Biomarkers for Kidney Disease

Lessons From Modeling Lupus Nephritis

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DISCLOSURE STATEMENTS

Dr. Rovin has the following relevant financial relationships. Any real or apparent conflicts of interest related to the content of this presentation have been resolved.

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The Current LN Biomarker Landscape…

- **Papers published on LN biomarkers over the last decade: 266***
- **Unique biomarkers identified: At least 45**
- **Categories of biomarkers: cytokines, growth factors, hormones, adhesion molecules autoantibodies, serum components, cell types**
- **Novel LN biomarkers in routine clinical use: 0**

source-PUBMED; key words-lupus nephritis, human, biomarkers, disease activity, since early spring

What Have the Barriers Been?

There are a number of contributing factors to the lack of novel biomarkers in clinical use, but among the most important are:

- **A biomarker must be validated in an independent set of patients (*discovery is easier than validation*)**
  - **Validation cohorts must be of sufficient size, not a simple task in a rare disease**
  - **Validation cohorts must be well-phenotyped and prospective, especially if the biomarker is to represent a time-dependent clinical feature like flare or outcome**

- **A novel *clinical* biomarker must perform *better than* or *add to* existing clinical markers that are readily available and relatively inexpensive**

So many markers, so little time…

Focus on Biomarker Studies that Break Through These Barriers*

Apologies if your favorite biomarkers are not discussed…

Biomarker Studies with Validation Cohorts

A Urine Biomarker Panel of Active LN in Children

- **Surveyed literature for individual biomarkers that seemed to reflect LN activity**
- **Tested these biomarkers in all combinations to see if they could derive a biomarker panel that reflected active LN**
- **Tested the panel in a discovery cohort; validated the panel in an independent cohort**
- **Biomarkers tested were: MCP-1, NGAL, VCAM-1, TF (transferrin), CP (ceruloplasmin), LPGDS (lipocalin-type prostaglandin D synthase), AGP (alpha-1 acid glycoprotein)**

Smith et al, Ped Nephrol, 2017

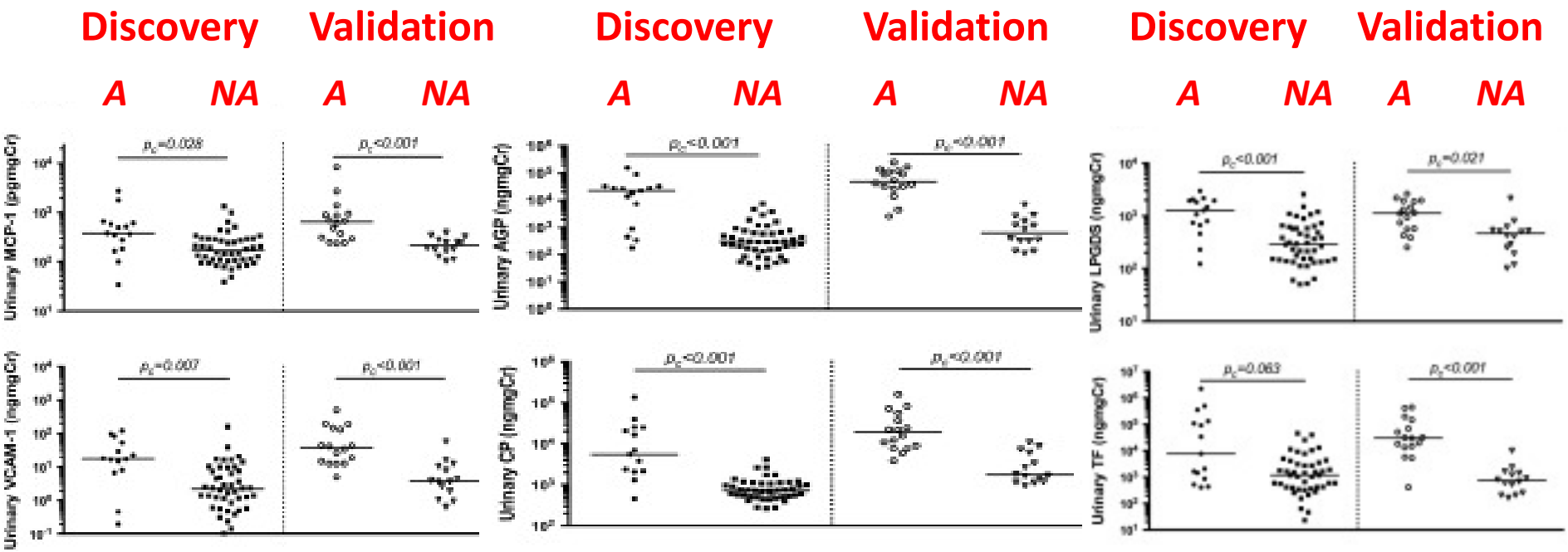
A Urine Biomarker Panel of Active LN in Children (cont.)

- Urines collected from patients with biopsy proven LN or no LN, with or without active extra-renal disease
- Urines were *not* collected at the time of kidney biopsy
- Active LN defined by renal BILAG plus history of LN on biopsy

Variable	Discovery Cohort		Validation Cohort	
	Active LN	Non-LN	Active LN	Non-LN
N	15	46	16	14
Caucasian (%)	13	50	0	0
African Descent (%)	20	11	69	36
Hispanic (%)	0	0	31	57
Carribean (%)	13	4	0	0
Mixed Race (%)	20	0	0	0
Asian-Indian (%)	21	24	0	7
Asian-Chinese (%)	13	11	0	0

Smith et al, Ped Nephrol, 2017

Analyte Levels in Active and Non-Active LN



- **For most of these analytes there is considerable overlap in levels between active and non-active LN**
- **This suggests that no single analyte is sufficient to accurately differentiate active LN from inactive disease**

Building the Panel

- **From these analytes binary logistic regression models using two variables at a time and all combinations of analytes were developed and the model that best differentiated active LN from inactive LN was used as a starting point**
- **In a step-wise fashion the other analytes were added to see if the ability to differentiate active from inactive LN improved based on AUC of ROC**

Analyte Model	AUC Discovery	AUC Validation
AGP+CP	0.881	0.982
AGP+CP+LPGDS	0.900	0.982
AGP+CP+LPGDS+TF	**0.920**	**0.991**
AGP+CP+LPGDS+TF+ VCAM-1	0.920	0.987
AGP+CP+LPGDS+TF+VCAM-1+MCP-1	0.920	-

Smith et al, Ped Nephrol, 2017

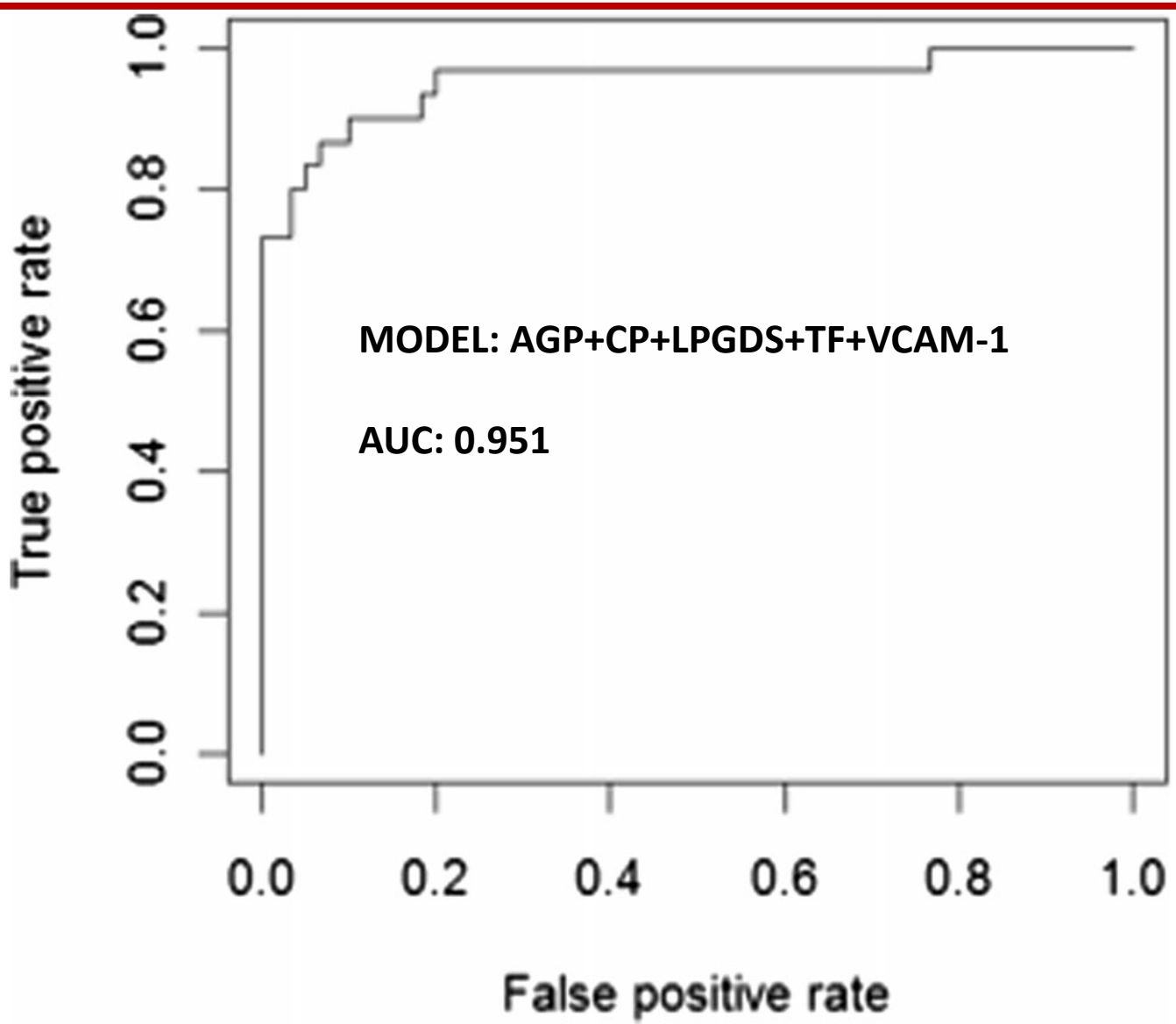
Effect of Traditional Biomarkers

- **Renal function, proteinuria and urine sediment could not be tested because they make up the standard of comparison for this study (BILAG2004)**
- **Tested other traditional Lupus and LN biomarkers used in clinical practice alone and in combination**

Analyte Model	AUC Discovery	AUC Validation
dsDNA	0.617	0.643
C3	0.645	0.638
C4	0.593	0.482
ESR	0.796	-
dsDNA+C3+C4+ESR	0.783	0.670 (no ESR)
AGP+CP+LPGDS+TF+ESR	0.910	-

Smith et al, Ped Nephrol, 2017

Final Model Including Discovery and Validation Cohorts



Smith et al, Ped Nephrol, 2017

Strengths and Weaknesses

STRENGTHS

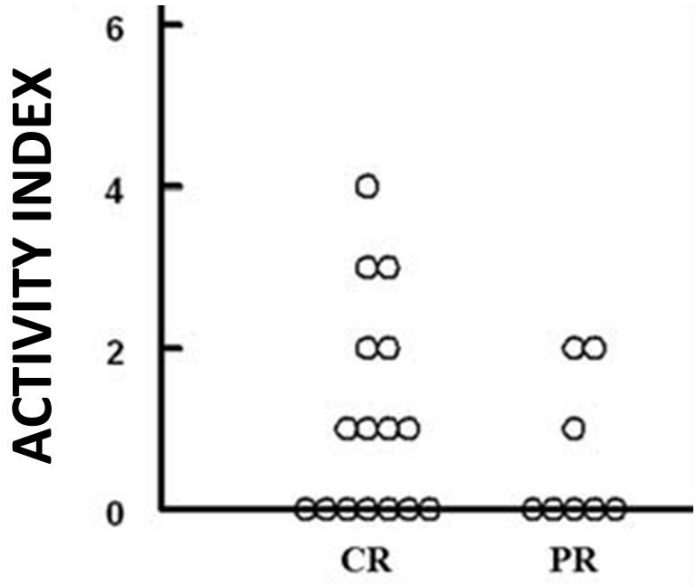
- **Based on promising biomarkers already shown to have some value in the literature**
- **Panel approach-no single biomarker is good enough**
- **Discovery cohort plus a completely independent validation cohort**
- **Considerable patient diversity**

WEAKNESSES

- **This model reflects BILAG (clinical) not histologic LN activity**
- **BILAG is easily measured and inexpensive compared to a novel biomarker panel**
- **Active LN was compared to non-renal lupus; it would be more robust if active LN was compared to non-active LN**
- **Model was developed on cross-sectional data; it would be more robust if built using longitudinal data from patients who have active LN, are treated, and remit**

In Assessing LN Activity What is the *Gold Standard* for Biomarker Development-Biopsy or Clinical Information?

A cohort of Hispanic LN patients who were re-biopsied after at least 42 months of immunosuppressive treatment and 30 months of clinical inactivity/stability



- **Complete Renal Response (CR) Normal SCr and Up < 500 mg/d**
- **Partial Renal Response (PR) Stable SCr and 500 ≤ Up ≤1000 mg/d**
- **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**

Clinical Data *Does Not* Reflect Histologic Activity

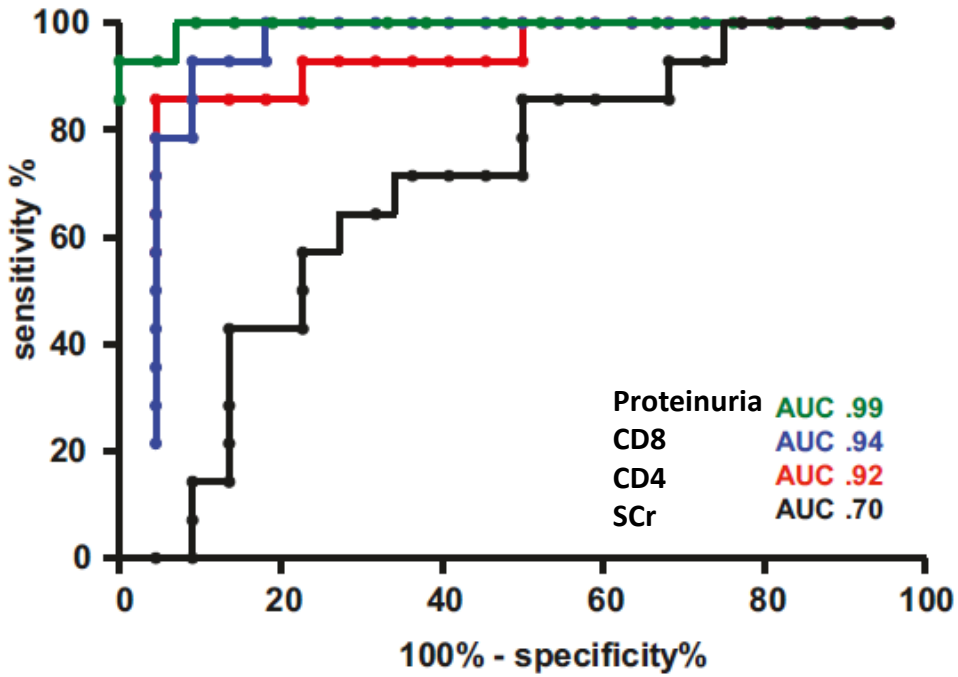
Malvar et al, Lupus, 2014

Urinary Leukocytes as Biomarkers of LN Activity

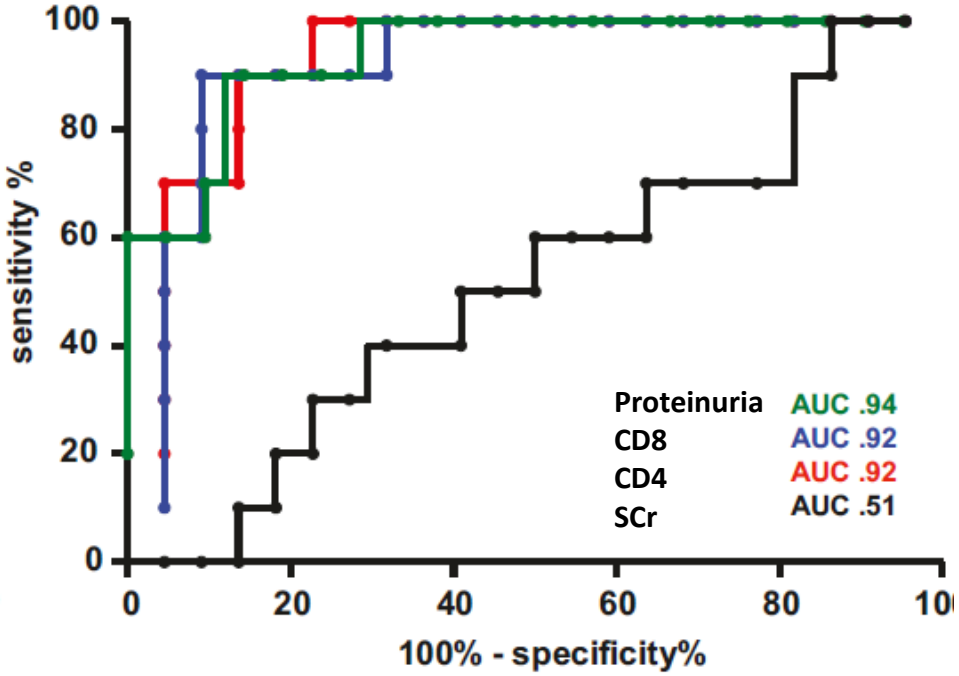
- Observed urine mononuclear leukocytes were increased in patients with new LN and not present in patients with recent LN who had been treated
- Wanted to determine if urine T cells could differentiate patients with active LN
- Active LN was defined as having two of the following criteria:
  - Active urine sediment reflecting glomerular injury
  - New onset proteinuria >0.5g/d
  - Kidney biopsy showing active nephritis
- 21 of 22 patients with active LN had a biopsy at the time of urine collection

Dolff et al, Arth Res Ther, 2013

ROC Analysis of Urine T Cells as Activity Biomarkers



Patients with active LN (n=22) compared to patients with lupus but no history of LN (n=14)

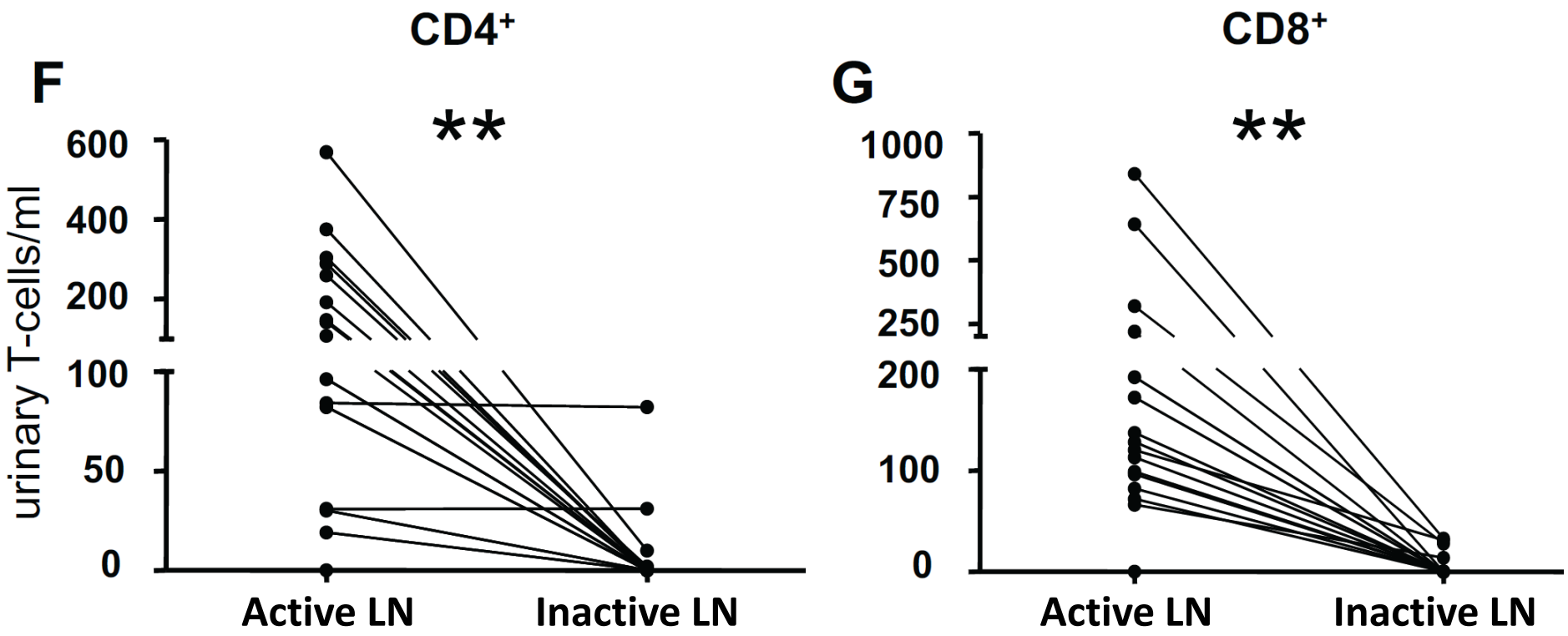


Patients with active LN (n=22) compared to patients with history of LN but not currently active (n=8)

Dolff et al, Arth Res Ther, 2013

Urine T Cells Over Time

Patients with active LN (n=16) were followed until resolution of LN and urine for T cell analysis was obtained; T cell levels were compared during active and inactive disease



Dolff et al, Arth Res Ther, 2013

Strengths and Weaknesses

STRENGTHS

- **LN activity based at least partially on kidney biopsy with urine obtained at the time of biopsy**
- **Longitudinal follow-up**

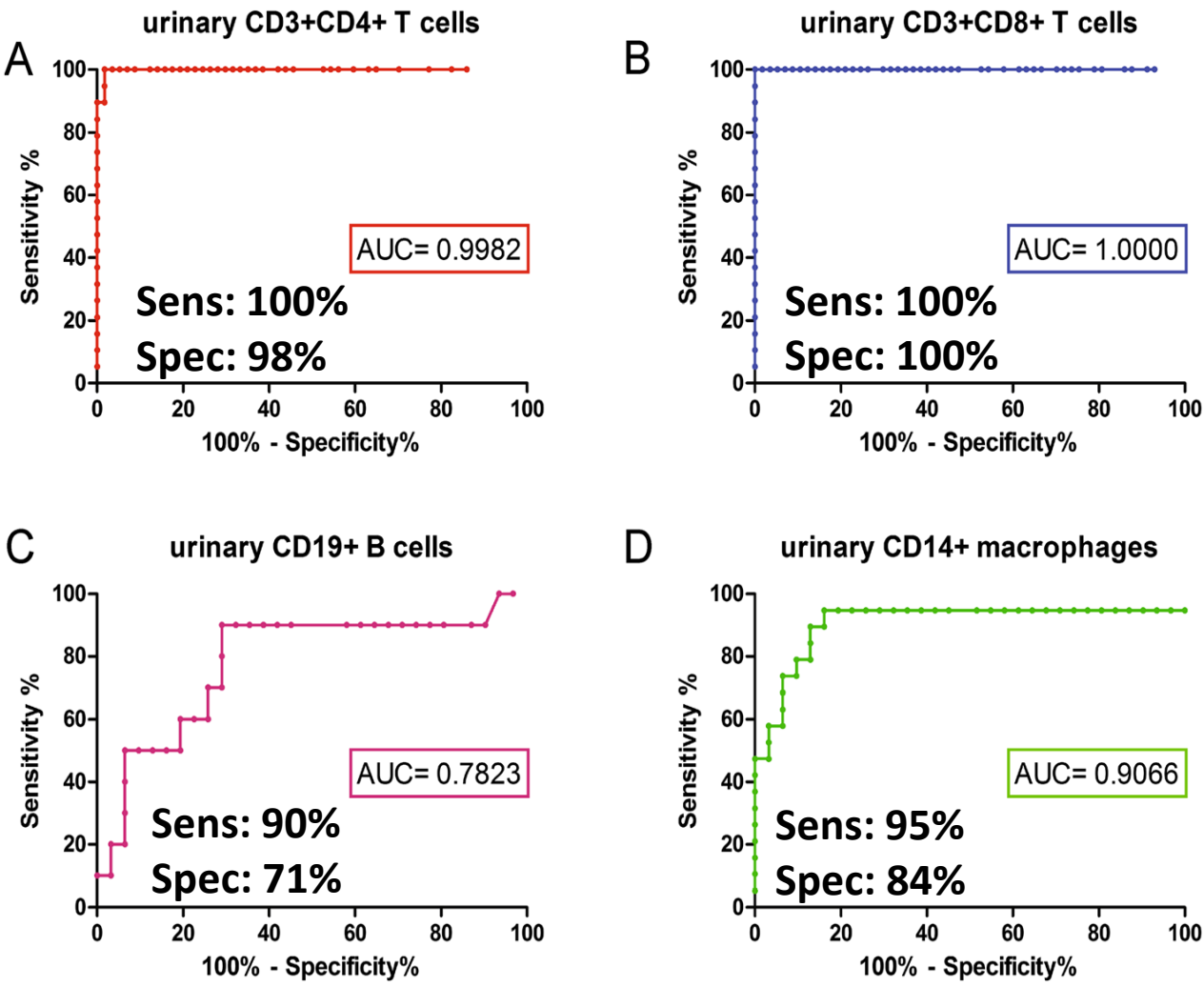
WEAKNESSES

- **Very small cohort; not diverse**
- **No validation**
- **Inactive LN is not proven histologically**
- **Small improvement over proteinuria alone**

-Proteinuria is a good biomarker of LN activity during the initial episode of LN
-Later it is hard to determine if proteinuria is due to active LN or residual scar
-Would like to see this study done with the comparator being histologic activity and the biomarker being urine T cells plus proteinuria

Validation of Urinary Leukocytes

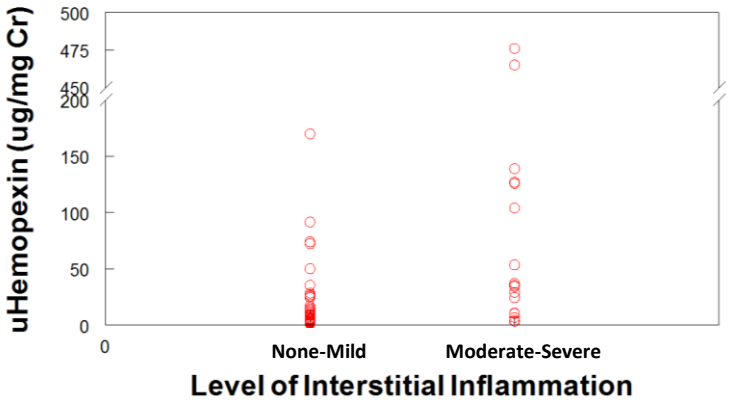
- ROC curves were generated for urinary WBC to differentiate active LN from inactive LN and non-renal lupus
- The AUC for proteinuria was 0.92 and SCr 0.60
- Caveats
  - Small number of patients: LN n=19; SLE n=55
  - Active LN confirmed by biopsy only in 14 patients; in the rest active LN was defined by SLEDAI
  - Inactive LN not verified by biopsy



Kopetschke et al, Arth Res Ther, 2015

Getting More Granular: Biomarkers of Specific Histologic Lesions in LN

- Using an agnostic approach (urine proteomics), several analytes were found to be differentially-expressed when patients who had significant interstitial inflammation were compared to those who did not
- Analyte levels were quantified by ELISA; uHPX is an example
- While the average level of uHPX was significantly different between levels of interstitial inflammation, there was considerable overlap among individual patients
- This degree of overlap precluded use as a biomarker



Solution: Combine Individual Biomarkers with Limited Accuracy Alone into a Composite Biomarker that Predicts Kidney Pathology Accurately and Reliably

How: Linear Discriminant Modeling to Develop and Test Biomarker Panels

Linear Discriminant Modeling

- **The composite biomarker takes the following form:**
  - $Y_{\text{kidney lesion}} = X_1 \ln(\text{analyte}_1) + X_n \ln(\text{analyte}_n)$
    $X_1 \ldots X_n$ are the weighting factors for the n test biomarkers

- **Measured biomarker values are put into the equation to calculate $Y_{\text{kidney lesion}}$**

- **If Y ≥ pre-determined threshold the presence and/or intensity of a pathologic lesion can be diagnosed**

Birmingham et al, Neph Dial Trans Suppl 1, 2017

Analytes Studied

- Osteopontin
- Hemopexin
- Endothelial protein C receptor

} Agnostic Discovery

- MCP-1

} Biologically Plausible

- SCr
- Proteinuria

} Established Clinical Biomarkers

Birmingham et al, Neph Dial Trans Suppl 1, 2017

Training Set: Tubulointerstitial Inflammation or Fibrosis

- Analytes were measured in an adult LN cohort (n=81)
- All combinations of analytes were tested to find the combination that maximized the sum of sensitivity and specificity and gave the fewest number of misclassifications
- The composite biomarkers were tested for their ability to discriminate between:
    - Interstitial Inflammation ≤25% and >25%*
    - Interstitial Fibrosis ≤25% and >25%

**Fibrosis and/or inflammation above 25% has been associated with poor long-term kidney outcomes (ESRD) and below 25% better long-term renal survival*

Results-Training Set

Fibrosis

Y = 2.3*log(Scr) + 1.4*log(uHPX) – 2.1*log(uEPCR) + 7.42*

If Y<0 fibrosis is ≤25%; If Y≥0 fibrosis is >25%

- **Misclassification Rate: 23%**

Inflammation

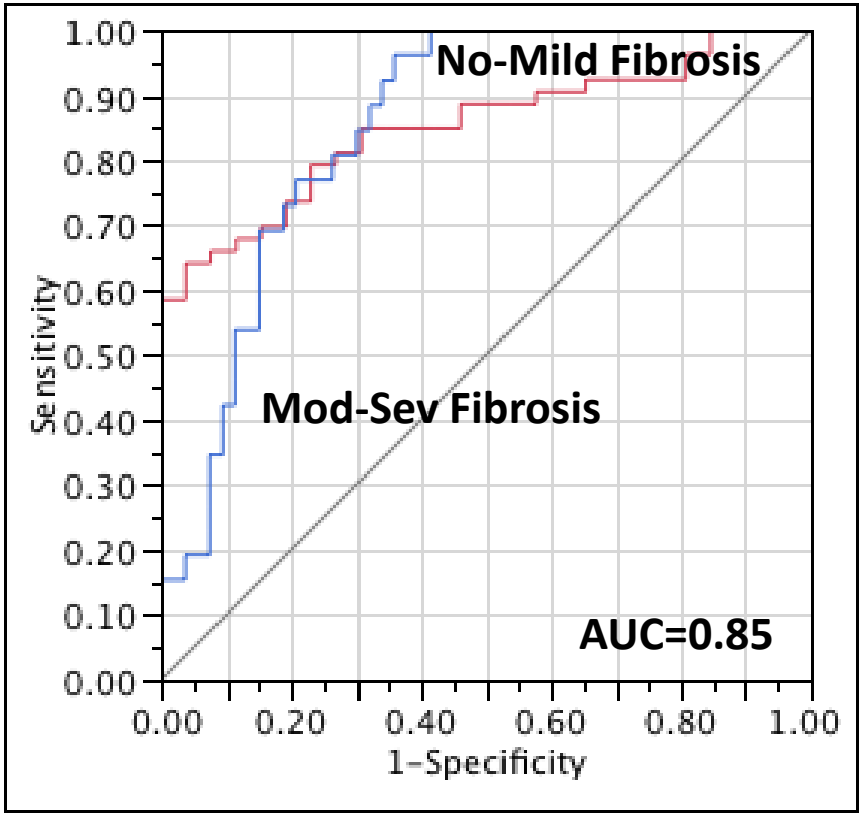
Y = 3.7*log(SCr) + 1.2*log(uHPX) + 1.2*log(uMCP-1) – 3.6*

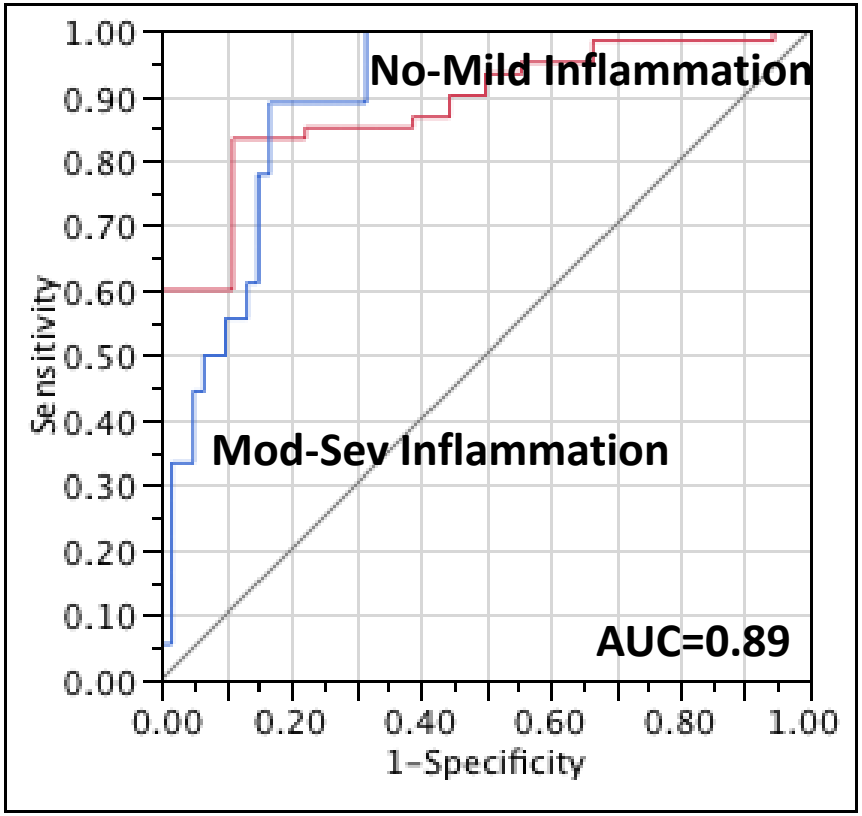
If Y<0 inflammation is ≤25%; If Y≥0 inflammation is >25%

- **Misclassification Rate: 18%**

Birmingham et al, Neph Dial Trans Suppl 1, 2017

Biomarker Performance-Training Set





Birmingham et al, Neph Dial Trans Suppl 1, 2017

Biomarker Performance-Validation Set

Parameter	Interstital Fibrosis Biomarker	Interstitial Inflammation Biomarker
Training Set		
Sensitivity	70%	72%
Specificity	81%	79%
Misclassification Rate	22.7%	22.5%
Validation Set (n=53)		
Sensitivity	44%	80%
Specificity	91%	92%
Misclassification Rate	17.6%	9.4%

- Misclassifications: The interstitial inflammation equation over-classified patients as >25% inflammation. The fibrosis equation tended to over and under-classify equally
- Misclassification rate for percutaneous needle kidney biopsies (gold standard) are ≈25% if 10 glomeruli are present in biopsy and ≈15% if 20 glomeruli in biopsy; difficult to estimate interstitial disease misclassifications
- A non-invasive misclassification rate of 10-20% may be acceptable

Strengths and Weaknesses

STRENGTHS

- **Biomarker compared to biopsy**
- **Urine collected at time of biopsy**
- **Multi-biomarker panel**
- **Validation set**

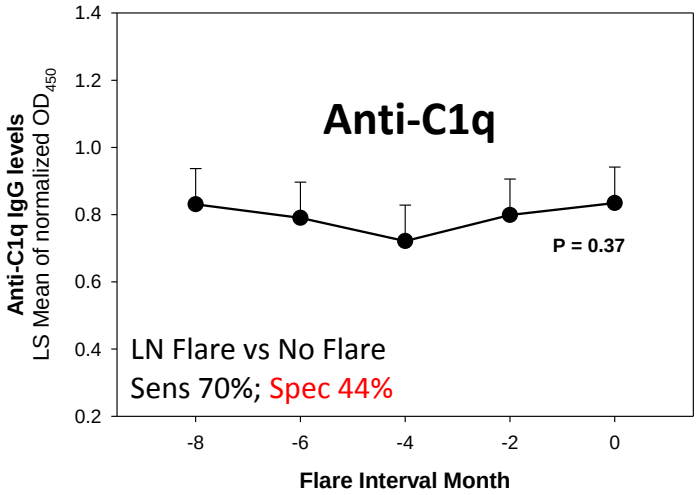
WEAKNESSES

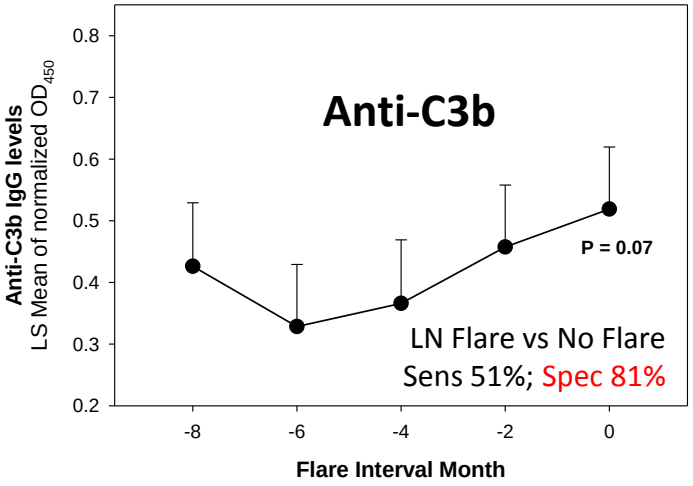
- **Modest cohort sizes**
- **Semi-quantitative estimate of fibrosis/inflammation**
- **No longitudinal data to demonstrate that the biomarkers can be used to follow response to treatment (for example, resolution of inflammation; no progression of fibrosis)**
- **Longitudinal data would ideally require repeat biopsies**

Longitudinal Biomarker Studies

The Importance of Longitudinal Data

- **Anti-complement autoantibodies are seen in SLE but not healthy controls**



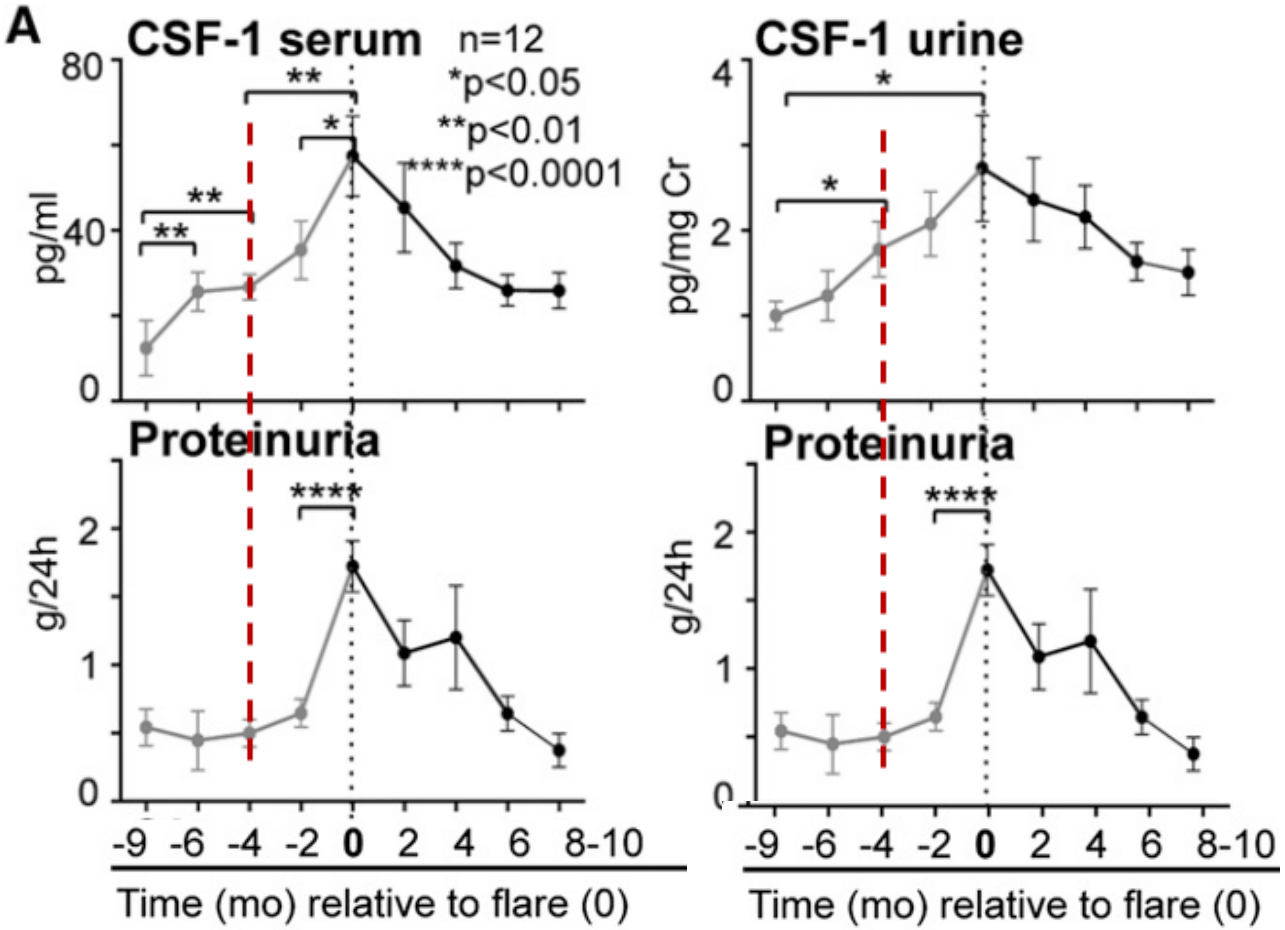


- **Anti-C1q levels *do not* change during periods leading to LN flare**
- **Cross-sectional studies done at LN flare give the impression Anti-C1q is a marker for active LN**
- **However longitudinal analysis suggests anti-C1q does not change much in LN patients who move from quiescent disease to flare; does not discriminate well**
- **Anti-C3b levels ↑ at LN flare, discriminates active from inactive LN better than anti-C1q**
- **Longitudinal data allow analytes to be assessed as predictors of future events (flare)**

Birmingham et al, Clin J Am Soc Nephrol, 2016

Colony Stimulating Factor-1 During LN Flare

- CSF-1 is made by tubular epithelial cells in LN mice and mediates the expansion of M1 macrophages in the tubulointerstitial compartment and subsequent tubular cell apoptosis
- Urine and serum CSF-1 reflect intra-renal, tubulointerstitial CSF-1 expression
- Urine and serum CSF-1 were measured longitudinally during LN flare cycles



The increase in CSF precedes and therefore may predict impending flare, allowing preventative treatment

Menke et al, J Am Soc Nephrol, 2015

Strategy for Biomarker Development in LN

- **Biomarkers should be assessed prospectively and longitudinally in appropriately sized cohorts**
- **Biomarkers should be verified in independent cohorts that represent the racial and ethnic diversity of SLE**
- **Clinical biomarkers should perform better than conventional clinical criteria**
- **To perform better than conventional markers novel LN biomarkers will often need to be measured against the kidney biopsy**

The People That Do the Work…

Ohio State Wexner Medical Center

- Dan Birmingham
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- Michael Merchant

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