to previously reported findings. Serum levels of IFNα and IFNγ were associated with autoantibodies, consistent with their roles in B lymphocyte regulation. While IFNs were associated with overall disease activity and renal disease, skin disease activity was not associated with IFNα and was instead associated with lower levels of IFNγ, consistent with a regulatory function. If confirmed in future studies, these findings suggest that cytokine levels have potential to identify patients with significantly high organ-specific disease activity.

REFERENCE

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305 USING ACR-COMPONENT BASED UNSUPERVISED CLUSTERING WITH OLINK PROTEOMICS TO RESOLVE SLE HETEROGENEITY

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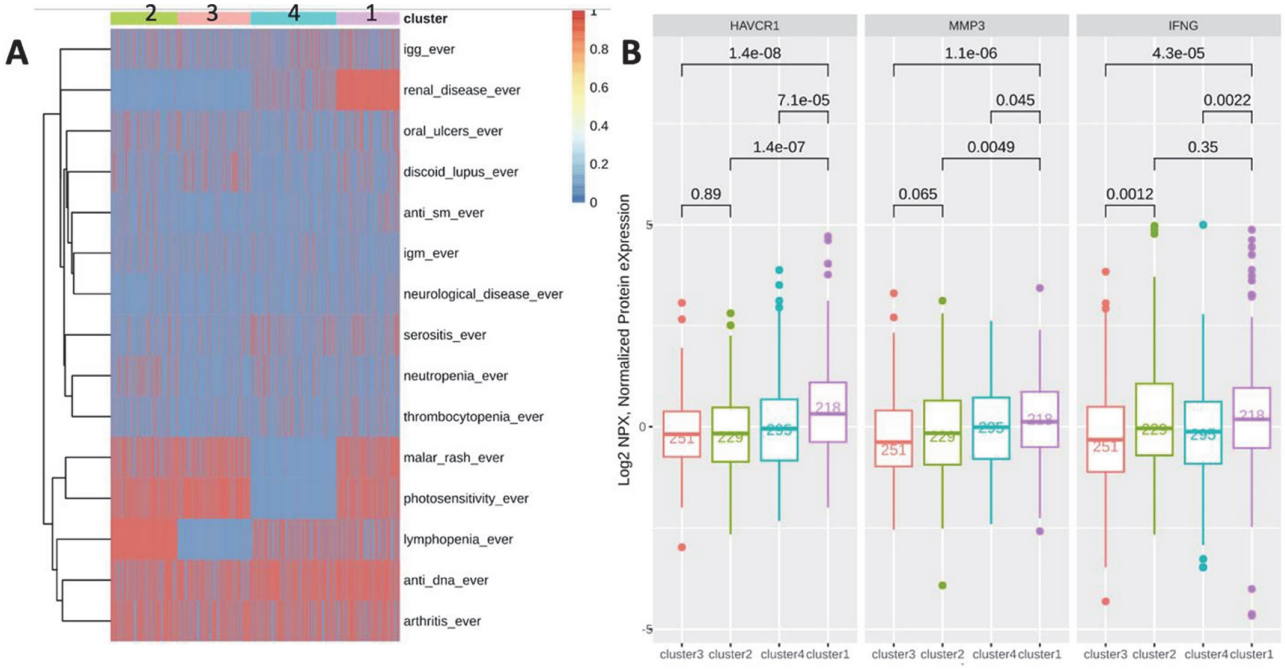
Background The high heterogeneity in systemic lupus erythematosus (SLE) implies that differential treatment may be required for different patient strata, hence there is an increased need of accurate patient stratification to increase treatment success.

Methods Patient serum samples from a Genuity Science cross-sectional cohort of SLE patients ranging from minimal to high disease activity (total SLEDAI range 0 - 32) on current standard-of- care treatment, sampled at a single timepoint, were profiled on the Olink Explore proteomic panel. The output of 1428 protein analyte relative concentrations was normalized and corrected for differences in site, sex and age. Low variance proteins were filtered out with median absolute deviation above 1. Unsupervised k-means clustering was performed on ACR components ('ever' criteria). The association of proteins with these clusters was evaluated with Kruskal-Wallis tests, random forest and limma differential expression analysis.

Results Unsupervised k-means clustering yielded four patient clusters as the optimal solution, characterized in figure 1A and in the table 1 below. Approximately two thirds of the patients had reported anti-dsDNA antibodies and arthritis. Half had malar rash and lymphopenia, and one third had renal manifestations. The clusters in the table 1 are arranged left to right in the order of increasing disease severity. The disease severity was determined based on clusters association with SLEDAI and number of renal manifestations. Patients in Cluster3 had the least severe disease, displaying the lowest symptom burden with only joint/skin manifestation. Patients in Cluster2 had minimal renal disease, whereas cluster4 has no malar rash but increased numbers of renal cases. Patients in Cluster1 had the most severe disease, with all four organ manifestations. The differences between clusters were statistically significant according to chi-squared test for all parameters except arthritis.

Kruskal-Wallis tests of the Olink proteomic dataset identified 10 proteins that were significantly (FDR < 0.05) associated with these four clusters. The top three proteins with FDR less than 0.01 were: Kidney injury molecule 1 (HAVCR1), interferon-γ (IFNG) and matrix metalloproteinase 3 (MMP3) (figure 1B). HAVCR1 was significantly upregulated in cluster1 compared to all others. IFNG was significantly upregulated in cluster2 and cluster1. MMP3 was also increased in severe clusters2,4,1. The other seven significant identified proteins were: CXCL13, FABP1, SSC4D, DDX58, BST2, MEP1B, GH2. Top significant proteins were also confirmed by random forest and differential expression analysis.



Abstract 305 Figure 1 A) Results of unsupervised k-means clustering; B) Boxplots showing expression of top 3 discovered proteins with respect to clusters

Abstract 305 Table 1

	Overall (n=1009)	*cluster3 (n=255)*	*cluster2* (n=234)	*cluster4* (n=299)	*cluster1* (n=221)	*p-value*
Renal disease ever	326	0	1	104	221	<0.0001
Malar rash ever	502	175	156	2	169	<0.0001
Lymphopenia ever	523	0	234	168	121	<0.0001
Arthritis ever	703	169	174	205	155	0.259
Anti-dsDNA ever	769	170	163	241	195	<0.0001

Conclusions Using ACR component criteria in an unsupervised fashion, we were able to stratify the patients into four clusters with different levels of severity based on their historical symptomatology. These clusters have significant associations with serum protein markers derived from the Olink platform. The top three identified proteins (HAVCR1, MMP3, IFNG) were associated with SLE disease manifestation and could potentially be used to classify patients into different severity clusters. Further study is needed to validate the findings in additional cohorts and test for prognostic value.

306 PERSISTENCE OF URINARY BIOMARKERS OF INTRARENAL INFLAMMATION PRECEDES LOSS OF KIDNEY FUNCTION IN LUPUS NEPHRITIS

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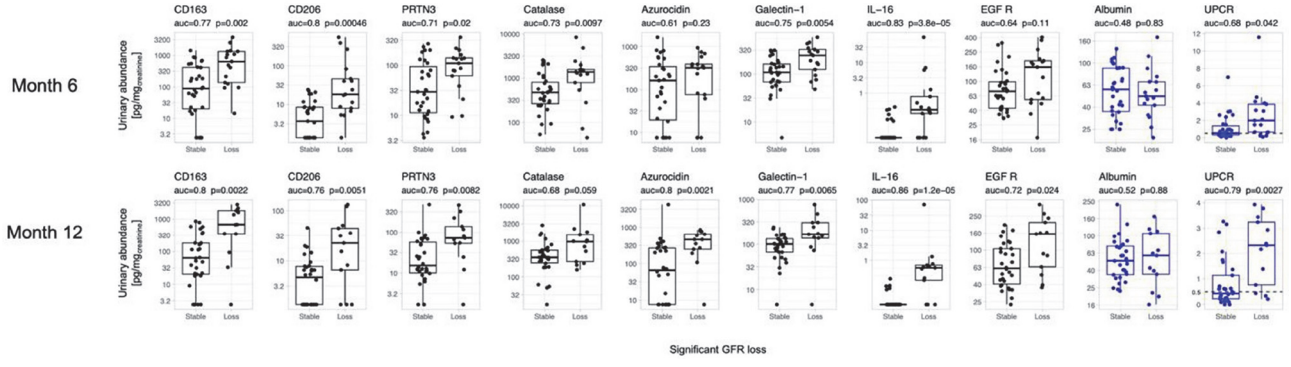
Introduction One third of lupus nephritis (LN) patients develop irreversible kidney damage despite achieving a clinical response based on resolution of proteinuria. Furthermore, per protocol kidney biopsies showed clinically significant histological activity in 50% of patients with proteinuria < 0.5 g/g. We hypothesized that persistence of intrarenal inflammation (=LN histological activity) after treatment leads to accrual of kidney damage. We have previously identified several urinary biomarkers that correlate with the NIH Activity Index (histological activity). Here, we tested whether the elevation of these candidate biomarkers of LN immunological activity at 6 and 12 months from the diagnostic kidney biopsy predict loss of kidney function at 3 years.

Methods We quantified 1200 biomarkers (Kiloplex, RayBiotech) in urine samples collected on the day of (73%) or within 3 weeks (27%) of kidney biopsy and week 12, 24, or 52 in LN patients (ISN class III, IV, V, or mixed) with proteinuria > 1 g/d. Glomerular filtration rate (GFR) was estimated using the CKD-EPI equation. Significant GFR loss was defined as a decline of >15 ml/min below 90 ml/min at 3 years from biopsy or end-stage kidney disease (ESKD) by year 3 requiring dialysis or transplant.

Results We included 73 patients: 85% female, 46% identified as Black, 40% as White, 10% as Asian, and 4% as Other. ISN classification included 25% pure proliferative (III or IV), 40% pure membranous (V), and 35% mixed (III or IV + V). Mean GFR at biopsy was 85 (SD 34.7) ml/min. There were 32/73 (44%) patients who developed significant GFR loss. Figure 1 shows the associations of candidate urinary biomarkers at 6 and 12 months with significant GFR loss at 3 years. Most urinary biomarkers of histological activity were higher at 6 and 12 months in patients who ultimately lost GFR at 3 years. For example, IL-16 outperformed UPCR both at 6 and 12 months (figure 1) and was independent of proteinuria (not shown). UPCR at 12 months predicted 3-year GFR loss with AUC 0.79, but albuminuria did not. In a multivariable model, the combination of CD163 (macrophage activation), PRTN3 (degranulation), and IL-16 (cellular inflammation in LN) urinary levels at 12 months predicted GFR loss at 3 years with an AUC of 0.96.

Conclusions Elevation of urinary biomarkers of histological activity after 6 or 12 months of treatment predict GFR loss at 3 years better than proteinuria, especially IL-16. These findings suggest that insufficient immunosuppression results in persistent intrarenal immunological activity in LN that increases the risk of kidney function loss. The ultimate treatment goal in LN is long term preservation of kidney function. Therefore, clinical trials endpoints should include response definitions that best associate with GFR preservation. Because noninvasive urinary biomarkers of immunological activity parallel intrarenal



Abstract 306 Figure 1 Association of candidate urinary biomarkers of histological activity with significant GFR loss. Urinary abundance (pg/$mg_{creatinine}$) of urinary biomarkers selected a priori based on their correlation with histological activity (NIH Activity Index) in a matching kidney biopsy of LN according to GFR loss at 3 years. Urine protein and albumin to creatinine ratios are reported for reference as clinically used biomarkers.