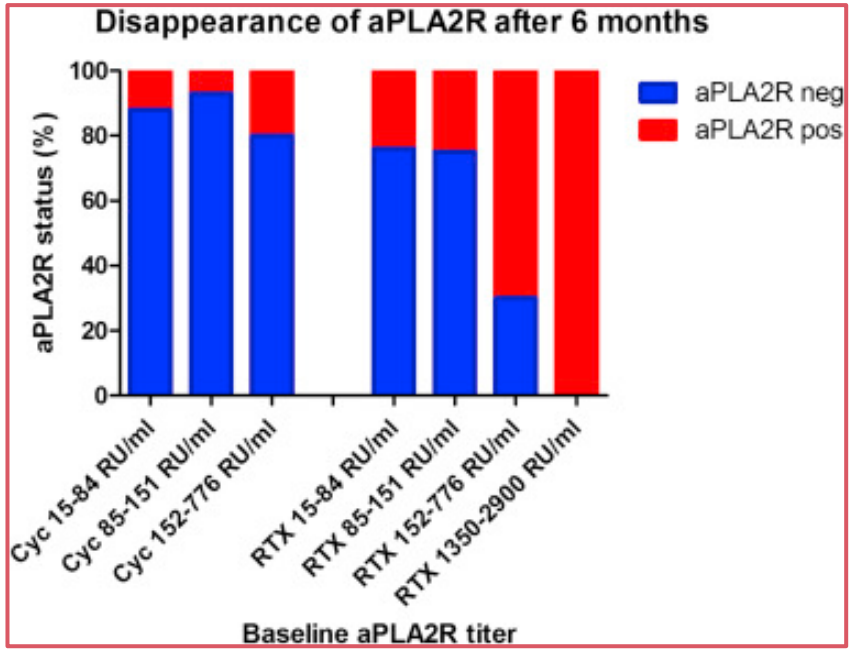
(Rituximab)

Ruggenenti et al. JASN 2015.

©2015 by American Society of Nephrology

A comparison of rituximab and cyclophosphophamide

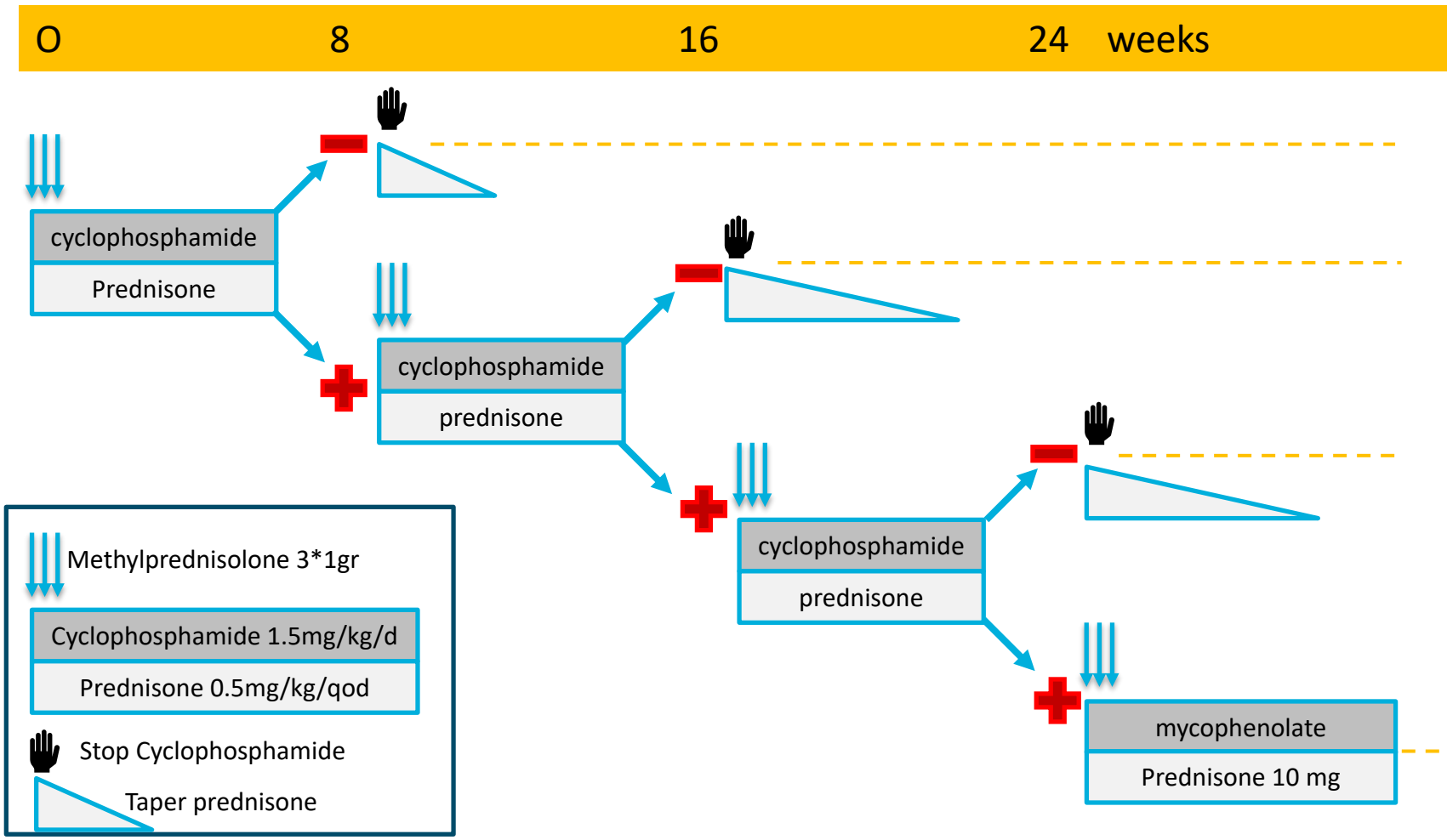




Cyc = cyclophosphamide
RTX = rituximab

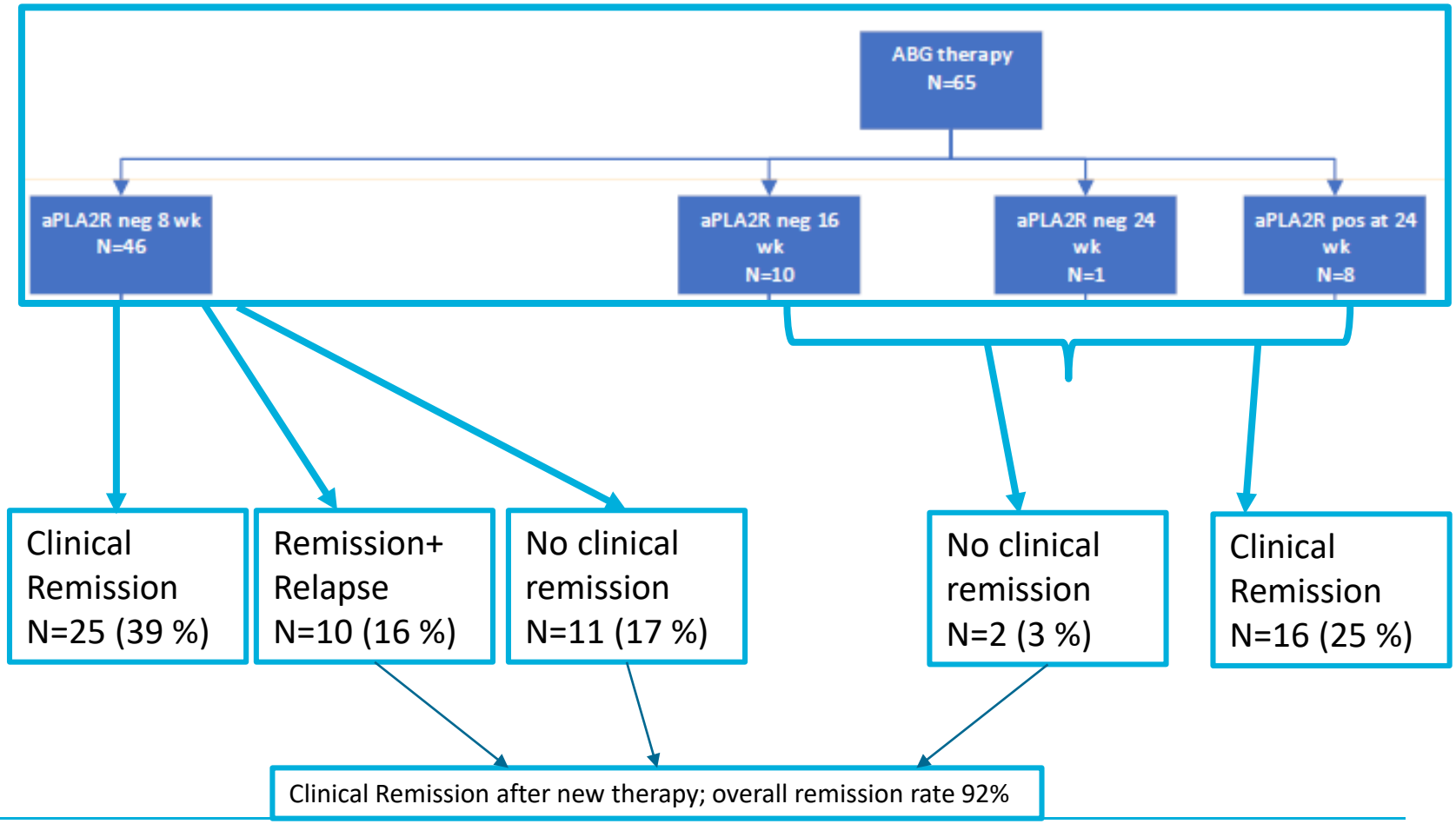
Van den Brand J et al. J Am Soc Nephrol. 2017.

Van de Logt AE et al. Kidney International 2018.

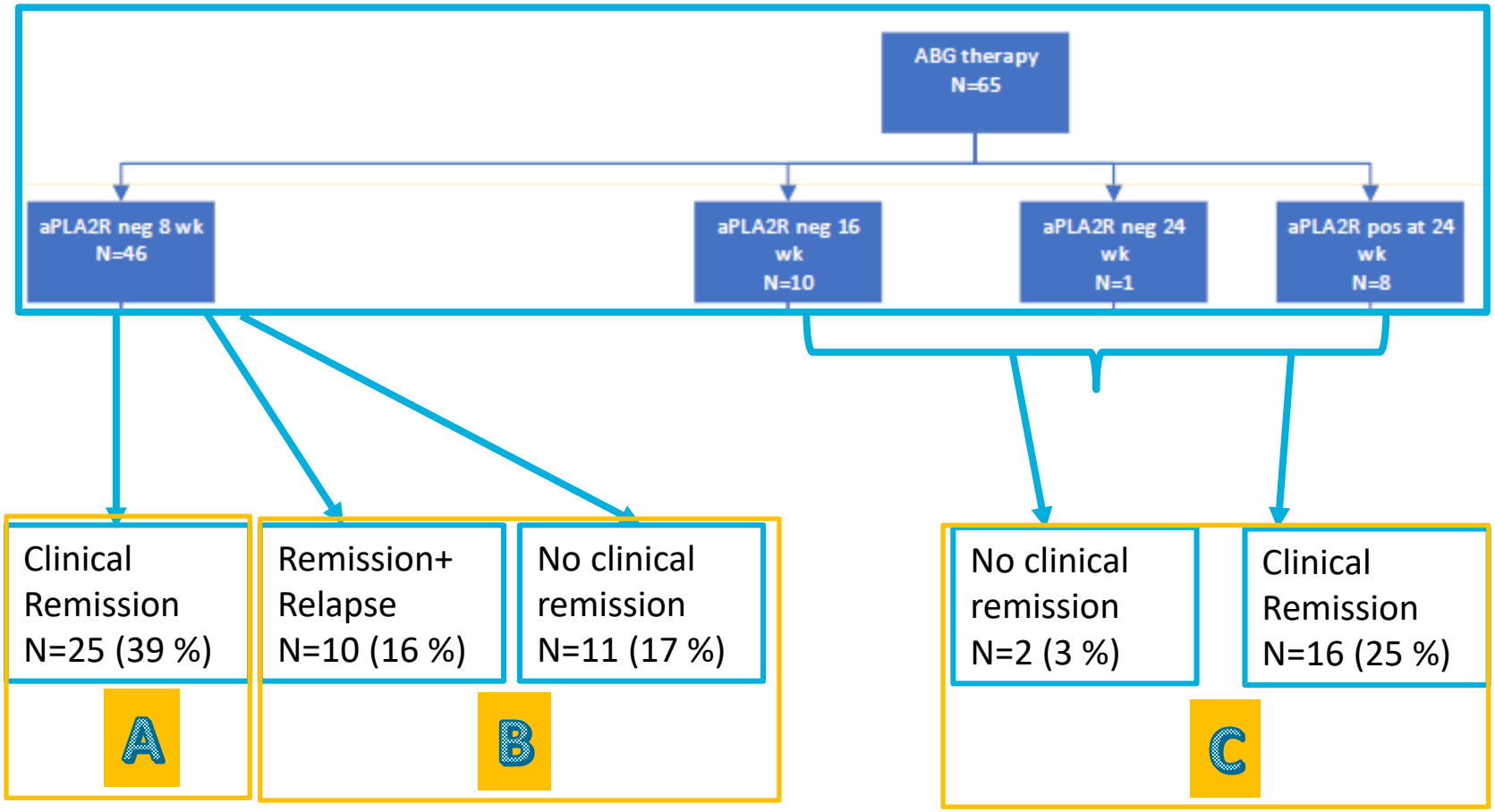


C.Vink & AE van de Logt, Kidney Int Reports 2022

Antibody-guided therapy: RESULTS



Antibody-guided therapy: can we predict response?



aPLA2Rab levels and response

Baseline: ELISA is less sensitive than IFT, cut-off < 14 RU/ml preferred
After therapy: ELISA is even less sensitive: cut-off should be < 2RU/ml?

Baseline aPLA2Rab < 80 RU/ml
→ Immunologische remissie EN persisterende klinische remissie met therapieduur van 8 weken

Daling aPLA2Rab van 0 → 8 weken voorspelt niet klinische remissie na 8 weken behandeling

Daling aPLA2Rab van 0 → 8 weken voorspelt niet totale duur behandeling

Absolute waarde aPLA2Rab na 8 weken therapie voorspelt wel totale duur behandeling, MAAR niet gevalideerd!

MN PLA2Rab: what have we learned?

- ❑ We do not need a kidney biopsy to diagnose membranous nephropathy
- ❑ PLA2Rab levels can be used as predictive biomarker
- ❑ Disappearance of PLA2Rab can guide therapy
(no evidence that change in PLA2Rab levels are reliable)
- ❑ PLA2Rab can be absent → staining of biopsy helpful

→ Identification and detection of other antigens/antibodies might be equally relevant

MN: new antigens

- ❑ 2009: PLA_2R: M-type phospholipase A_2 receptor
- ❑ 2014: THSD7A:Thrombospondin type 1 Domain 7A
- ❑ 2019: EXT1/2: exostosin1/2
- ❑ 2020: NELL-1:Neural epidermal growth factor like 1 protein
- ❑ 2020: SEMA3B: Semaphorin 3B
- ❑ 2021: PCDH7:protocadherin 7
- ❑ 2021: HTRA1: high temperature recombinant protein A1
- ❑ 2021: NCAM1: neutral cell adhesion molecule1
- ❑ 2022: NTNG1: Netrin G1
- ❑ 2022: CTN1: Contactin 1
- ❑ 2022: FAT1: Protocadherin FAT1
- ❑ 2022: TGFBR3: TGF-beta receptor 3
- ❑ 2022: FCN3, CD206, EEA1, SEZ6L1, NPR3, MSP1, VASN (abstract ASN 2022): WB??
- ❑ 2022: NDNF: Neuron Derived Neurotropic Factor (abstract ASN 2022) Syphilis? IgG1 IgG3 WB ongoing
- ❑ 2022: PCSK6: Proprotein convertase subtilisin/kexin type 6 (abstract ASN 2022) NSAIDs, WB positive

FCN3 = Ficolin3; CD206 = mannose receptor; EEA1 early endosome antigen 1; SEZ6L1 Seizure related 6 homolog like; NRP3 natriuretic peptide receptor 3; MSP1 Merozoit Surface protein 1; VASN Vasorin

MN: new antigens

Relevant questions:

- Is the antigen expressed on the podocyte?
- Are there detectable antibodies?
- Is the dominant antibody of the IgG4 subclass?
- Is there an association with underlying disease?

- Is there an assay to detect antibodies?
- Can the antigen detected by staining of kidney biopsy?

Limitations: clinical data of discovery cohort not always available; validation cohort lacking; few patients

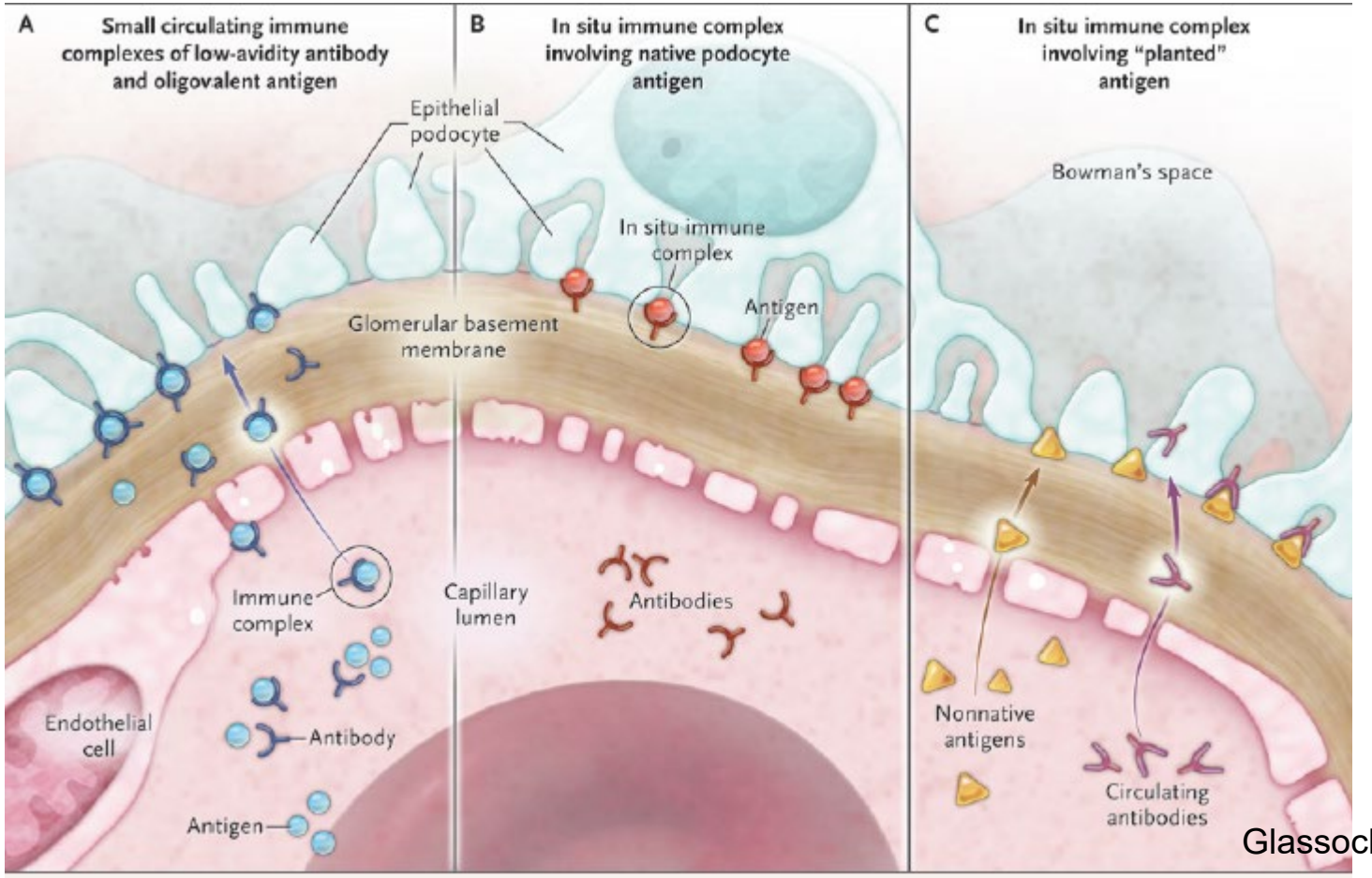
MN: new antigens

Relevant questions:

- Is the antigen expressed on the podocyte?

→ Pathophysiology of MN

MN: an immunecomplex disease (IgG4 in primaryMN)



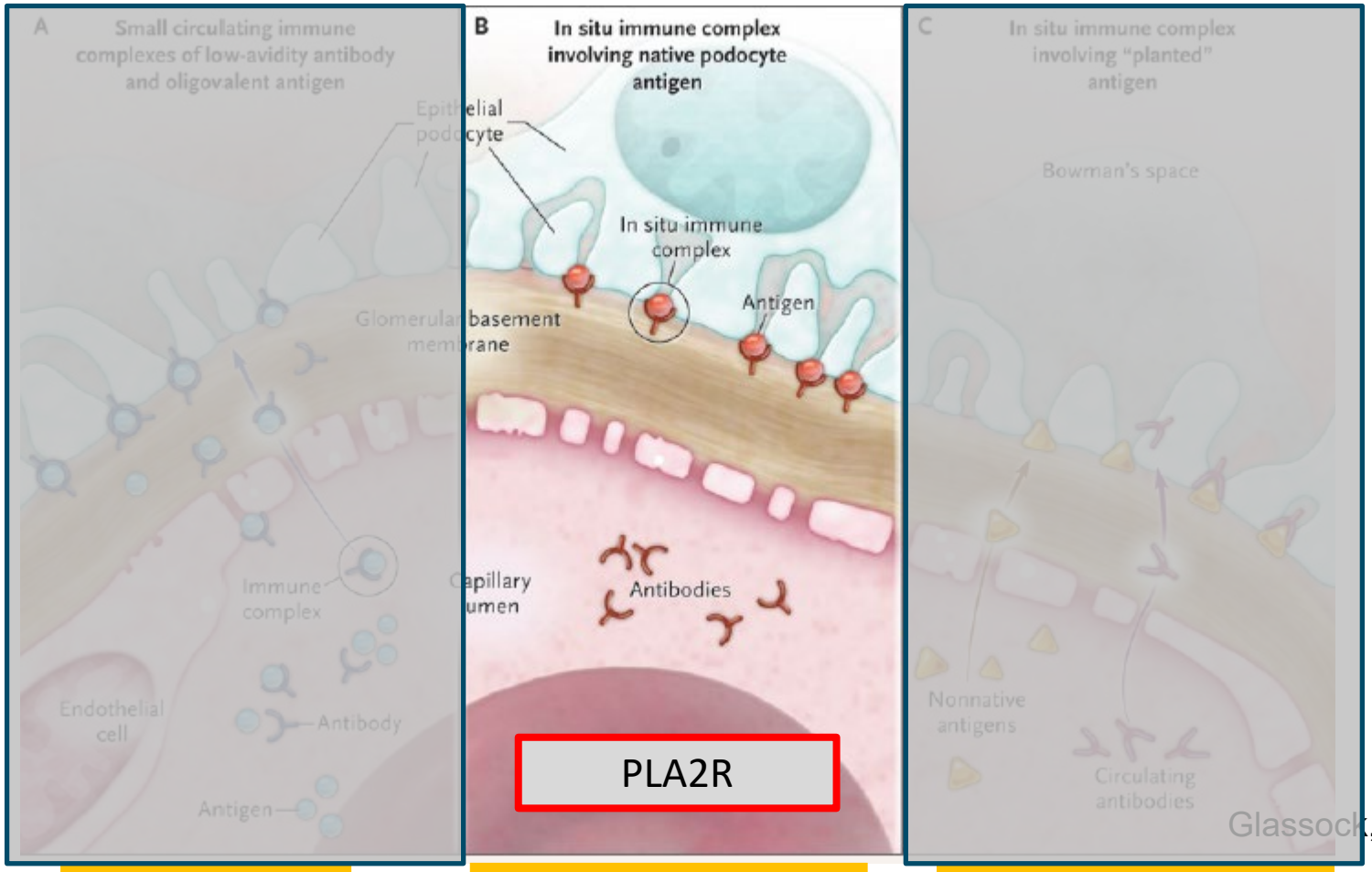
Glassock, NEJM 2009

Circulating IC

Antibody binding to intrinsic antigens

Antibody binding to 'Planted antigen'

MN: an immunecomplex disease (IgG4 in primaryMN)



Glassock, NEJM 2009

Circulating IC

Antibody binding to intrinsic antigens

Antibody binding to 'Planted antigen'

MN: new antigens

Relevant questions:

- Are there detectable antibodies?

→ No antibodies: is the antigen relevant?

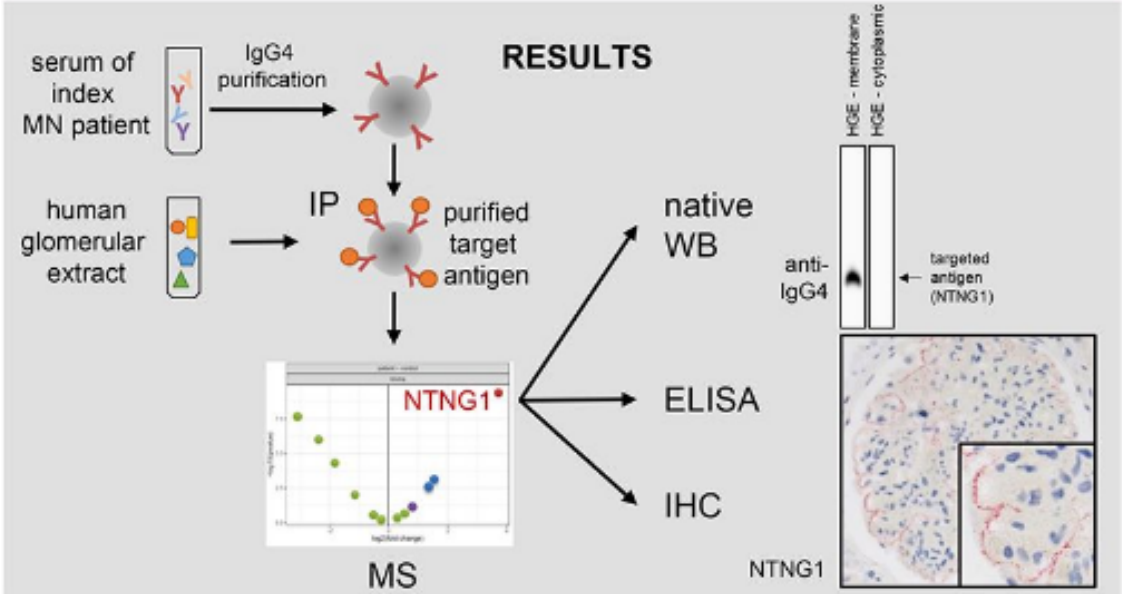
MN: antibody detection

Netrin G1 is a novel target antigen in primary membranous nephropathy

JASN
JOURNAL OF THE AMERICAN SOCIETY OF NEPHROLOGY

METHODS

- Four cohorts with 1271 patients
- Native Western blot (WB)
- Immunoprecipitation (IP)
- Mass spectrometry (MS)
- Immunohistochemistry (IHC)
- Enzyme-linked immunosorbent assay (ELISA)



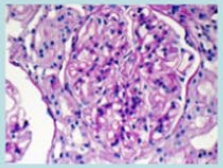
MN: antigen detection

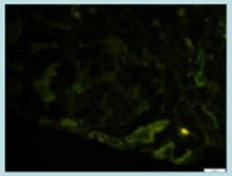
Semaphorin 3B-associated membranous nephropathy is a distinct type of disease predominantly present in pediatric patients.

Patient Selection

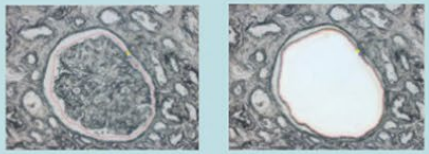
→ **Laser Microdissection and Mass Spectrometry** → **Immunohistochemistry /Immunofluorescence** → **Western Blot Analysis**

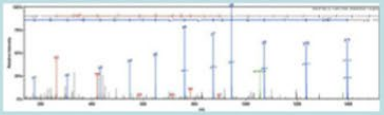
PLA2R-negative MN



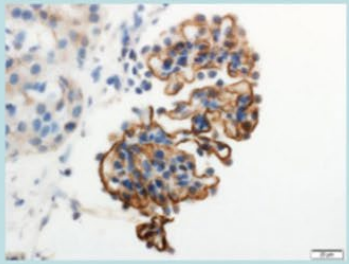


Identification of novel protein Sema 3B

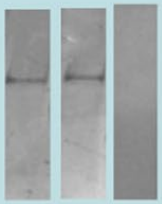




Granular anti-Sema3B GBM staining



Serum antibodies to Sema3B



CONCLUSION:

Semaphorin 3B defines a distinct type of membranous nephropathy

ISN kidney INTERNATIONAL

OFFICIAL JOURNAL OF THE INTERNATIONAL SOCIETY OF NEPHROLOGY

Sethi et al, 2020

MN: new antigens

Relevant questions:

- Are there detectable antibodies?

→ No antibodies: is the antigen relevant?

Examples:

Exostosin1/2 in lupus membranous nephropathy

TGFBR3 in lupus membranous nephropathy

LIMP2 in membranous nephropathy

MN: new antigens

Relevant questions:

- Is the dominant antibody of the IgG4 subclass?

→ IgG4 in primary MN

- Is there an association with underlying disease?

→ Secondary MN
→ Patient care: detailed search for underlying disease

MN: antigens and disease associations

- NELL1: malignancy, tradional drugs, supplements, e.g lipoic acid; Siddha and Ayurvedic drugs mercury (IgG1)
- NCAM1: SLE (IgG1)
- FAT1: post allogeneic stem cell transplant (IgG4)
- Contactin1: Chronic inflammatory demyelinating neuropathy (IgG4)
- NDNF: syphilis (IgG1/IgG3)
- PCSK6: NSAIDs (IgG??)

Membranous Nephropathy: new antigens

	PLA2R1	**THSD7A**	PCDH7	HTRA1	**Netrin G1**
compartment	membrane	membrane	membrane	secreted	membrane
expression by podocyte	Moderate	Moderate	??? Not tested in detail	moderate	yes
Presence in deposits	Yes	Yes	Yes	yes	Yes
Circulating Ab	Yes	Yes	Yes	yes	Yes
Deposited Ab	Yes	Yes	Yes	yes	Yes
Predominant IgG subclass	IgG4	IgG4	IgG4	IgG4	IgG4
Remarks	Prototype for primary MN	Malignancy in few cases (Expression by tumor)	autoimmune diseases and malignancy (C3+/-?)	Primary MN?	Primary MN?

PLA_2R: phospholipase A_2 receptor; THSD7A:Thrombospondin type 1 Domain 7A; PCDH7 Protocadherin 7; HTRA1 High temperature recombinant protein A1

Ronco, P., Debiec, H. Membranous nephropathy: current understanding of various causes in light of new target antigens. Curr. Opin. Nephrol. Hypertens. 2021 Jan 21. doi: 10.1097/MNH.0000000000000697.

2022: new antigens

	PLA2R1	NCAM-1	Sema3B	NELL-1
compartment	membrane	membrane	Secreted	Secreted
expression by podocyte	Moderate	Weak if any	No (yes Sema3A)	Weak
Presence in deposits	Yes	Yes	Yes	Yes, often segmental
Circulating Ab	Yes	Yes	Yes, reduced Ag	Yes
Deposited Ab	Yes	Yes	Not tested	Yes
Predominant IgG subclass	IgG4	IgG1	IgG1	IgG1
Remarks	Prototype for primary MN	Lupus	Pediatric MN; early onset	Malignancy (±30%) (Expression in tumor)

PLA_2R: phospholipase A_2 receptor; THSD7A:Thrombospondin type 1 Domain 7A; NELL-1:Neural epidermal growth factor like 1 protein; SEMA3B: Semaphorin 3B; NCAM1 Neural cell adhesion molecule 1

Ronco, P., Debiec, H. Membranous nephropathy: current understanding of various causes in light of new target antigens. Curr. Opin. Nephrol. Hypertens. 2021 Jan 21. doi: 10.1097/MNH.0000000000000697.

MN: new antigens

Relevant questions:

- Is there an assay to detect antibodies?
- Can the antigen detected by staining of kidney biopsy?

Commercially available assays: PLA2Rab (IFT, ELISA), THSD7A (IFT)

Other antibodies: no assay (WB in research settings)

Low prevalence of “new”antigens → expensive for laboratories, no profit for companies?

MN: prevalence of “new”antigens

Low in primary MN: 0.4 – 3% (NELL1 most frequent?)

Note of caution:

Few studies

Description of cohort unclear

Often selection bias:

- Inclusion/exclusion associated disease (e.g lupus)
- Inclusion/exclusion PLA2R/THSD7A/NELL1/EXT positive biopsies

MN: new antigens

Relevant questions:

- Is there an assay to detect antibodies?
- Can the antigen detected by staining of kidney biopsy?

Is staining of kidney biopsy an option?

It is possible (at least shown in the discovery cohorts)

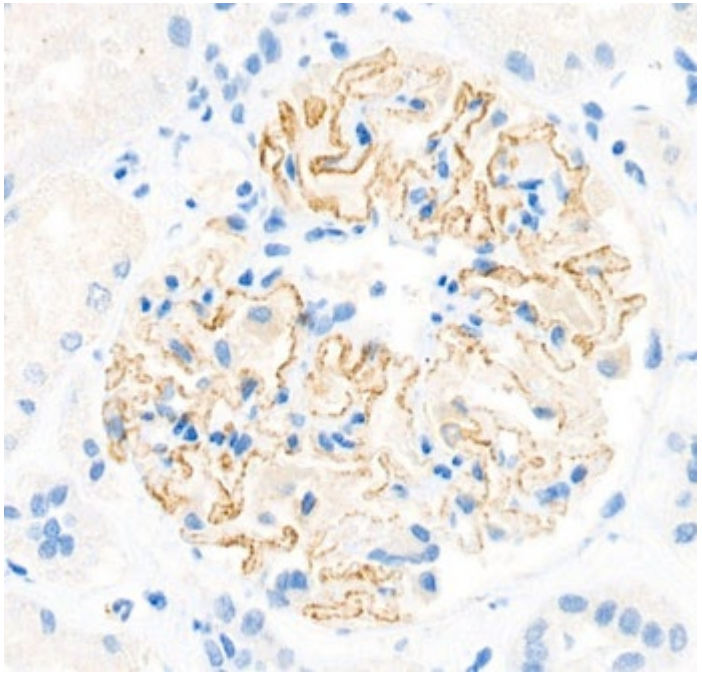
It is done in routine practice for PLA2Rab

It could be developed in routine practice, example NELL1

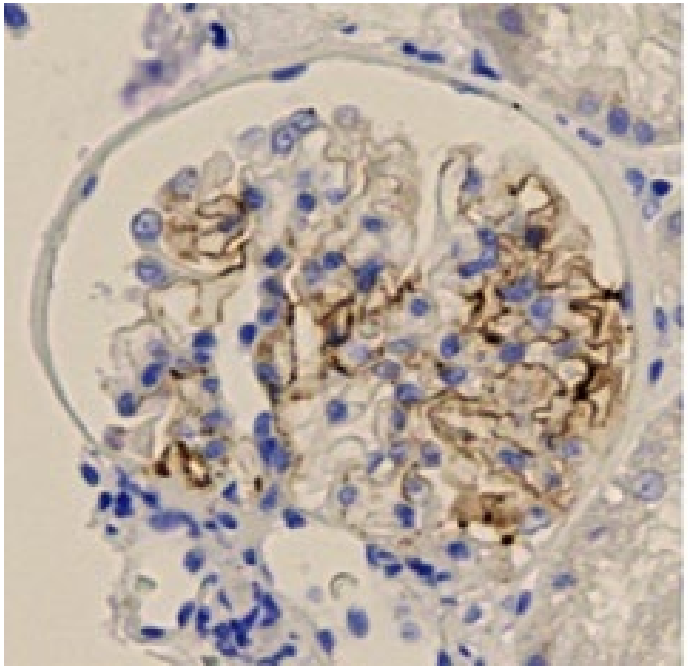
MN: new antigens

- Can the antigen detected by staining of kidney biopsy?

PLA2R



NELL1



CONCLUSIONS

Qualitative detection of PLA2Rantibodies/antigen: very important (diagnosis, guide therapy)

Quantitative detection of PLA2Rantibodies: important as predictor of therapy

Detection of other antigens/antibodies: likely important, but no data yet

Commercial assays: will they be developed? High costs?

Detection of antigen in kidney biopsy: diagnostic value – associated disease

Questions