Finally! Biomarkers of Kidney Pathology for Patients with Lupus Nephritis

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***No conflict of interest with the materials in this presentation**

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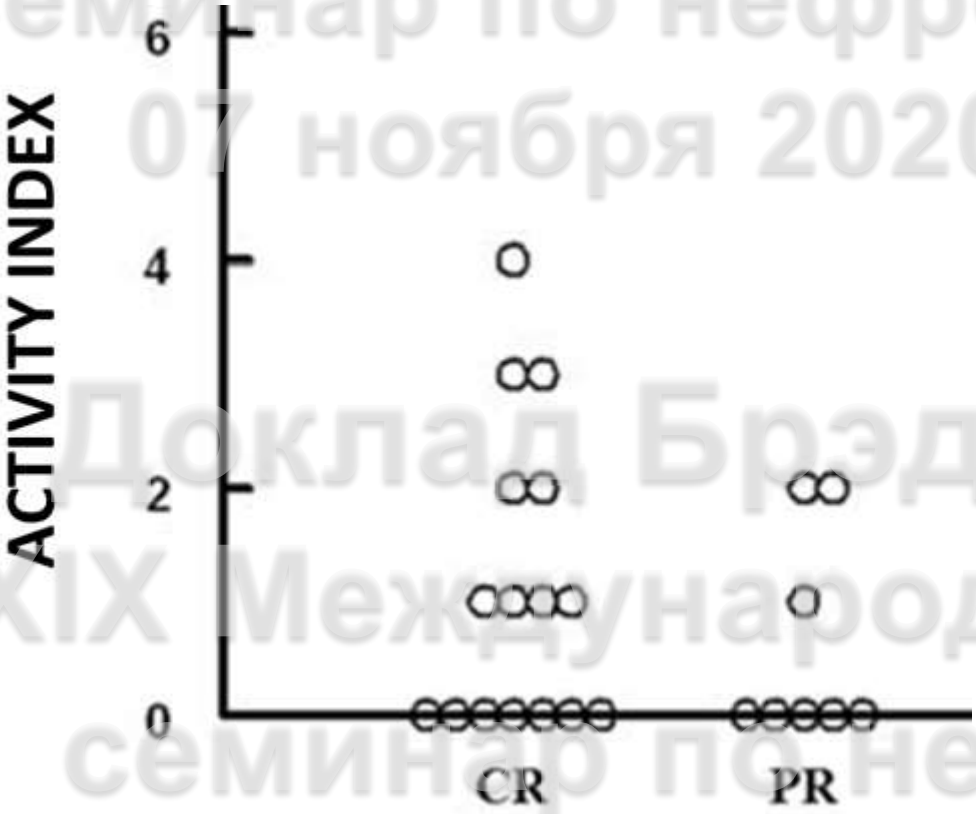
Clinical and serologic data do not sufficiently reflect renal histologic activity or chronicity in lupus nephritis

For Example…

Histologic and Clinical Remission are Discordant

Patients induced for 6 months, ≥30 months of maintenance immunosuppression, ≥12 months of clinical response



- **Only 44% of complete clinical responders had complete histologic remission (AI = 0)**
- **62.5% of the patients with persistent proteinuria (PR) had complete histologic remission**

Alvarado et al, Lupus, 2014

Serology and Histology are Discordant

C3	C4	ds-DNA	Activity Index
170	24	Absent	5
82	14	Present	3
107	14	P	3
66	5	A	2
95	19	A	2
134	11	A	3
124	24	A	1
100	15	P	3
83	18	P	3
122	28	A	4
55	3	A	0

De Rosa et al, Kidney Int, 2018

Without knowing what is going on in the kidney, critical medical management questions arise

For Example…

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XIX Международная школа-семинар по нефрологии (СМЕ)

With Respect to Immunosuppression in LN, We Know That…

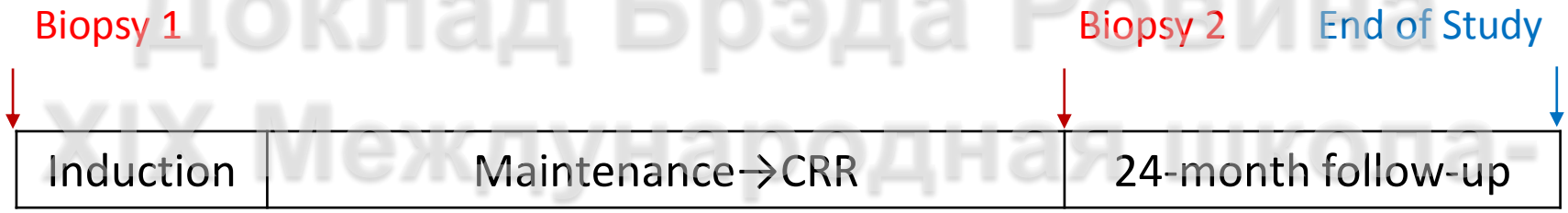
- It would be desirable not to keep patients on life-time immunosuppression for LN
- Presently no one knows how long to maintain immunosuppression in patients who have achieved a complete clinical response
- A complete clinical response does not necessarily mean immune activity within the kidneys has resolved; A partial clinical response does not necessarily mean immune activity within the kidneys is ongoing

We Postulate That…

LN patients in complete clinical remission but who have not achieved complete histologic remission during treatment, defined as persistent immunologic activity on a kidney biopsy done during maintenance immunosuppression, are more likely to experience a flare of LN after withdrawal of maintenance therapy

So We Designed a Study to Test this Hypothesis…

Study Design



Biopsy 1

Variable	Entire Cohort (n=36)	Flare Group (n=11)	No Flare Group (n=25)	P
Age (years)	31.6±11.3	30.0±6.5	32.0±13	0.92
% Male	16.7	9.1	20	0.64
Duration of SLE (mo)	54 (1-240)	120 (12-240)	48 (1-240)	0.03
% With History of Renal Flare	33.3	55	24	0.12
Proteinuria (g/d)	2.1 (0.2-20)	2.1 (0.27-4.6)	2.0 (0.3-20)	0.66
SCr (mg/dl)	0.78 (0.46-2.80)	0.74 (0.55-1.00)	0.80 (0.46-2.80)	0.51
C3 (mg/dl)	84 (25-140)	89 (40-116)	68 (25-140)	0.36
C4 (mg/dl)	12 (0-32)	12 (3-23)	11 (0-32)	0.78
Activity Index	8 (3-16)	11 (4-16)	7 (3-13)	0.23
Chronicity Index	3 (0-6)	2 (0-4)	3 (0-6)	0.24

Biopsy 2

Variable	Entire Cohort (n=36)	Flare Group (n=11)	No Flare Group (n=25)	P
Duration of Treatment (months)	38 (36-54)	38 (36-48)	38 (36-54)	0.61
Time to Remission (mos)	24 (12-40)	24 (16-36)	24 (12-40)	0.75
Duration of Remission (mos)	12 (12-30)	12 (12-20)	13 (12-30)	0.43
Proteinuria (g/d)	0.11 (0.03-0.48)	0.16 (0.06-0.48)	0.07 (0.03-0.48)	0.06
SCr (mg/dl)	0.70 (0.50-1.12)	0.66 (0.60-0.90)	0.70 (0.50-1.12)	0.70
C3 (mg/dl)	112 (55-188)	100 (55-170)	116 (64-188)	0.19
C4 (mg/dl)	19 (3-51)	15 (3-28)	20 (6-51)	0.20
ΔC3	1 (-36-77)	-7 (-30-26)	10 (-36-77)	0.07
Activity Index	0 (0-5)	3 (0-5)	0 (0-2)	<0.0001
% Endocapillary Hypercellularity	30.6	90.9	4	<0.0001
% Subendothelial Deposits	38.9	90.9	16	<0.0001
% Glomerular Leukoctyes	25	45.5	16	0.075
Chronicity Index	3 (0-5)	3 (0-4)	2 (0-5)	0.13

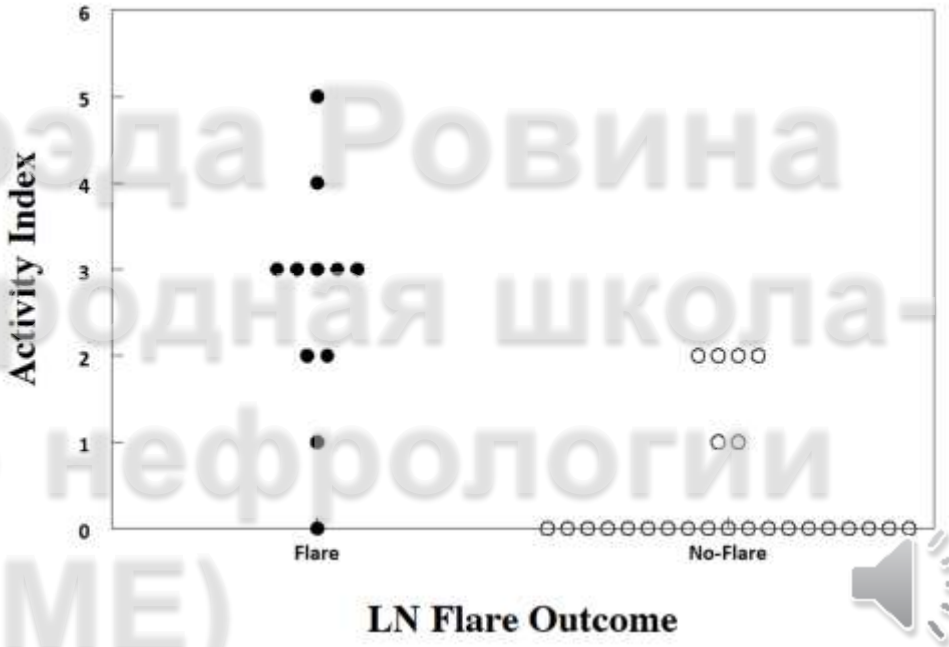
De Rosa et al, Kidney Int, 2018

At Biopsy 2…

- 56% of patients achieved complete histologic remission with an AI of 0
- 44% of patients had persistent histologic activity despite complete clinical renal remission

After Maintenance Immunosuppression Was Withdrawn…

- 31% of patients had an LN flare
- 91% of flares occurred in patients with persistent histologic activity on bx 2
- 100% of patients with an AI>2 flared



De Rosa et al, Kidney Int, 2018

Logistic Regression Models to Predict Future LN Flare

Predictor	Model P-Value	Misclassification Rate	Sen/Spec	AUC
AI BX2 + log duration	<0.0001	0.083*	100%/88%	0.98
Log duration + Endocap Prolif	<0.0001	0.056*	100%/92%	0.99

Unanswered Questions

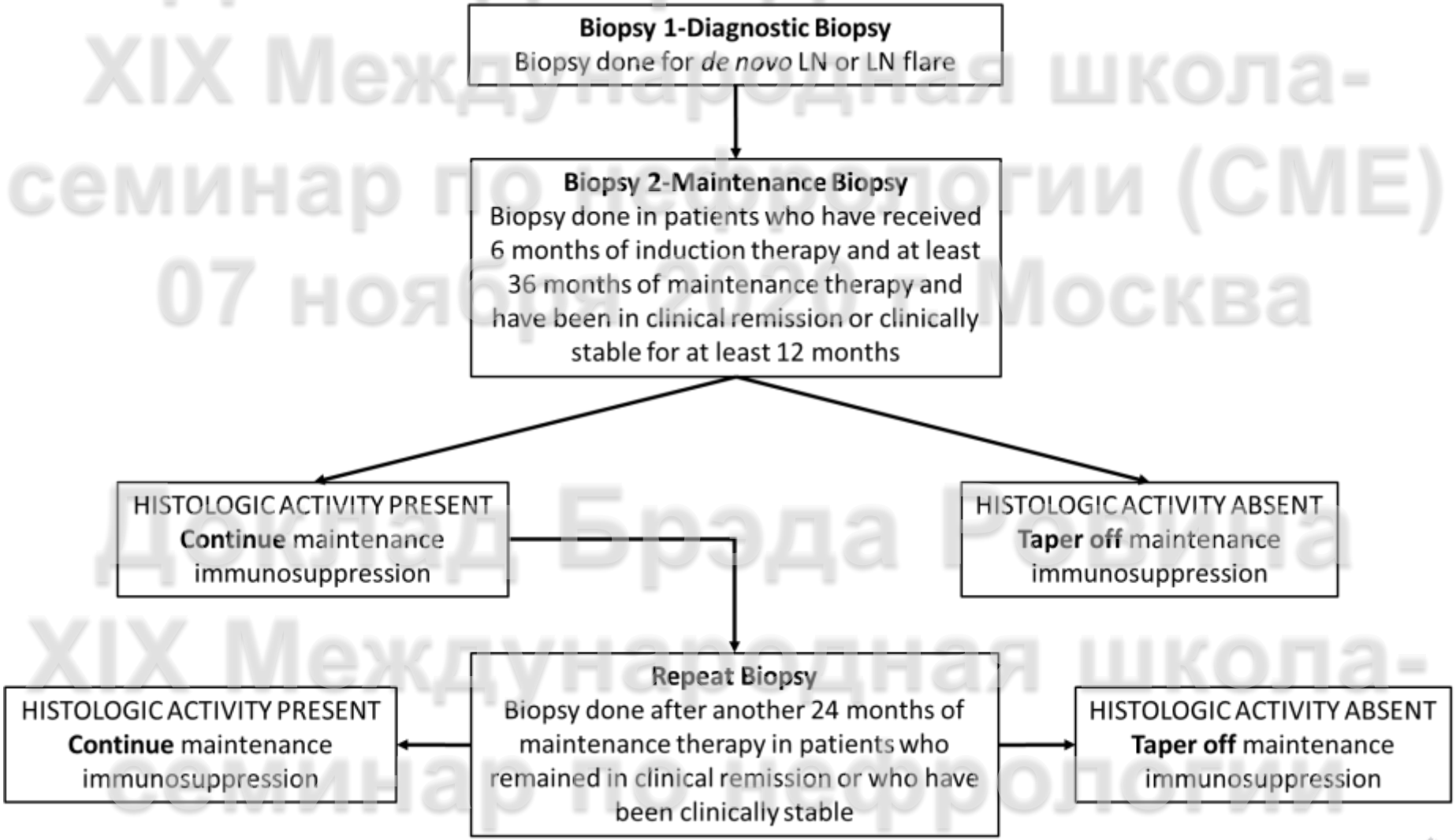
This study did not address:

- Whether continuation of therapy would have prevented flares
- Whether therapy should simply be continued or changed to an alternative immunosuppressant

To address these questions we managed patients using a multiple kidney biopsy protocol…

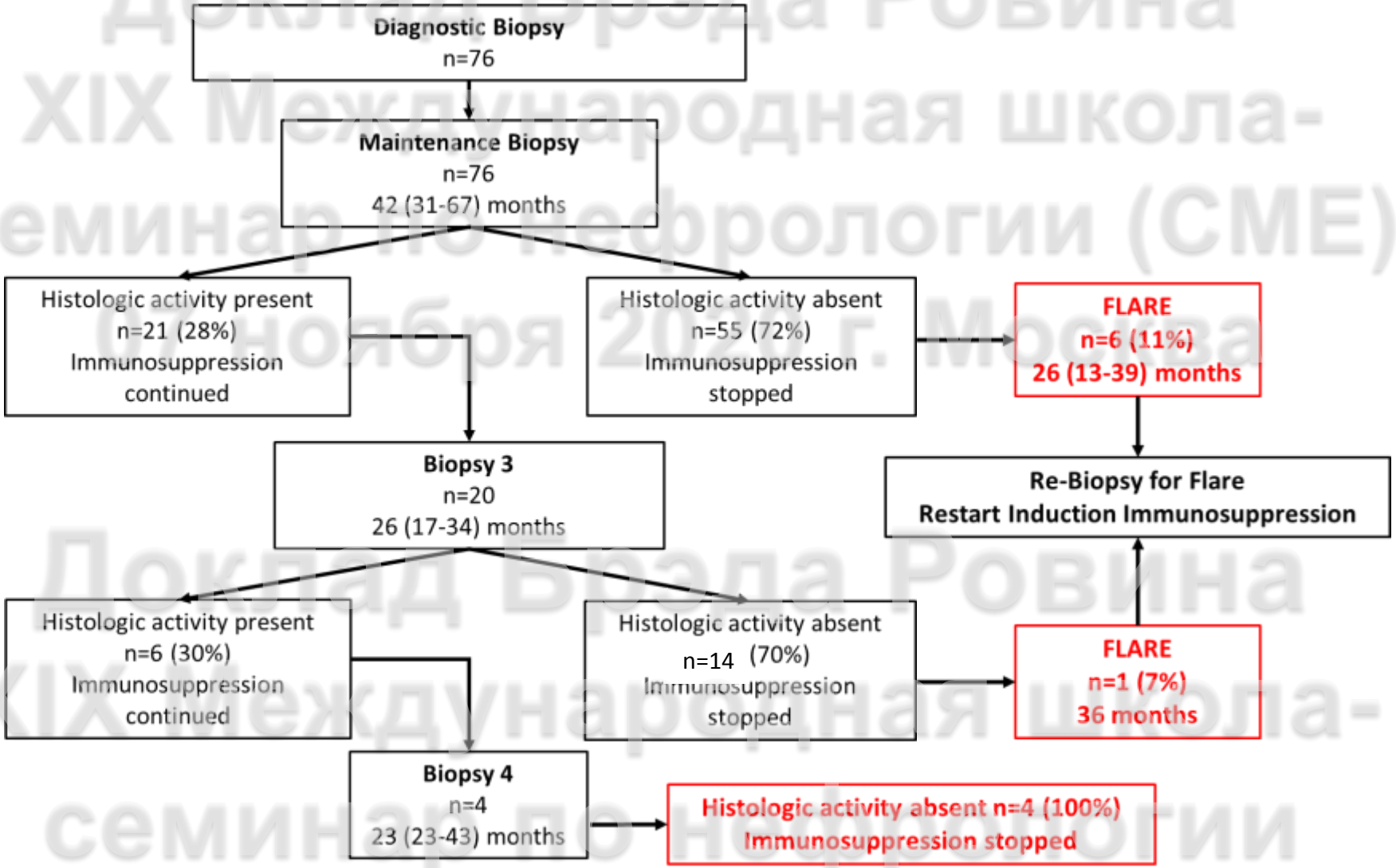
De Rosa et al, Kidney Int, 2018

Protocol Flow



Malvar et al, Kidney Int, 2020

Flare Outcomes



Malvar et al, Kidney Int, 2020

Summary

BIOPSY MANAGED COHORT

 # Flares: 7 (9.2%)

Duration of Follow-Up:

96 (53-165) months

Flare Rate: 0.012/patient-year

USUAL MANAGED COHORT

 # Flares: 11 (31%)

Duration of Follow-up:

62 (60-78) months

Flare Rate: 0.059/patient-year

5-fold lower flare rate in Biopsy-Managed Cohort

Using these flare rates the Number Needed to Treat using the Biopsy Managed Protocol to Prevent One Flare is about 10 Patients

Malvar et al, Kidney Int, 2020

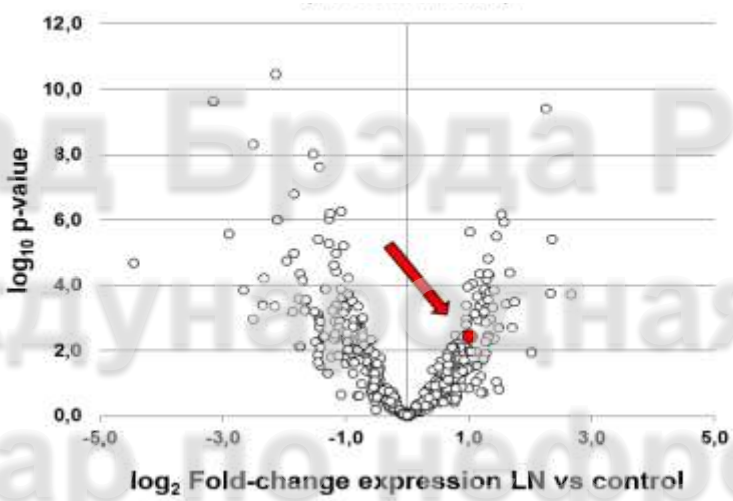
Histology-based management of LN may be effective but it is unattractive; no one really wants to undergo a bunch of kidney biopsies

The solution is non-invasive biomarkers of kidney histopathology!

CD163: A Biomarker of Histologic Activity

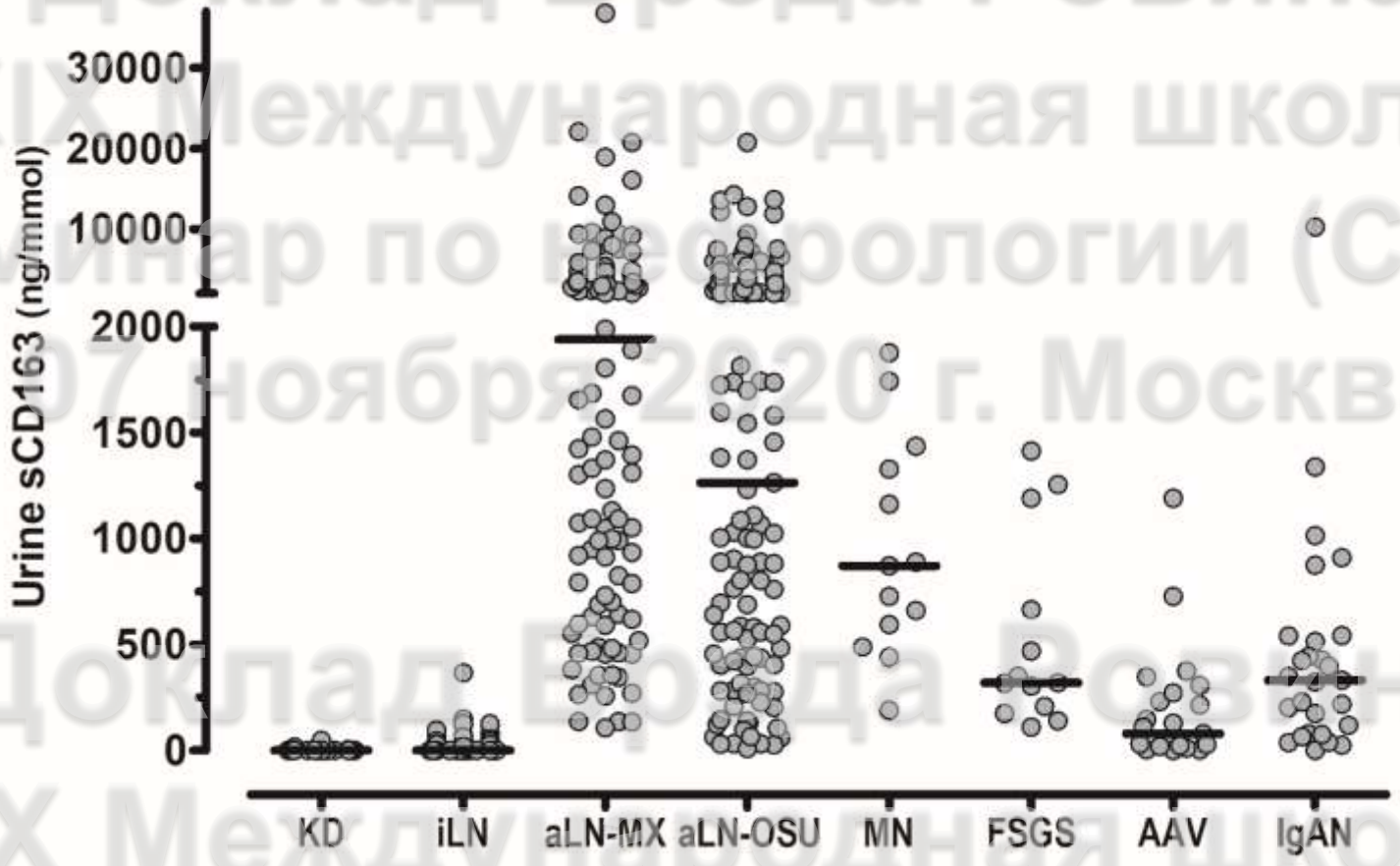
- This biomarker was chosen as biologically plausible candidate (an inefficient but occasionally successful approach to biomarker development)
- CD163-scavenger receptor expressed on tissue infiltrating M2 macrophages
- CD163+ cells have been found in crescents, proliferative GN and acute interstitial nephritis
- We found CD163 gene expression is increased in glomeruli from LN patients
- CD163 cells that infiltrate the kidney in LN may be inflammatory, as opposed to the traditional idea of an M2 macrophage as a clean up cell

Glomerular Transcript Expression



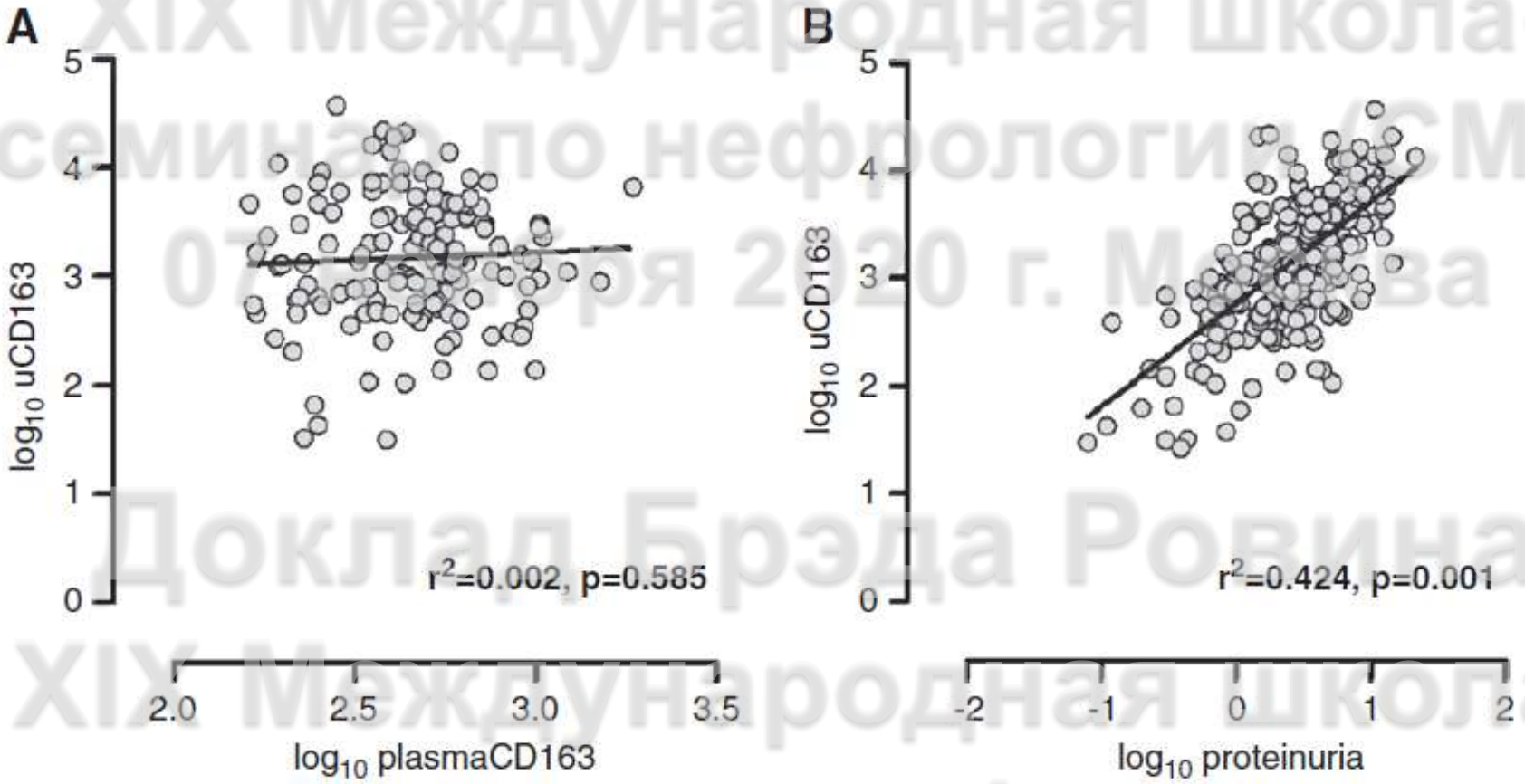
Mejia-Vilet et al, NDT 2019; Dutertre et al, Immunity, 2019

Soluble CD163 is Found in Urine



Mejia-Vilet et al, JASN 2020

Correlation of uCD163 with plasma CD163 and Proteinuria



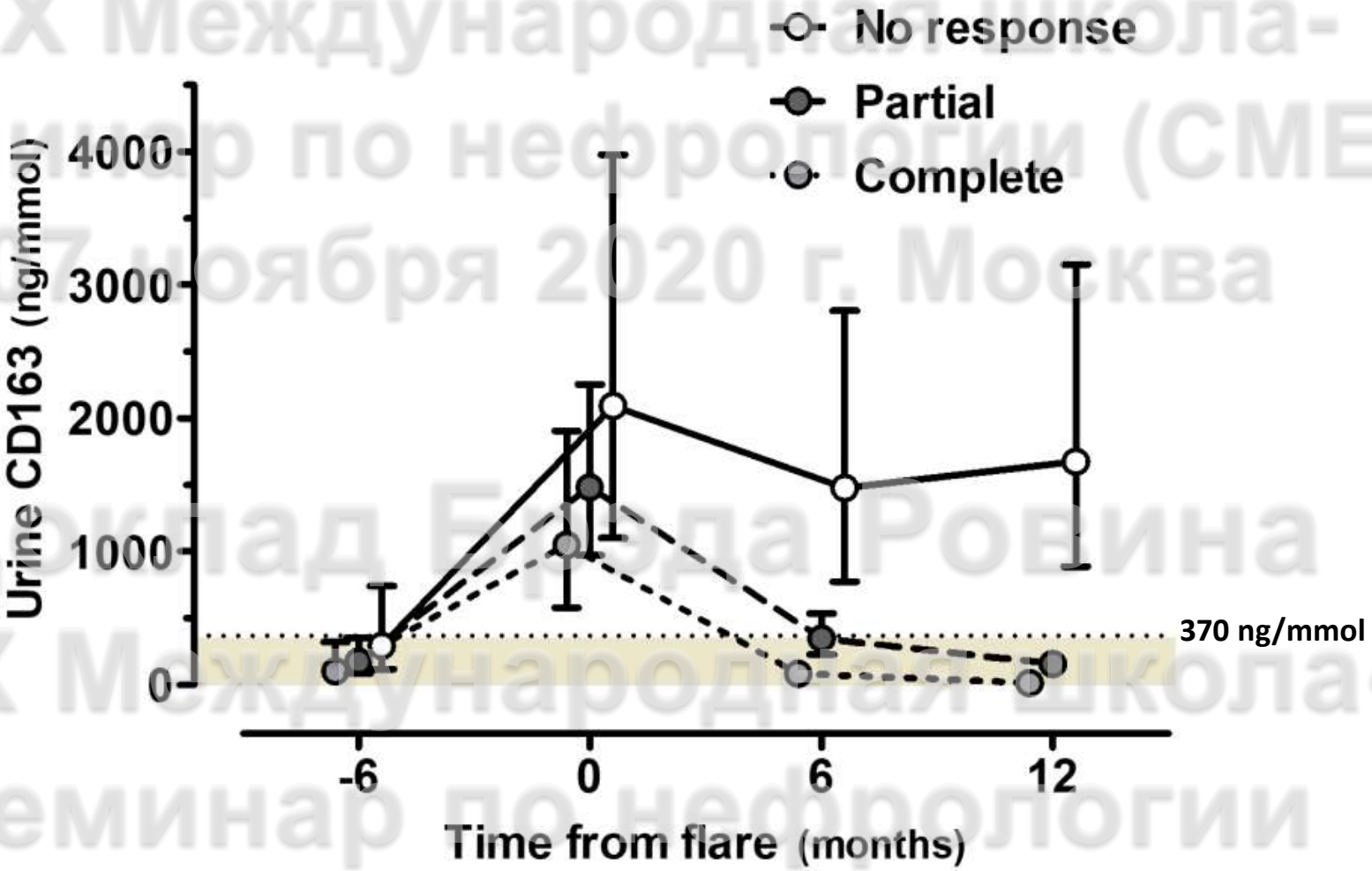
Mejia-Vilet et al, JASN 2020

Urine CD163 and Kidney Histology

	r	P
Activity Index	0.53	<0.001
Chronicity Index	0.11	0.10
Endocap Hypercell	0.37	<0.001
Cellular Crescents	0.34	<0.001
Fibrinoid Necrosis	0.33	<0.001
Wire Loops	0.42	<0.001
Interstitial Inflammation	0.28	<0.001
Glomerulosclerosis	0.19	0.76
Interstitial Fibrosis	0.07	0.30
Tubular Atrophy	0.07	0.24

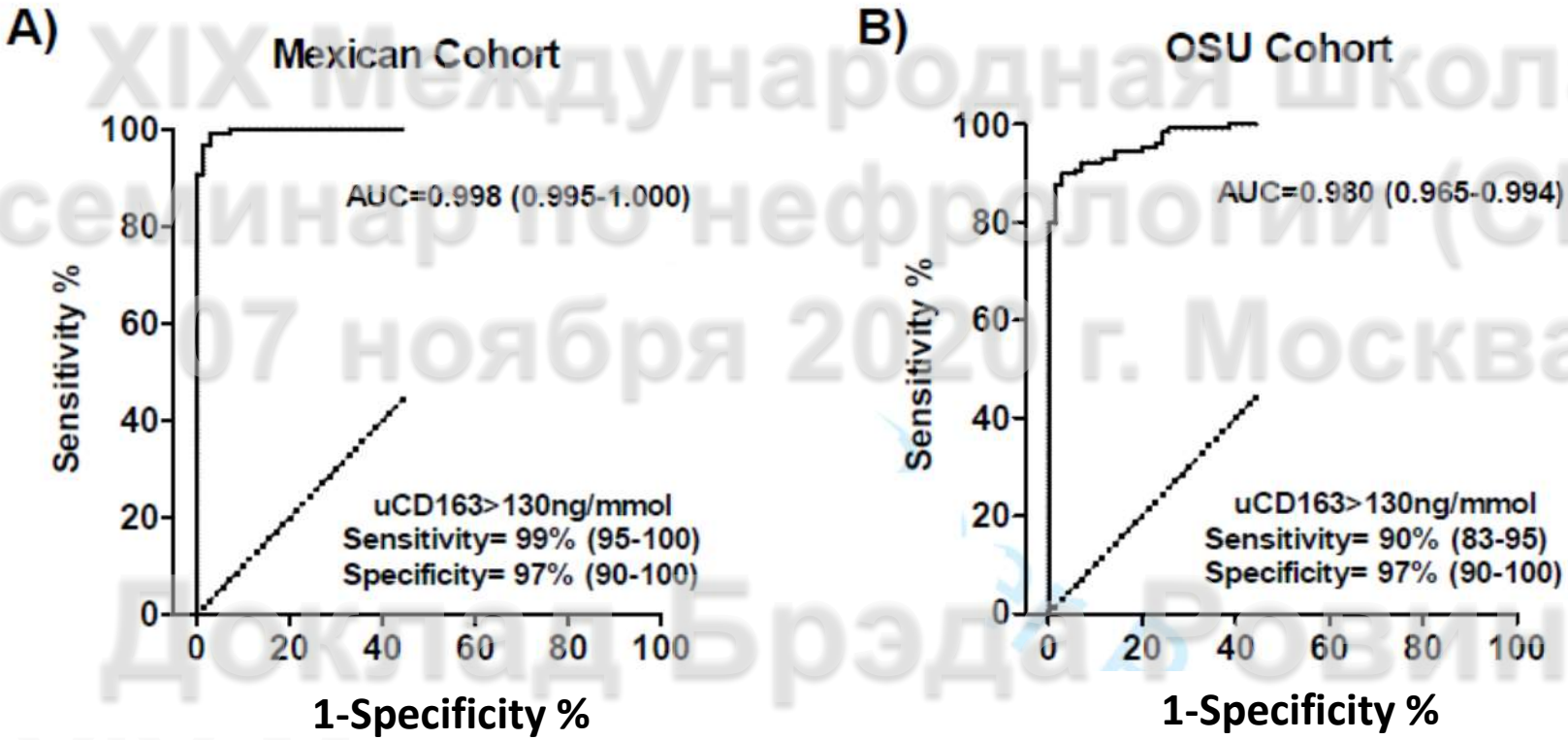
Mejia-Vilet et al, JASN 2020

Longitudinal Measurement of Urine CD163



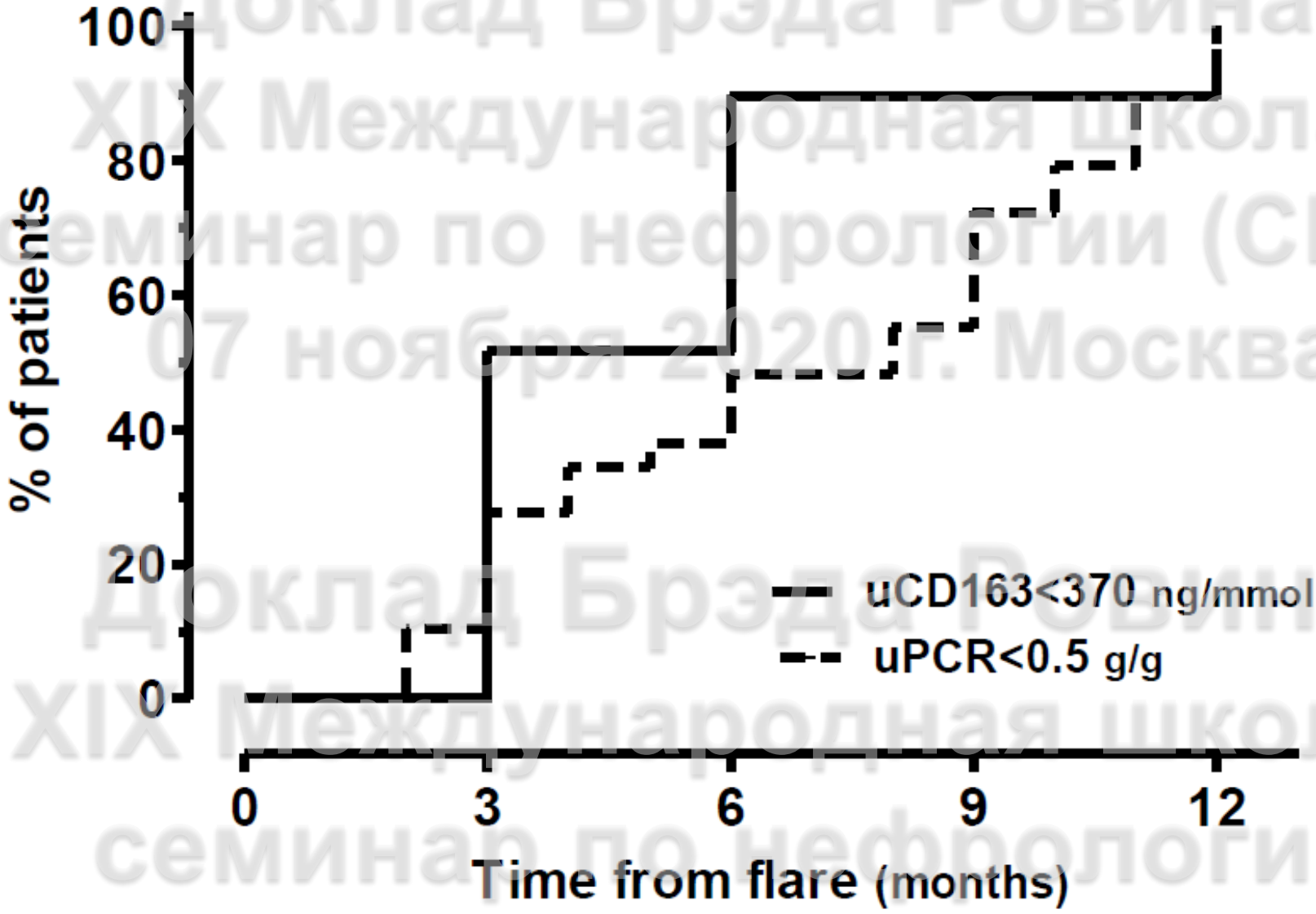
Mejia-Vilet et al, JASN 2020

uCD163 can Differentiate Active from Inactive LN



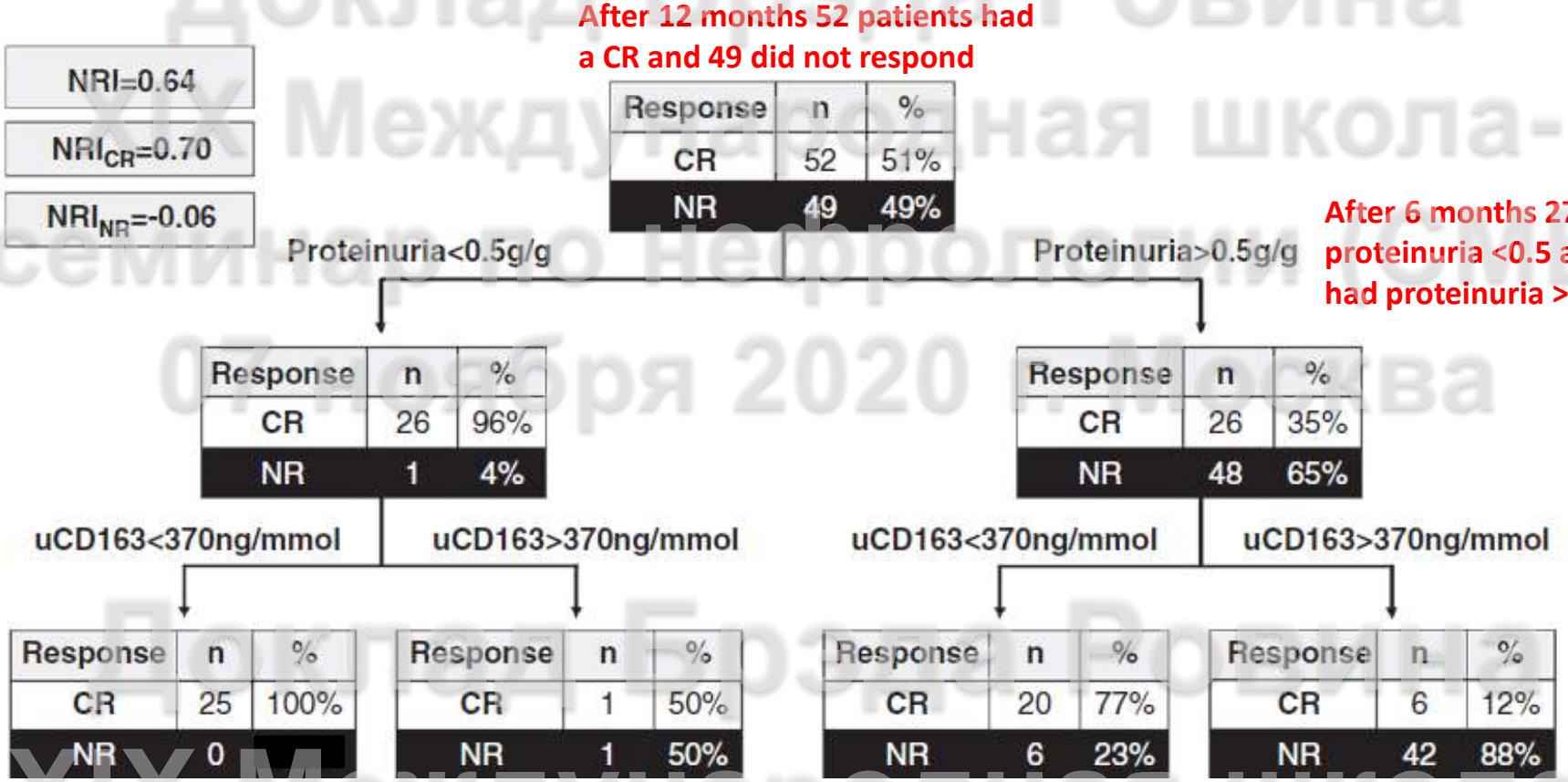
Mejia-Vilet et al, JASN 2020

uCD163 Falls Earlier than Proteinuria as LN Resolves



Mejia-Vilet et al, JASN 2020

Net Reclassification Index Adding uCD163

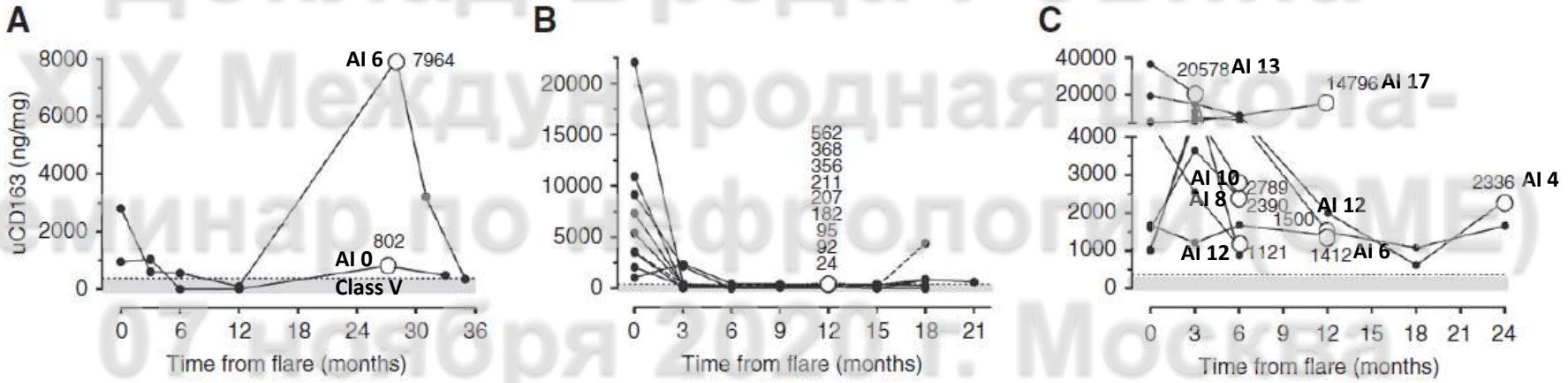


Measuring uCD163 at 6 months did not add value in predicting who would be a CR and who would be a NR at 12 months from patients with low proteinuria at 6 months

Measuring uCD163 at 6 months helped differentiate who would go on to a CR and who would go on to an NR at 12 months from patients with high proteinuria at 6 months

Mejia-Vilet et al, JASN 2020

uCD163 in Patients who were Re-biopsied



Patients who Relapsed | **Protocol Bx in Patients who Responded** | **NR**

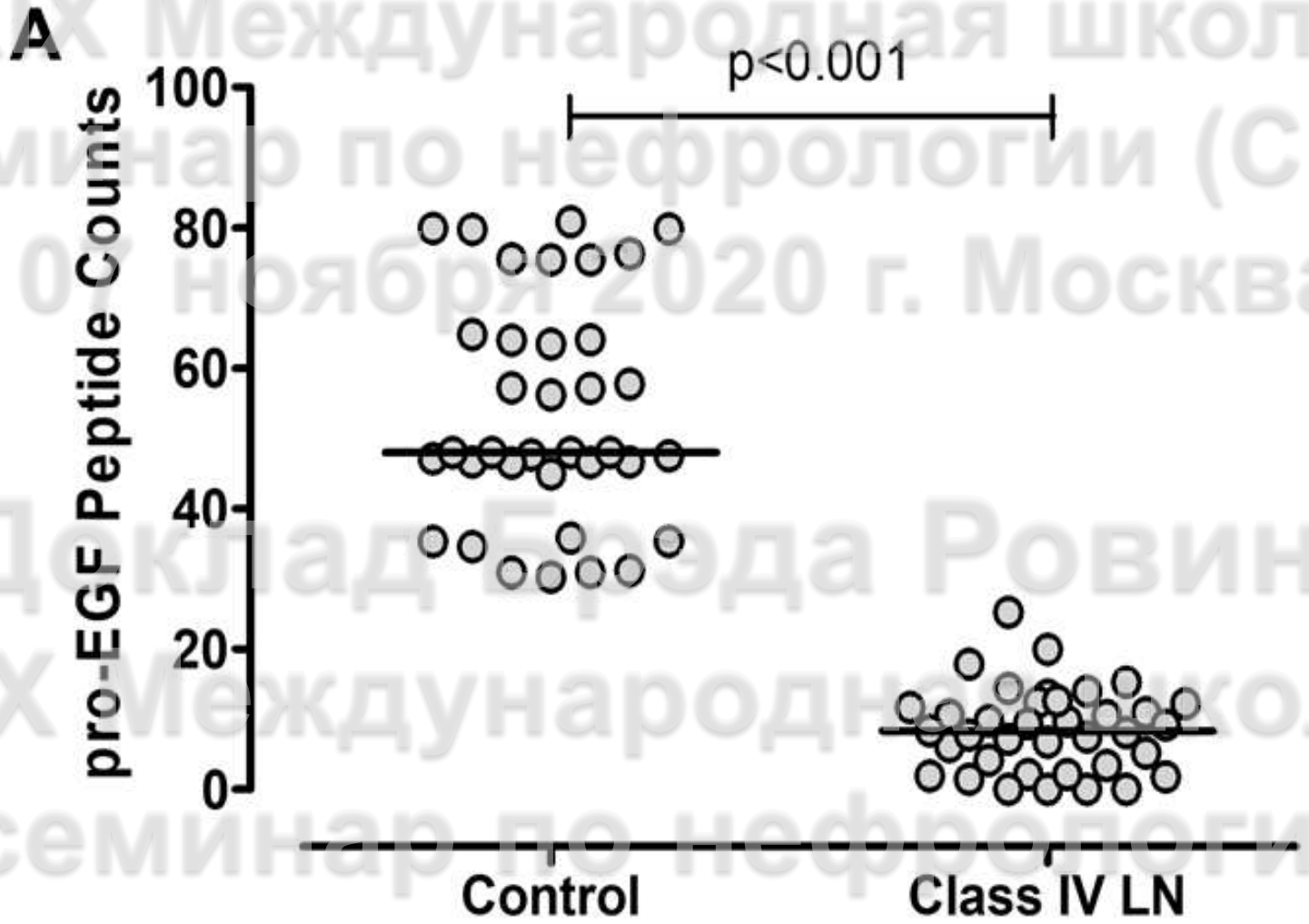
	At Biopsy 1 (at Flare)		At Biopsy 2 (at month 12)	
Patient	AI	uCD163 (ng/mmol)	AI	uCD163 (ng/mmol)
1	8	1075	1	368
2	11	22,072	1	211
3	10	3581	4	562
4	16	10,889	1	92
5	3	3492	0	24
6	10	2082	1	356
7	4	7338	1	95
8	4	9169	1	207
9	14	5401	1	182

The AI was 1 or below in all patients below the uCD163 threshold of 370 and was above 1 in the single patient above the threshold

Mejia-Vilet et al, JASN 2020

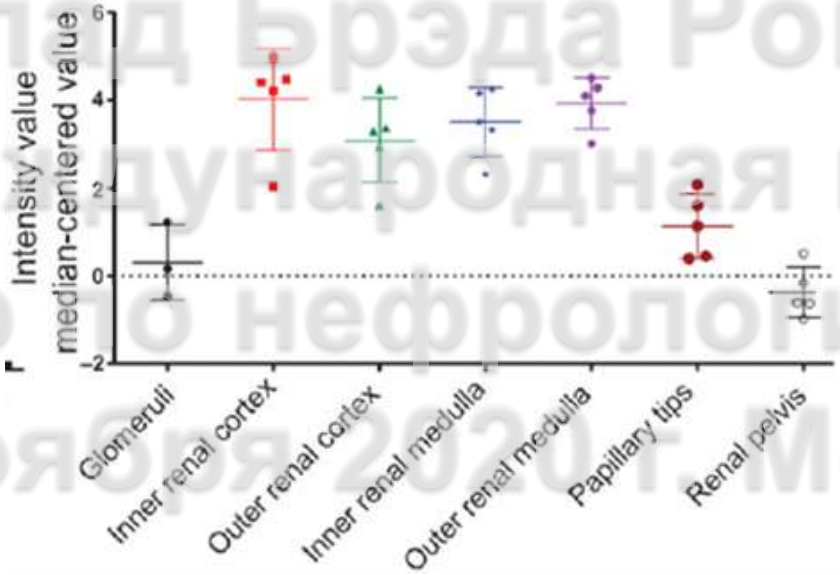
A Biomarker of Histologic Chronicity

Unbiased proteomic analysis of urine showed uEGF to be significantly ↓ in LN



Mejia-Vilet et al, Arth Rheum 2020

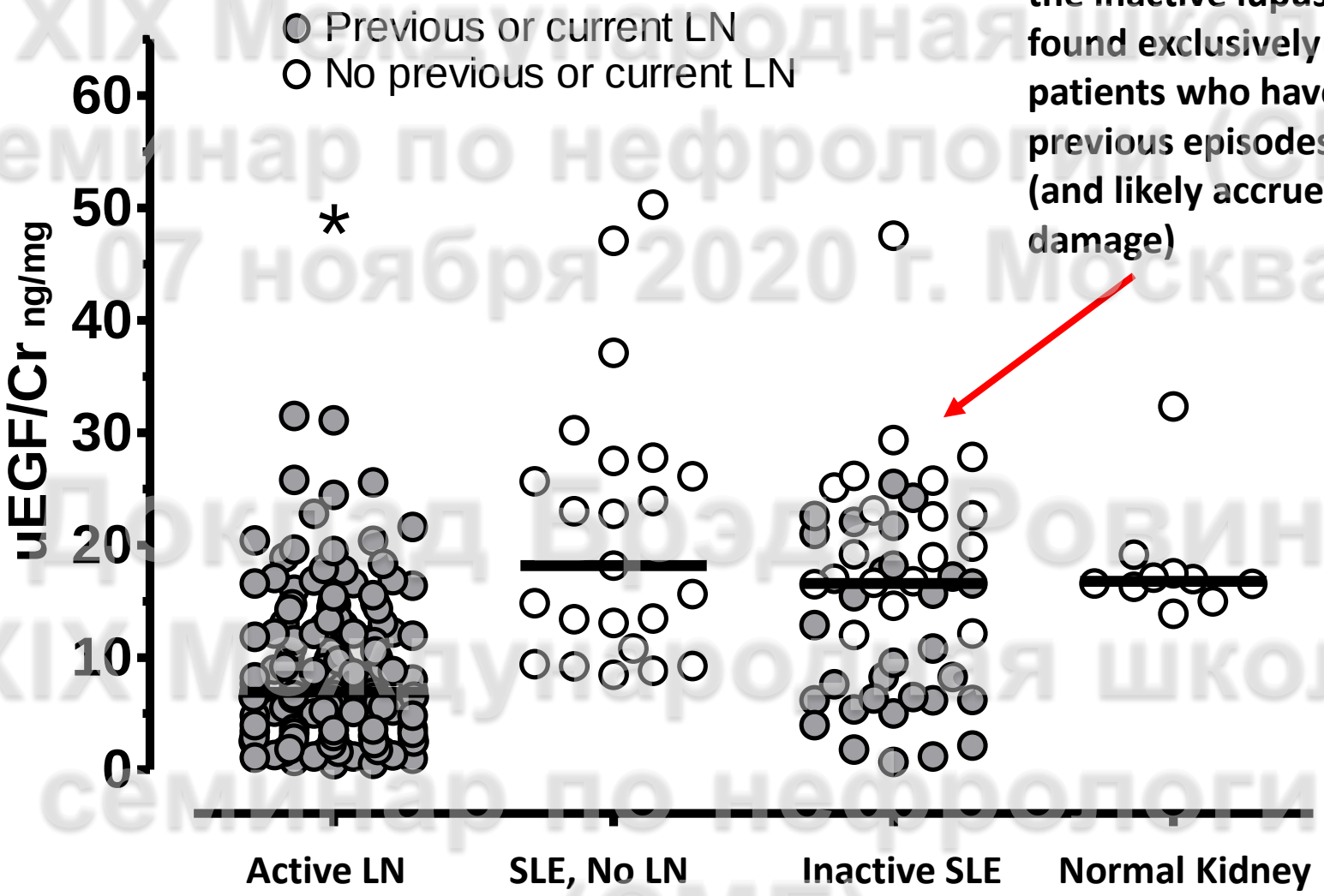
EGF is Highly Expressed in the Kidney



uEGF and Histology	r	P
Activity Index	-	0.66
Global/Seg Glomerulosclerosis	-	>0.10
Interstitial Fibrosis	-0.79	<0.0001
Tubular Atrophy	-0.61	0.002

Mejia-Vilet et al, Arth Rheum 2020

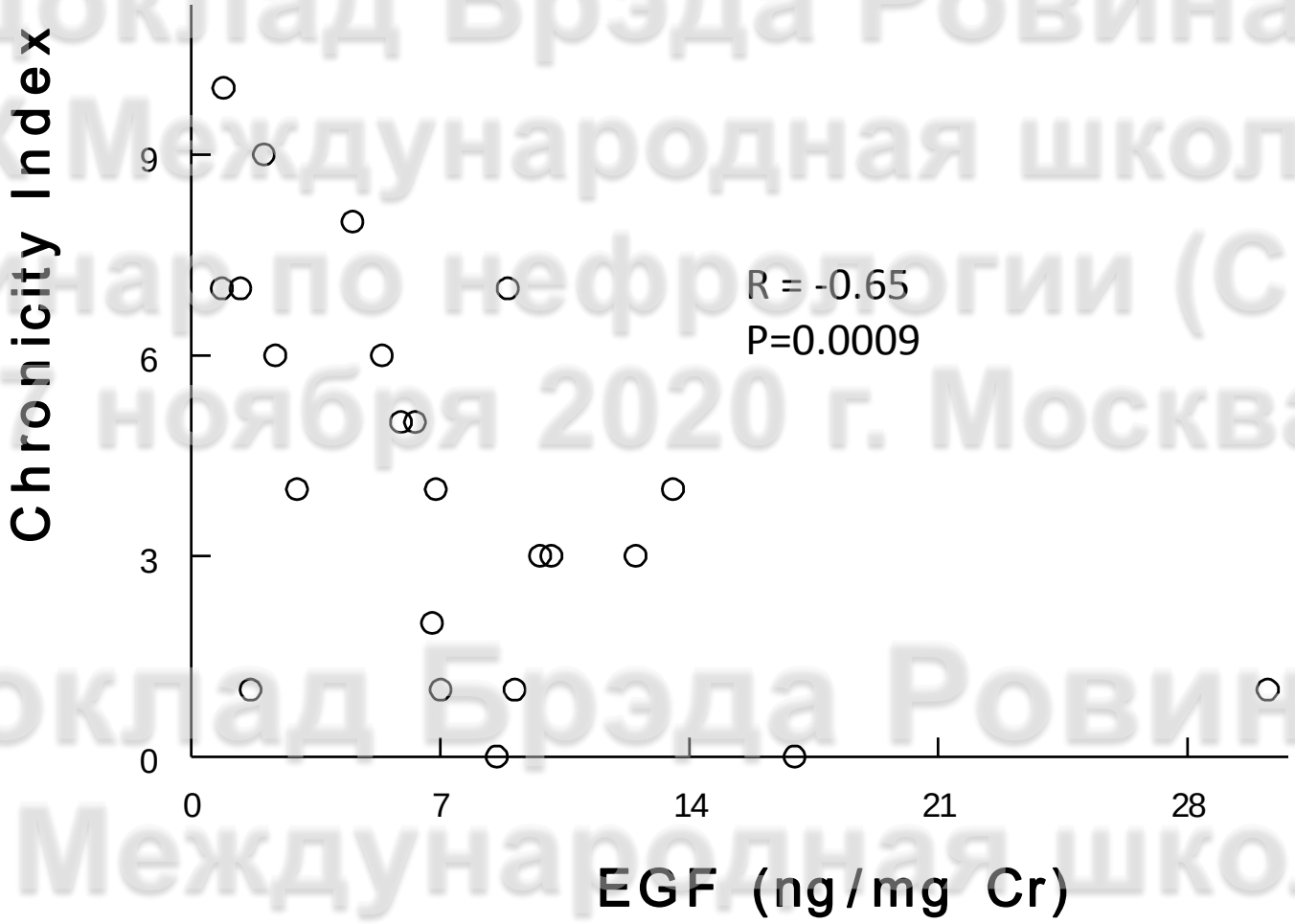
Urine EGF and Lupus Disease Phenotype



The lowest uEGF values in the inactive lupus group are found exclusively in those patients who have had previous episodes of LN (and likely accrued chronic damage)

Mejia-Vilet et al, Arth Rheum 2020

Relationship between CI and uEGF



Mejia-Vilet et al, Arth Rheum 2020

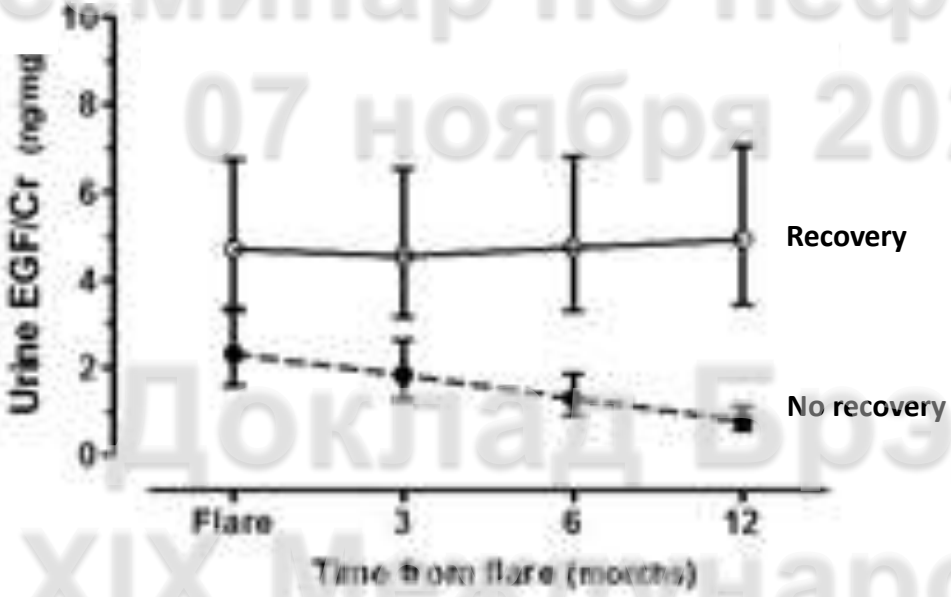
uEGF Detects Chronic Damage Earlier than eGFR/SCr

Chronicity index	Creatinine	eGFR	EGF/Cr	n
0	0,70	110,4	15,5	9
1	0,63	130,5	16,6	9
2	0,79	103,8	9,96	15
3	0,91	93,1	8,41	28
4	1,12	67,8	7,3	26
5	1,02	73,1	7,49	17
6	1,68	42,1	5,36	14
7	1,96	39,7	2,67	11
8	1,89	36,3	3,13	6
9	2,41	30,1	2,06	8
10	3,81	15,5	1,63	10
11	na	na	na	na
12	na	na	na	na

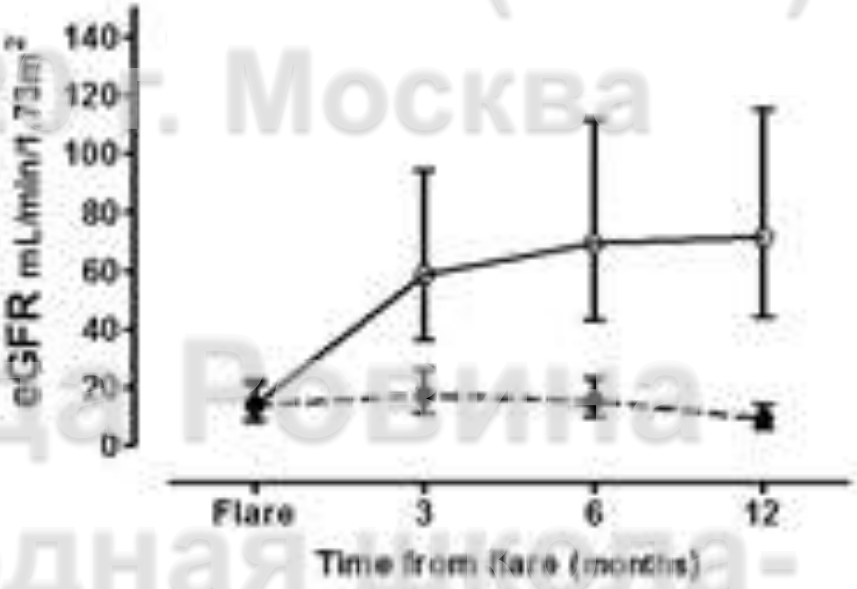
Mejia-Vilet et al, Arth Rheum 2020

Urine EGF and Kidney Function

Time Course of uEGF after treatment in patients with severely impaired kidney function at LN flare



Time Course of eGFR after treatment in patients with severely impaired kidney function at LN flare



Patients who recover (at least some) kidney function (eGFR) have higher levels of uEGF at flare and these levels increase (or at least do not decline further) over time with treatment; Conversely, patients who never recover kidney function have lower levels of uEGF at flare that decline over time as eGFR declines

Mejia-Vilet et al, Arth Rheum 2020

Summary

- For the first time we have two biomarkers that track histologic activity and histologic chronicity
- The biomarkers have biologic plausibility
- The biomarkers have been tested in independent cohorts for validation
- The biomarkers have been examined in cross section and longitudinally, and in relation to biopsy findings

- We envision uCD163 as a non-invasive way to follow the resolution of kidney inflammation during treatment, and may be used to prolong (and maybe shorten) duration of immunosuppression in individual patients

- We envision uEGF as a non-invasive way to follow the development of chronic damage to the kidney that is a more sensitive and earlier marker than SCr and eGFR; after SCr is obviously abnormal, uEGF and SCr/eGFR reflect progression similarly