Identification of highly active systemic lupus erythematosus by combined type I interferon and neutrophil gene scores vs classical serologic markers

François Chasset, Camillo Ribi, Marten Trendelenburg, Uyen Huynh-Do, Pascale Roux-Lombard, Delphine S. Courvoisier, Carlo Chizzolini

Identification of highly active systemic lupus erythematosus by combined type I interferon and neutrophil gene scores versus classical serologic markers

François Chasset[1,2,3], Camillo Ribi[4], Marten Trendelenburg[5], Uyen Huynh-Do[6], Pascale Roux-Lombard[1], Delphine S. Courvoisier[7], Carlo Chizzolini[1,2] for the Swiss SLE Cohort Study (SSCS)

[1] *Division of Immunology and Allergy, University Hospital and School of Medicine, Geneva, Switzerland*

[2] *Department of Pathology and Immunology, School of Medicine, Geneva, Switzerland*

[3] *Faculté de Médecine Sorbonne Université, AP-HP, Service de Dermatologie et Allergologie, Hôpital Tenon, F-75020 Paris, France*

[4] *Division of Immunology and Allergy, University Hospital Center of Lausanne, Switzerland*

[5] *Laboratory for Clinical Immunology, Department of Biomedicine and Division of Internal Medicine, University Hospital of Basel, Switzerland*

[6] *Division of Nephrology and Hypertension, Inselspital, Bern University Hospital, Switzerland*

[7] *Division of Rheumatology, University Hospital and School of Medicine, Geneva, Switzerland*

Running title: Exploiting simplified interferon and neutrophil scores in SLE

Corresponding author & reprint requests:

Carlo Chizzolini, Immunology & Allergy, University Hospital, rue Gabrielle Perret Gentil 4, 1211 Geneva 14, Switzerland

Phone number: +41 22 372 9370; Fax Number: +41 22 372 9369

E-mail: carlo.chizzolini@unige.ch; ORCID: 0000-0003-4849-6335

Funding: The work was partially supported by funds provided to CC by an unrestricted research grant by GSK, a grant from Fondation Fleurette Wagemakers, Sion (Switzerland), a grant from La Société Académique de Genève (Switzerland).

Disclosures: The authors declare no conflict of interest.

Abstract

Objectives: In systemic lupus erythematosus (SLE), heterogeneous clinical expression and activity may reflect diverse pathogenic and/or effector mechanisms. We investigated SLE heterogeneity by assessing the expression of three gene sets representative of type I interferon (IFN-I), polymorphonuclear neutrophil (PMN) and plasmablast (PB) signatures in a well-characterized, multidisciplinary cohort of SLE patients. We further assessed whether individual gene products could be representative of these three signatures.

Methods: Whole blood, serum and clinical data were obtained from 140 SLE individuals. Gene expression was assessed by NanoString© technology, using a panel of 37 probes to compute six IFN-I, one PMN and one PB scores. Protein levels were measured by ELISA.

Results: Depending on the score, 45% to 50% SLE individuals showed high IFN-I gene expression. All six IFN-I scores were significantly associated with active skin involvement, and two of six were associated with arthritis. IFN-induced Mx1 protein (MX1) level was correlated with IFN-I score ($p<0.0001$) and associated with a similar clinical phenotype. In all, 25% of SLE individuals showed high PMN gene expression, associated with SLE fever, serositis, leukopenia and glucocorticoid use. PB gene expression was highly affected by immunosuppressant agents, with no association with SLE features. Combined IFN-I and PMN gene scores were significantly associated with high disease activity and outperformed anti-dsDNA and anti-C1q autoantibody and complement levels for predicting SLE activity.

Conclusion: IFN-I and PMN gene scores segregate with distinct SLE clinical features, and their combination may identify high disease activity. MX1 protein level performed similar to IFN-I gene expression.

Key words: Systemic lupus erythematosus, type-I interferon, polymorphonuclear cells, gene expression, score

Key messages:

- **In SLE, high IFN-I gene scores are strongly associated with active skin lesions**
- **MX1 protein levels perform similarly to IFN-I gene scores**
- **Combined IFN-I and PMN gene scores outperform serological markers for predicting SLE activity**

Introduction

Systemic lupus erythematosus (SLE) is characterized by a wide spectrum of clinical, laboratory and immunological abnormalities with marked individual variability (1). Dissecting the heterogeneity of SLE in terms of clinical expression and pathophysiology remains an important unmet need (2). Furthermore, classical laboratory tests, including anti-double-stranded DNA (anti-dsDNA) antibody titers and complement component levels have limited accuracy in assessing disease activity or predicting flares (3,4).

Unbiased gene expression studies allowed for the identification of subgroups of patients with distinct blood transcriptional signatures associated with SLE activity and clinical features (5–9). In particular, increased expression of type-I interferon (IFN-I)-regulated genes was associated with SLE disease activity as well as immunological and clinical phenotypes (8,10,11). Several lines of evidence support the role of IFN-I as central mediator in the pathogenesis of SLE, and IFN-I targeting is emerging as a novel therapeutic strategy (12). Polymorphonuclear neutrophils (PMNs) also play a central role in the pathogenesis of SLE (12). Genes expressed in PMNs were found in 45% to 65% of SLE patients, and their presence was strongly associated with lupus nephritis (5,14,15). Moreover, plasmablasts (PBs) and autoantibody production are hallmark of SLE (15). Genes expressed in PBs were preferentially expressed in about 20% of pediatric SLE patients (5) and associated with renal involvement in adult SLE (7).

Beside high-resolution, unbiased techniques (5–7), simplified approaches involving sets of genes to generate scores have been proposed to assess IFN-I (17–21), PMNs (15) and PBs signatures (22) in peripheral blood. For instance, high IFN-I gene scores were found correlated with Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) and

autoantibody production (8,9,23,24) and have been used to stratify patients with or without a high IFN-I signature in clinical trials (25,26).

We investigated simultaneously IFN-I, PMN and PB gene signatures in a multidisciplinary, well-characterized cohort of Swiss SLE patients (SSCS) (27) and 1) we measured their respective prevalence 2) we assessed potential associations of these signatures with clinical and immunological SLE phenotypes, 3) we tested whether the combination of these three gene signatures rather than a single signature correlated with disease activity compared with classical serologic markers and 4) whether individual gene products could be representative of these three signatures

Material and methods

Biological samples were collected from 140 consecutive SLE patients included in the Swiss SLE Cohort Study (SSCS) (27) and 35 age- and sex-matched healthy controls (HCs) between November 2017 and December 2018. Inclusion criteria were age ≥ 18 years, diagnosis of SLE according to the updated American College of Rheumatology classification criteria (28) or the SLICC 2012 (29). The cohort study was approved by the SwissEthics review board (PB_2017-01434) and all patients gave written informed consent. Detailed information on disease activity, experimental methodology and statistical methods is available in **supplementary data S1**. Briefly, patients were classified in 2 groups according to their SELENA-SLEDAI: inactive SLE (SLEDAI < 4) and active SLE (SLEDAI ≥ 4). In order, to compare gene scores to classical serologic markers, we used clinical SLEDAI (cut-off ≥ 2 for active disease), which does not take into consideration the two biological items: low serum complement and high dsDNA autoantibodies. Gene expression was assessed by mRNA expression profiling by using a NanoString nCounter gene expression system (NanoString Technologies, Seattle, WA) using a custom panel of 37 genes allowing the computation of six IFN-I scores (17–21,25), one PMN (15) and one PB score (22). Then IFN-I, PMN, and PB scores were calculated as the median of the relative expression of all genes contributing to the score. The mean plus 2 SD of HC values was used as a threshold to define high scores. Chi-square test and Fisher's exact test were used to compare categorical variables; Mann-Whitney test was used to compare non-paired variables, and Spearman correlation analysis (rs) was used for correlations. When more than 2 groups were compared, statistical correction for multiple comparisons was performed using Dunn's test for all pair by joint ranking. Unsupervised hierarchical clustering analysis was used to identify clusters enriched in active SLE. In order to compare the potential role of gene scores versus classical serologic markers (complement levels, ds-DNA autoantibodies) to predict SLE activity, we used a bootstrap

forest model with 10 000 decision trees to identify potential predictors and their respective contributions (33). To validate the results of the bootstrap forest model multivariable logistic analyses were performed.

Results

Characteristics of SLE patients

The clinical characteristics of the 140 SLE patients included are in **Table 1**. Median age was 45.6 (range 19-78.8); 118 (84%) were women and 101 (72%) were Caucasians. At the time of sampling, median SLEDAI was 3 (range 0-46), and 69 patients (50%) had active disease (SLEDAI $\geq$ 4).

IFN-I-, PMN- and PB-gene scores in SLE individuals and HCs

To identify IFN-I-, PMN-, and PB-related genes expressed in the peripheral blood of SLE individuals and HCs, we used scores previously published grouping the expression of several genes representative of each signature (**Supplementary Table S1**). For IFN-I, we tested six distinct scores (17–21,25). All IFN-I scores were significantly higher for SLE individuals than HCs (**Figure 1A)**. The performance of individual IFN-I scores was similar but not identical. High IFN-I scores ranged from 45% as defined by Khamastha *et al*. (25) to 50% by Rice et al. (21) in SLE but were consistently about 1% in HCs. For simplicity, in the following paragraphs we describe the IFN-I score (25) as defined by Khamastha *et al*., unless otherwise stated. The PMN score was higher but not significantly in SLE individuals than HCs (median 1.48 [IQR 0.69-3.95] vs 1.0 [IQR 0.54-1.89], p=0.053), and a high PMN score was found significantly more frequently in SLE individuals than HCs (25% vs 6%, p=0.017). In contrast, PB scores did not differ when comparing SLE and HC scores both as absolute values and as frequency of positivity. Among individual PB genes, only CD38 was differentially expressed in SLE individuals and HCs (median normalized values 796 [IQR 25-75; 590-1114] vs 569 [420-772], p<0.001 (**supplementary Figure S1**). A total of 85 SLE (61%) patients had at least one positive score, the IFN-I gene scores being most commonly positive (**Figure 1B**). In total, 35 (25%) and 21 (15%) SLE individuals had high PMN and PB

scores, respectively (**Figure 1B)**. Among the 63 SLE individuals with high IFN-I scores, 33 (52%) were positive for only IFN-I and 30 (48%) were positive for IFN-I and PMN and/or PB scores (**Figure 1B**).

We found a high positive correlation within the expression of IFN-I–induced genes, those contributing to the PMN score, and those contributing to the PB score, with the exception of *CAMK1G*, which was poorly detected in both SLE individuals and HCs (**Figure 1C**). We found lower levels of correlation or inverse correlation between IFN-I and PMN or PB-expressed genes (**Figure 1C**). Overall, these data confirm that IFN-I genes are frequently overexpressed in SLE but that PMN and PB genes are also present with or without the concomitant overexpression of IFN-I genes.

IFN-I-, PMN- and PB-gene scores related to SLE clinical characteristics

We then asked whether the expressed genes could segregate with SLE clinical and classical laboratory features. Overall, nine IFN-I–related genes and five PMN-related genes but no PB-related genes showed a weak but statistically significant positive correlation with the SLEDAI (**Supplementary Table S2**). Moreover, the IFN-I score (25) and PMN score but not PB score were significantly higher with active than inactive disease (SLEDAI ≥ 4 vs < 4) (**Figure 2A**). Similar results were observed using as additional definitions of active SLE: clinical SLEDAI ≥2, or SLEDAI ≥6, or a composite definition using SLEDAI≥4 in association with physician's global assessment (PGA)>1 **(supplementary Figure S2)** as well for the other IFN-I scores (p-values from 0.057 to 0.10, data not shown). The presence and number of SLE-related autoantibodies, including U_1RNP, SSA, ds-DNA and C1q, significantly impacted the IFN-I score (**Figure 2B**). Moreover, the IFN-I score was significantly higher with positivity than negativity for anti-U_1RNP (p=0.004) and anti-SSA (p=0.0069) autoantibodies (**Figure 2C**). It was higher but not significantly with anti-dsDNA autoantibodies (p=0.08) and

did not differ with anti-C1q autoantibodies (p=0.51) We found no association between the presence and number of autoantibodies for PMN and PB scores (**Figure 2B**).

Concerning active clinical features given by SELENA-SLEDAI at the time of sampling, univariable analysis revealed that as compared with a low score, a high IFN-I score was associated with SLE fever: 8% vs 0% (p=0.02); active arthritis: 31% vs 9% (p=0.0014), active skin involvement: 35% vs 10% (p=0.004), leukopenia: 10% vs 0% (p=0.006), and GC use: 52% vs 33% (p=0.03) (**Figure 2D** and **supplementary Table S3**). On multivariable analysis, a high IFN-I score was significantly associated with active skin involvement (OR: 4.60 [95% CI 1.87-11.38] p=0.01) and active arthritis (OR: 4.29 [95% CI 1.66-11.06], p=0.01). On both univariable and multivariable analyses, the frequency of high IFN-I score was lower without than with treatment (3% *vs* 16%, p=0.02); (OR: 0.17 [95% CI 0.04-0.80], p=0.04). Notably, when the six IFN-I scores were compared, some differences were observed, with only active skin involvement remaining significantly associated with high IFN-I score on multivariable analysis for all scores (**Figure 2D**).

Of note, a high PMN score segregated with different clinical features compared to high IFN-I score. High versus low PMN score was associated with SLE fever: 11% vs 1% (p=0.015), active serositis: 11% *vs* 2% (p=0.037), leukopenia: 11% *vs* 2% (p=0.039) and use of GC: 65% vs 33% (p=0.0007). On multivariable analysis, a high PMN score remained significantly associated with active serositis (OR: 6.45 [95% CI 1.13-36.9], p=0.04) and use of GC (OR: 3.83 [95% CI 1.7-8.62], p=0.02) (**Figure 2D**). A high PB score was associated with AM therapy only (48% *vs* 20%, p=0.01) and negatively with IS therapy (24% *vs* 54%, p=0.008) but not with the use of GC independent of activity (**Figure 2D, E**). At the individual gene levels, CD38 mRNA levels were significantly higher in SLE with leukopenia (p=0.049) and with positive anti-dsDNA autoantibodies (p=0.04) and were associated but not significantly with arthritis (p=0.06) and SLEDAI ≥4 (p=0.09) (**Supplementary Table S4**). Further, CD38

expression was not significantly decreased by IS therapy (p= 0.16). Overall, these data indicate that the IFN-I and PMN scores are associated with specific SLE clinical features, but the PB score is highly affected by the use of IS agents.

Because GC may induce lymphopenia and promote intravascular redistribution of circulating PMN, we assessed the scores in terms of PMN and lymphocyte counts. Indeed, in our cohort, we observed a low positive correlation between GC dose and PMN count ($r_s = 0.21$, $p = 0.02$) and low negative correlation with lymphocyte count ($r_s = -0.20$, $p = 0.02$). High PMN count was associated with high PMN and low IFN-I scores (**supplementary Figure S2A**), whereas low lymphocyte count was associated with high IFN-I and high PMN scores (**supplementary Figure S2B**). Thus, the relation between the dose of GC and IFN-I and PMN scores have a counterpart in PMN and lymphocyte counts, which may reflect both disease activity and treatment.

Combined high IFN-I and high PMN scores identify very active SLE

We performed unsupervised hierarchical clustering analysis to assess how individual gene expression segregated across individuals with active and inactive SLE, and HCs (**Figure 3A**). Three main clusters were readily evident. In cluster 1, which grouped only active SLE patients, all had high PMN score and 88% had high IFN-I score. Furthermore, the SLEDAI was significantly higher in cluster 1 than 2 and 3 (median: 10.5 [IQR 6.5-27] vs 2 [IQR 0-6], p=0.001, and 2 [IQR 0-7.5], p=0.0009) (**Figure 3B**). Moreover, 29% of SLE individuals in cluster 1 had proteinuria > 0.5 g/24 h versus 3% in clusters 2 and 3 (p=0.03). Cluster 2 was characterized by a high frequency (74%) of high IFN-I score. While cluster 3, mostly of individuals with inactive SLE and HCs, was characterized by increased frequency (76%) of no high scores (**Figure 3A**).

Patients with the joint presence of high IFN-I and high PMN scores, highly enriched in cluster 1, had higher SLEDAI than those with low IFN-I score (median 6 [IQR 3-10.5] vs 2 [0-6], p=0.0058) and scores tended to be higher than those with high IFN-I score only (6 [IQR 3-10.5] vs 3 [0-8], p=0.06). Thus, gene expression clustering distinguished individuals with active SLE, inactive SLE and HCs. Furthermore, individuals with both high IFN-I and PMN scores had high disease activity.

Performance of gene scores compared to classical biological markers to predict SLE disease activity

Next, we assessed how upregulated genes could contribute to and perform as compared with classical biological markers to predict disease activity. In addition to the IFN-I, PMN, and PB scores, we computed a composite score adding all PMN genes to the four IFN-I genes by Khamastha et al. (25). Use of bootstrap forest models revealed that the composite IFN-I plus PMN and IFN-I scores best predicted SLE clinical activity (clinical SLEDAI ≥ 2) and performed better than classical immunological markers (**Table 2)**. To substantiate these findings, we used multivariable logistic regression models. After adjustment for age, sex, ethnicity, and current use of GC or IS, the composite IFN-I plus PMN and the IFN-I scores were significantly associated with SLE activity; no association was found with anti-dsDNA and anti-C1q autoantibodies or complement levels (**Table 2)**. Thus, by machine-learning techniques and multivariable analysis, the composite IFN-I plus the PMN and IFN-I scores were found highly associated with disease activity.

MX1 protein levels as surrogate for the IFN-I gene scores.

Because the techniques needed to assess gene expression are not readily available in routine laboratories, we aimed to identify whether gene products could be used as surrogates reflecting IFN-I-, PMN- and PB-gene scores. We selected one protein for each gene group

chosen on the basis of high correlation between that specific gene and the whole score. The mRNA level of IFN-induced GTP-binding MX1 showed high positive correlation with the IFN-I score ($r_s = 0.97$, $p<0.0001$) (**Figure 4A**). MX1 protein level showed moderate positive correlation with the IFN-I score ($r_s = 0.40$, $p<0.0001$). Moreover, MX1 protein level was higher in SLE individuals than HCs (p=0.0002) and was higher with active (SLEDAI ≥ 4) than inactive SLE (p=0.03). Similar results were observed using other definitions for active SLE **(supplementary Figure S4).** A receiver operating characteristic curve identified a threshold of 133.7 pg/ml defining high MX1 level associated with SLEDAI ≥ 4 (**supplementary Figure S3)**. High MX1 level was associated with clinical features similar to those observed with the IFN-I score except for lack of association with leucopenia (**Figure 4B and supplementary Table S5)**.

Myeloperoxidase (*MPO*) serum levels discriminated SLE individuals and HCs but not active and inactive SLE (**Figure 4C**). Similar to the PB score, TNFRS17 protein level did not discriminate SLE individuals and HCs nor active and inactive SLE (**Figure 4D**). Thus, MX1 protein level appears to perform similar to IFN-I–induced genes and could be used as a biomarker to identify patients with overexpressed IFN-I genes; MPO protein level did not approach the performance of the PMN gene score.

Discussion

To delve into SLE heterogeneity, we simultaneously assessed gene expression signatures related to IFN-I, PMN and PB responses. Our aim was to investigate, by using a minimal number of probes, whether we could identify distinct subgroups of patients with specific clinical characteristics. Confirming and extending previous reports, we identified distinct clinical and immunological phenotypes associated with IFN-I and PMN signatures and, most novel, with their combination. Strikingly, this combination outperformed all classical immunological parameters associated with disease activity. Furthermore, we established that MX1 protein level could be a surrogate for the gene signature to identify SLE patients with high IFN-I expression.

To detect the presence of IFN-I, several scores have been developed based on the expression of 3 to 34 genes (8,9,20,23,24,35–38). By studying the expression of 22 IFN-I–related genes, we were able to compute six distinct IFN-I scores (17–21,25) (**Supplementary Table S1**). In our cohort, 45% to 50% individuals had a high IFN-I score depending on the score used. Of note, the highest frequency of positive samples was observed with a score consisting of only six genes (21). Furthermore, the score most strongly associated with disease activity from the statistical point of view consisted of only four genes (25). Both observations suggest that the sensitivity and clinical relevance may not increase when increasing the number of genes composing a score.

In previous reports, the frequency of high IFN-I score ranged from 50% to 84% (5,8,35). Thus, the frequency of positive samples in our cohort is among the lower range of the reported positivity, likely due to the large number of patients with inactive disease in our cohort but also their ethnicity and age (39). From a clinical point of view, all IFN-I scores were significantly associated with active skin disease on multivariable analysis. This finding

agrees with studies showing an increased IFN-I signature in peripheral blood of individuals with active cutaneous lupus and the extent of lesions (10,37) with or without associated systemic features (40), which supports a specific role for IFN-I in the development of skin disease. Unfortunately, in our study, cutaneous lesions were recorded according to SELENA-SLEDAI definitions and no systematic information on cutaneous lupus erythematosus (CLE) subtypes was available. Of note, we found an association between the number of positive autoantibody specificities and IFN-I score, which was not found with PMN and PB scores. Furthermore, high IFN-I score was strongly associated with the presence of anti-SSA and anti-U_1RNP but not anti-C1q autoantibodies. This may suggest that RNA-associated nucleoproteins, when complexed with autoantibodies, are particularly strong IFN-I inducers (39,41).

We found high PMN score in about 25% of SLE individuals. High PMN score was associated with disease activity, and individuals with high PMN score showed a specific phenotype associated with fever, serositis and leukopenia (**Figure 2D**). Previous studies assessed the role of PMN gene expression in SLE (5,6,14,15,42); three explicitly found an association between PMN signature and lupus nephritis. Although in our study only six patients had active proteinuria, remarkably they were enriched in cluster 1, characterized by very high expression of PMN genes.

In agreement with previous data (14,15), we found an association between GC use and high PMN score, which remained significant on multivariable analysis (**Figure 2D**). The impact of GC on PMN gene expression may be an important confounding factor. Indeed, after adjusting for GC use, the PMN score was no longer associated with clinical SLEDAI (**Table 2**). However, 15% of patients with high PMN score were not receiving GC, and conversely about 60% of patients receiving GC did not have high PMN score, similar to previous observations (14). Moreover, we found that patients receiving GC had higher PMN count and that PMN

score and PMN count were correlated, which confirms previous data (15). Altogether, the use of GC appears to affect the PMN score, at least in part by increasing PMN count, but high PMN score seems also to be associated with a distinct phenotype of SLE likely to be treated with GC.

Previous reports of adult (7,22) and pediatric SLE (5) provided evidence for a correlation between PB gene expression, disease activity, and kidney involvement. We found a high PB score, defined by five genes (22), in 15% of SLE patients. However, we did not find differences in PB score (22) between SLE individuals and HCs nor between active and inactive SLE. This may be explained by a treatment effect. Indeed, we found the PB score significantly decreased in patients receiving IS agents and the highest PB scores for patients without treatment or AM therapy only. In agreement with our findings, a study of adult SLE did not find genes associated with PB signatures differentially expressed between active and inactive SLE (43). Moreover, longitudinal PB gene expression did not segregate with disease activity when re-analyzing longitudinal adult and pediatric SLE data (6). However, in our cohort, CD38 gene expression significantly differed among SLE individuals and HCs and was associated although not significantly with active disease and some SLE features including leukopenia and anti-dsDNA autoantibody positivity. However, no statistical correction for multiple comparisons was performed. Thus, further work is needed to ascertain whether PB gene expression could provide substantial information for SLE subgrouping.

The direct detection of IFN-I in biological fluids represents a technical challenge. Although the new technology based on single-molecule array (SIMOA) may fulfill the required sensitivity across different IFN-α isotypes (44), simple tools to detect IFN-I activity are needed. We found the serum level of MX1 correlated with IFN-I score and associated with disease activity. Furthermore, patients with high MX1 levels had a clinical phenotype similar

to that associated with high IFN-I score. These data confirm and extend a recent report (45) supporting the use of MX1 protein level as a tool to assess IFN-I biological activity.

As limitation, the cross-sectional design of our study did not allow for assessing the potential role of gene expression as well as MX1 protein level to predict SLE flare or response to treatment. However, two longitudinal studies of 66 and 94 SLE individuals found that IFN-I score did not distinguish patients at risk for impending disease flare (18,46), but a post-hoc analysis of two phase III studies of 1700 patients receiving tabalumab showed high IFN-I gene score as an independent predictor of flare over 52 weeks (35). Similarly, high base-line IFN-I levels detected by SIMOA in not active SLE patients predicted relapses (47). Furthermore, a high IFN-I gene score predicted response to anti-IFN-I therapies (25,48). Another limitation is that whole-blood gene-expression analysis does not allow for identifying the cells contributing to the different signatures (49). However, IFN-I score was found to be higher in monocytes than in T and B cells in peripheral blood mononuclear cells (37). In addition, it is easier to collect whole blood rather than purified cell subset, which enhances clinical applicability. While type I IFNs are inducer of the genes selected for generating the IFN-I scores we used, type II IFN and other cytokines could also have a role in their induction (10,37). Whether this is relevant in clinical settings would require the analysis of samples before and after the use of IFN-I neutralizing agents. Finally, our choice of the IFN-I–induced genes may imprecisely define the severity of clinical manifestations; others have provided evidence that the modules of IFN-I–induced genes may be differentially regulated in severe versus non-severe SLE flare (10). However, when we used continuous values of the scores, we captured differences in active versus inactive SLE.

In our study, SLE patients with both high IFN-I and PMN scores had the most active and severe disease. Composite IFN-I plus PMN and IFN-I scores but no classical serologic markers significantly predicted SLE clinical activity and remained significantly associated

with clinical SLEDAI after adjusting for several confounding factors, particularly the use of GC and IS agents. Thus, the simultaneous assessment of IFN-I and PMN gene expression may substantially add to current biomarkers of activity and could be used in clinical practice and in clinical studies to stratify patients at risk.

Acknowledgement: We thank Dr. Mylène Docquier and Dr. Didier Chollet (Genetic Facility, University Medical Center, Geneva, Switzerland) for their help with the Nanostring methodology and analysis. Our thanks to Mrs Donatienne Wynar (Center for Clinical Research, University Hospital, Geneva, Switzerland) for collecting samples and clinical data, to Laura Smales for English editing. Our many thanks to the SLE individuals participating to the Swiss SLE cohort Study. FC was supported by a research travel grant from the French Society of Dermatology and from Institut Servier, Paris (France).

References

1. Ahearn JM, Provost TT, Dorsch CA, Stevens MB, Bias WB, Arnett FC. Interrelationships of HLA-DR, MB, and MT phenotypes, autoantibody expression, and clinical features in systemic lupus erythematosus. *Arthritis Rheum* 1982;25:1031–1040.

2. Felten R, Sagez F, Gavand P-E, Martin T, Korganow A-S, Sordet C, et al. 10 most important contemporary challenges in the management of SLE. *Lupus Sci Med* 2019;6:e000303.

3. Gensous N, Marti A, Barnetche T, Blanco P, Lazaro E, Seneschal J, et al. Predictive biological markers of systemic lupus erythematosus flares: a systematic literature review. *Arthritis Res Ther* 2017;19:238.

4. Wu H, Zeng J, Yin J, Peng Q, Zhao M, Lu Q. Organ-specific biomarkers in lupus. *Autoimmun Rev* 2017;16:391–397.

5. Banchereau R, Hong S, Cantarel B, Baldwin N, Baisch J, Edens M, et al. Personalized Immunomonitoring Uncovers Molecular Networks that Stratify Lupus Patients. *Cell* 2016;165:551–565.

6. Toro-Domínguez D, Martorell-Marugán J, Goldman D, Petri M, Carmona-Sáez P, Alarcón-Riquelme ME. Stratification of Systemic Lupus Erythematosus Patients Into Three Groups of Disease Activity Progression According to Longitudinal Gene Expression. *Arthritis Rheumatol Hoboken NJ* 2018.

7. Panousis NI, Bertsias GK, Ongen H, Gergianaki I, Tektonidou MG, Trachana M, et al. Combined genetic and transcriptome analysis of patients with SLE: distinct, targetable signatures for susceptibility and severity. *Ann Rheum Dis* 2019;78:1079–1089.

8. Baechler EC, Batliwalla FM, Karypis G, Gaffney PM, Ortmann WA, Espe KJ, et al. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *Proc Natl Acad Sci U S A* 2003;100:2610–2615.

9. Nikpour M, Dempsey AA, Urowitz MB, Gladman DD, Barnes DA. Association of a gene expression profile from whole blood with disease activity in systemic lupus erythaematosus. *Ann Rheum Dis* 2008;67:1069–1075.

10. Chiche L, Jourde-Chiche N, Whalen E, Presnell S, Gersuk V, Dang K, et al. Modular transcriptional repertoire analyses of adults with systemic lupus erythematosus reveal distinct type I and type II interferon signatures. *Arthritis Rheumatol Hoboken NJ* 2014;66:1583–1595.

11. Der E, Suryawanshi H, Morozov P, Kustagi M, Goilav B, Ranabathou S, et al. Tubular cell and keratinocyte single-cell transcriptomics applied to lupus nephritis reveal type I IFN and fibrosis relevant pathways. *Nat Immunol* 2019;20:915–927.

12. Chasset F, Arnaud L. Targeting interferons and their pathways in systemic lupus erythematosus. *Autoimmun Rev* 2017.

13. Smith CK, Kaplan MJ. The role of neutrophils in the pathogenesis of systemic lupus erythematosus. *Curr Opin Rheumatol* 2015;27:448–453.

14. Jourde-Chiche N, Whalen E, Gondouin B, Speake C, Gersuk V, Dussol B, et al. Modular transcriptional repertoire analyses identify a blood neutrophil signature as a candidate biomarker for lupus nephritis. *Rheumatol Oxf Engl* 2017;56:477–487.

15. Wither JE, Prokopec SD, Noamani B, Chang N-H, Bonilla D, Touma Z, et al. Identification of a neutrophil-related gene expression signature that is enriched in adult systemic lupus erythematosus patients with active nephritis: Clinical/pathologic associations and etiologic mechanisms. *PloS One* 2018;13:e0196117.

16. Jacobi AM, Mei H, Hoyer BF, Mumtaz IM, Thiele K, Radbruch A, et al. HLA-DRhigh/CD27high plasmablasts indicate active disease in patients with systemic lupus erythematosus. *Ann Rheum Dis* 2010;69:305–308.

17. Yao Y, Richman L, Higgs BW, Morehouse CA, Reyes M de los, Brohawn P, et al. Neutralization of interferon-alpha/beta-inducible genes and downstream effect in a phase I trial of an anti-interferon-alpha monoclonal antibody in systemic lupus erythematosus. *Arthritis Rheum* 2009;60:1785–1796.

18. Petri M, Singh S, Tesfasyone H, Dedrick R, Fry K, Lal P, et al. Longitudinal expression of type I interferon responsive genes in systemic lupus erythematosus. *Lupus* 2009;18:980–989.

19. McBride JM, Jiang J, Abbas AR, Morimoto A, Li J, Maciuca R, et al. Safety and pharmacodynamics of rontalizumab in patients with systemic lupus erythematosus: results of a phase I, placebo-controlled, double-blind, dose-escalation study. *Arthritis Rheum* 2012;64:3666–3676.

20. Kennedy WP, Maciuca R, Wolslegel K, Tew W, Abbas AR, Chaivorapol C, et al. Association of the interferon signature metric with serological disease manifestations but not global activity scores in multiple cohorts of patients with SLE. *Lupus Sci Med* 2015;2:e000080.

21. Rice GI, Forte GMA, Szynkiewicz M, Chase DS, Aeby A, Abdel-Hamid MS, et al. Assessment of interferon-related biomarkers in Aicardi-Goutières syndrome associated with mutations in TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, and ADAR: a case-control study. *Lancet Neurol* 2013;12:1159–1169.

22. Streicher K, Sridhar S, Kuziora M, Morehouse CA, Higgs BW, Sebastian Y, et al. Baseline Plasma Cell Gene Signature Predicts Improvement in Systemic Sclerosis Skin Scores Following Treatment With Inebilizumab (MEDI-551) and Correlates With Disease Activity in Systemic Lupus Erythematosus and Chronic Obstructive Pulmonary Disease. *Arthritis Rheumatol Hoboken NJ* 2018.

23. Kirou KA, Lee C, George S, Louca K, Peterson MGE, Crow MK. Activation of the interferon-alpha pathway identifies a subgroup of systemic lupus erythematosus patients with distinct serologic features and active disease. *Arthritis Rheum* 2005;52:1491–1503.

24. Feng X, Wu H, Grossman JM, Hanvivadhanakul P, FitzGerald JD, Park GS, et al. Association of increased interferon-inducible gene expression with disease activity and lupus nephritis in patients with systemic lupus erythematosus. *Arthritis Rheum* 2006;54:2951–2962.

25. Khamashta M, Merrill JT, Werth VP, Furie R, Kalunian K, Illei GG, et al. Sifalimumab, an anti-interferon-α monoclonal antibody, in moderate to severe systemic lupus erythematosus: a randomised, double-blind, placebo-controlled study. *Ann Rheum Dis* 2016.

26. Vollenhoven RF van, Hahn BH, Tsokos GC, Wagner CL, Lipsky P, Touma Z, et al. Efficacy and safety of ustekinumab, an IL-12 and IL-23 inhibitor, in patients with active systemic lupus erythematosus: results of a multicentre, double-blind, phase 2, randomised, controlled study. *Lancet Lond Engl* 2018;392:1330–1339.

27. Ribi C, Trendelenburg M, Gayet-Ageron A, Cohen C, Dayer E, Eisenberger U, et al. The Swiss Systemic lupus erythematosus Cohort Study (SSCS) - cross-sectional analysis of clinical characteristics and treatments across different medical disciplines in Switzerland. *Swiss Med Wkly* 2014;144:w13990.

28. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997;40:1725.

29. Petri M, Orbai A-M, Alarcón GS, Gordon C, Merrill JT, Fortin PR, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum* 2012;64:2677–2686.

30. Petri M, Kim MY, Kalunian KC, Grossman J, Hahn BH, Sammaritano LR, et al. Combined oral contraceptives in women with systemic lupus erythematosus. *N Engl J Med* 2005;353:2550–2558.

31. Pescarmona R, Belot A, Villard M, Besson L, Lopez J, Mosnier I, et al. Comparison of RT-qPCR and Nanostring in the measurement of blood interferon response for the diagnosis of type I interferonopathies. *Cytokine* 2019;113:446–452.

32. Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol* 2002;3:RESEARCH0034.

33. Breiman L. Random Forests. Machine Learning,.2001:5-32.

34. Courvoisier DS, Combescure C, Agoritsas T, Gayet-Ageron A, Perneger TV. Performance of logistic regression modeling: beyond the number of events per variable, the role of data structure. *J Clin Epidemiol* 2011;64:993–1000.

35. Hoffman RW, Merrill JT, Alarcón-Riquelme MME, Petri M, Dow ER, Nantz E, et al. Gene Expression and Pharmacodynamic Changes in 1,760 Systemic Lupus Erythematosus Patients From Two Phase III Trials of BAFF Blockade With Tabalumab. *Arthritis Rheumatol Hoboken NJ* 2017;69:643–654.

36. Brkic Z, Corneth OBJ, Helden-Meeuwsen CG van, Dolhain RJEM, Maria NI, Paulissen SMJ, et al. T-helper 17 cell cytokines and interferon type I: partners in crime in systemic lupus erythematosus? *Arthritis Res Ther* 2014;16:R62.

37. El-Sherbiny YM, Psarras A, Md Yusof MY, Hensor EMA, Tooze R, Doody G, et al. A novel two-score system for interferon status segregates autoimmune diseases and correlates with clinical features. *Sci Rep* 2018;8:5793.

38. Md Yusof MY, Psarras A, El-Sherbiny YM, Hensor EMA, Dutton K, Ul-Hassan S, et al. Prediction of autoimmune connective tissue disease in an at-risk cohort: prognostic value of a novel two-score system for interferon status. *Ann Rheum Dis* 2018;77:1432–1439.

39. Weckerle CE, Franek BS, Kelly JA, Kumabe M, Mikolaitis RA, Green SL, et al. Network analysis of associations between serum interferon-α activity, autoantibodies, and clinical features in systemic lupus erythematosus. *Arthritis Rheum* 2011;63:1044–1053.

40. Braunstein I, Klein R, Okawa J, Werth VP. The interferon-regulated gene signature is elevated in subacute cutaneous lupus erythematosus and discoid lupus erythematosus and correlates with the cutaneous lupus area and severity index score. *Br J Dermatol* 2012;166:971–975.

41. Eloranta M-L, Lövgren T, Finke D, Mathsson L, Rönnelid J, Kastner B, et al. Regulation of the interferon-alpha production induced by RNA-containing immune complexes in plasmacytoid dendritic cells. *Arthritis Rheum* 2009;60:2418–2427.

42. Bennett L, Palucka AK, Arce E, Cantrell V, Borvak J, Banchereau J, et al. Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. *J Exp Med* 2003;197:711–723.

43. Zollars E, Courtney SM, Wolf BJ, Allaire N, Ranger A, Hardiman G, et al. Clinical Application of a Modular Genomics Technique in Systemic Lupus Erythematosus: Progress towards Precision Medicine. *Int J Genomics* 2016;2016:7862962.

44. Mathian A, Mouries-Martin S, Dorgham K, Devilliers H, Barnabei L, Ben Salah E, et al. Monitoring disease activity in systemic lupus erythematosus with single-molecule array digital ELISA quantification of serum interferon-α. *Arthritis Rheumatol Hoboken NJ* 2018.

45. Huijser E, Helden-Meeuwsen CG van, Groot N, Bodewes ILA, Wahadat MJ, Schreurs MWJ, et al. MxA is a clinically applicable biomarker for type I interferon activation in systemic lupus erythematosus and systemic sclerosis. *Rheumatol Oxf Engl* 2019;58:1302–1303.

46. Landolt-Marticorena C, Bonventi G, Lubovich A, Ferguson C, Unnithan T, Su J, et al. Lack of association between the interferon-alpha signature and longitudinal changes in disease activity in systemic lupus erythematosus. *Ann Rheum Dis* 2009;68:1440–1446.

47. Mathian A, Mouries-Martin S, Dorgham K, Devilliers H, Yssel H, Garrido Castillo L, et al. Ultrasensitive serum interferon-α quantification during SLE remission identifies patients at risk for relapse. *Ann Rheum Dis* 2019.

48. Furie R, Khamashta M, Merrill JT, Werth VP, Kalunian K, Brohawn P, et al. Anifrolumab, an Anti-Interferon-α Receptor Monoclonal Antibody, in Moderate-to-Severe Systemic Lupus Erythematosus. *Arthritis Rheumatol Hoboken NJ* 2017;69:376–386.

49. Lyons PA, McKinney EF, Rayner TF, Hatton A, Woffendin HB, Koukoulaki M, et al. Novel expression signatures identified by transcriptional analysis of separated leucocyte subsets in systemic lupus erythematosus and vasculitis. *Ann Rheum Dis* 2010;69:1208–1213.

Figures Legends:

Figure 1. Prevalence and distribution of IFN-I, PMN and PB gene scores in systemic SLE. A. IFN-I, PMN and PB gene scores in SLE and healthy controls (HC). The dots represent for single individuals the median of the normalized values of each gene contributing to the score. The IFN-I, PMN, and PB scores were calculated as the median of the relative expression of all genes defining the signature. Statistical analysis performed using Mann-Whitney test. **B.** Venn diagram showing the prevalence of high IFN-I score according to (25), PMN and PB scores and their association. The mean plus 2 SD of HC values was used as threshold to define high scores. **C.** Matrix correlation diagram of individual IFN-I, PMN and PB gene expression. Correlation was based on Spearman correlation analysis (r_s) and values were condensed in a color scale.

Figure 2. Association between high IFN-I, PMN and PB scores with SLE activity, active features and treatment. A. SLE activity was based on the SELENA-SLEDAI (30). **B, C.** Testing for anti-dsDNA, anti-U_1RNP, anti-SSA and anti-C1q autoantibodies was performed in a central laboratory as described in Material and methods, supplementary material. **A, B, C, E**. Comparisons were performed using Mann-Whitney test. When more than 2 groups were compared * indicates that the p-values remains statistically significant after Dunn's tests for multiple comparisons. **D.** Regression analysis of factors associated with gene scores. Chi-square test and Fisher's exact test were used to compare categorical variables as appropriate. Variables with $p < 0.2$ on univariable regression were entered in multivariable logistic models. P-values are expressed using a color scale, and features that remained statistically significant on multivariable analysis are highlighted with a black dot.

Figure 3. The combined presence of high IFN-I- and high PMN-scores identify very active SLE. A. Unsupervised hierarchical clustering using Ward's agglomerative method,

passing the Euclidean distances between samples. Three clusters of at least five patients were identified, and the presence of active SLE (SLEDAI ≥4), inactive SLE (SLEDAI<4) and healthy controls (HCs) is highlighted in red, blue, and green bars, respectively. Cluster characteristics are summarized in the table**. B**. Association between SLEDAI score and patient clusters and gene scores. Comparison were performed using Mann-Whitney test. When more than 2 groups were compared * indicates that the p-values remains statistically significant after Dunn's tests for multiple comparisons.

Figure 4. MX1, MPO, and TNFRSF17 protein levels as surrogates for IFN-I, PMN and PB gene scores. A. Correlation between IFN-I score and *MX1* gene expression or MX1 protein expression. **B.** Matrix correlation diagram showing the association of SLE features and current treatment with IFN-I scores and MX1 protein levels. Chi-square test and Fisher's exact test were used to compare categorical variables as appropriate. Variables with p <0.2 on univariable regression were entered in multivariable logistic models. P-values are expressed using a color scale and features that remained statistically significant on multivariable analysis are highlighted with a black dot. **C,** Correlation between PMN score and *MPO* gene expression or MPO protein expression. **D.** Correlation between PB score and *TNFRS17* gene expression or TNFRS17 protein expression. Spearman correlation analysis (r_s) was used for correlation analysis and comparisons were performed using Mann-Whitney test.

Table 1. Baseline characteristics and active features at the time of sampling in SLE patients (n=140)

Features	
Female	118 (84)
Age, median (range), years	45.6 (19-78.8)
Ethnicity	
Caucasian	101 (72)
African	17 (12)
Asian	14 (10)
Others	8 (6)
Active manifestations at the time of study	
Fever	5 (4)
Arthritis	26 (19)
Cutaneous	30 (22)
Serositis	6 (4)
Neurological disorder	9 (7)
Leukopenia	6 (4)
Thrombocytopenia	9 (7)
Proteinuria > 0.5 g/24h	6 (4)
Immunological features (presence)	
ANA*	132 (95)
Anti-dsDNA	61 (45)
Anti-SSA	55 (41)
Anti-Sm*	38 (28)
Anti-U_1RNP	37 (28)
Anti-C1q	35 (26)
aPL*	55 (40)
Low complement	31 (23)
C3 (g/l) median, range*	0.89 (0.39-1.8)
C4 (g/l) median, range*	0.16 (0.01-0.45)
Activity	
SLEDAI, median (range)	3 (0-46)
SLEDAI $\geq$ 4	69 (50)

Current treatment	
No treatment	14 (10)
Antimalarials	102 (73)
Systemic glucocorticoids	57 (41)
Immunosuppressant agents	70 (50)
B cell targeted agents	10 (7)

* historical data: Antinuclear autoantibodies (ANA), Sm and aPL (antiphospholipid), C3 and C4. Double stranded DNA (ds-DNA), U_1 ribonucleoprotein (U1RNP), anti-C1q were determined by Q'Flash or ELISA as described in the methods section in supplementary material. SLEDAI, Safety of Estrogens in Lupus Erythematosus National Assessment–Systemic Lupus Erythematosus Disease Activity Index (SELENA–SLEDAI) (30).

Table 2. Comparisons between classical biomarkers and IFN-I, PMN, and composite scores to predict clinical SLEDAI ≥2

	Forest model[+]	**Logistic regression model**°			
	Contribution	Unadjusted		Adjusted*	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Composite score[#]	20.09%	**1.11 (1.02-1.21)**	**0.01**	**1.10 (1.01-1.21)**	**0.03**
IFN-I score	19.14%	**1.03 (1.007-1.048)**	**0.0069**	**1.03 (1.006-1.052)**	**0.01**
PMN score	14.06%	**1.08 (1.001-1.17)**	**0.04**	1.07 (0.99-1.16)	0.08
MX1 levels	9.79%	1.003 (1.000-1.007)	0.018	1.003 (1.000-1.007)	0.04
C4	8.64%	1.16 (0.01-88.0)	0.95	0.80 (0.008-75.4)	0.92
GC dose	6.90%	**1.09 (1.02-1.18)**	**0.02**	ND	
Anti-dsDNA	6.68%	1.001 (0.998-1.004)	0.35	1.001 (0.998-1.004)	0.42
C3	5.78%	0.53 (0.13-2.23)	0.39	0.47 (0.10-2.13)	0.32
PB score	5.16%	0.91 (0.77-1.07)	0.29	0.89 (0.73-1.09)	0.26
Anti-C1q	3.75%	1.01 (0.99-1.03)	0.15	1.01 (0.99-1.03)	0.13

GC, glucocorticoids; OR, odds ratio; 95% CI, 95% confidence interval; ND, not done

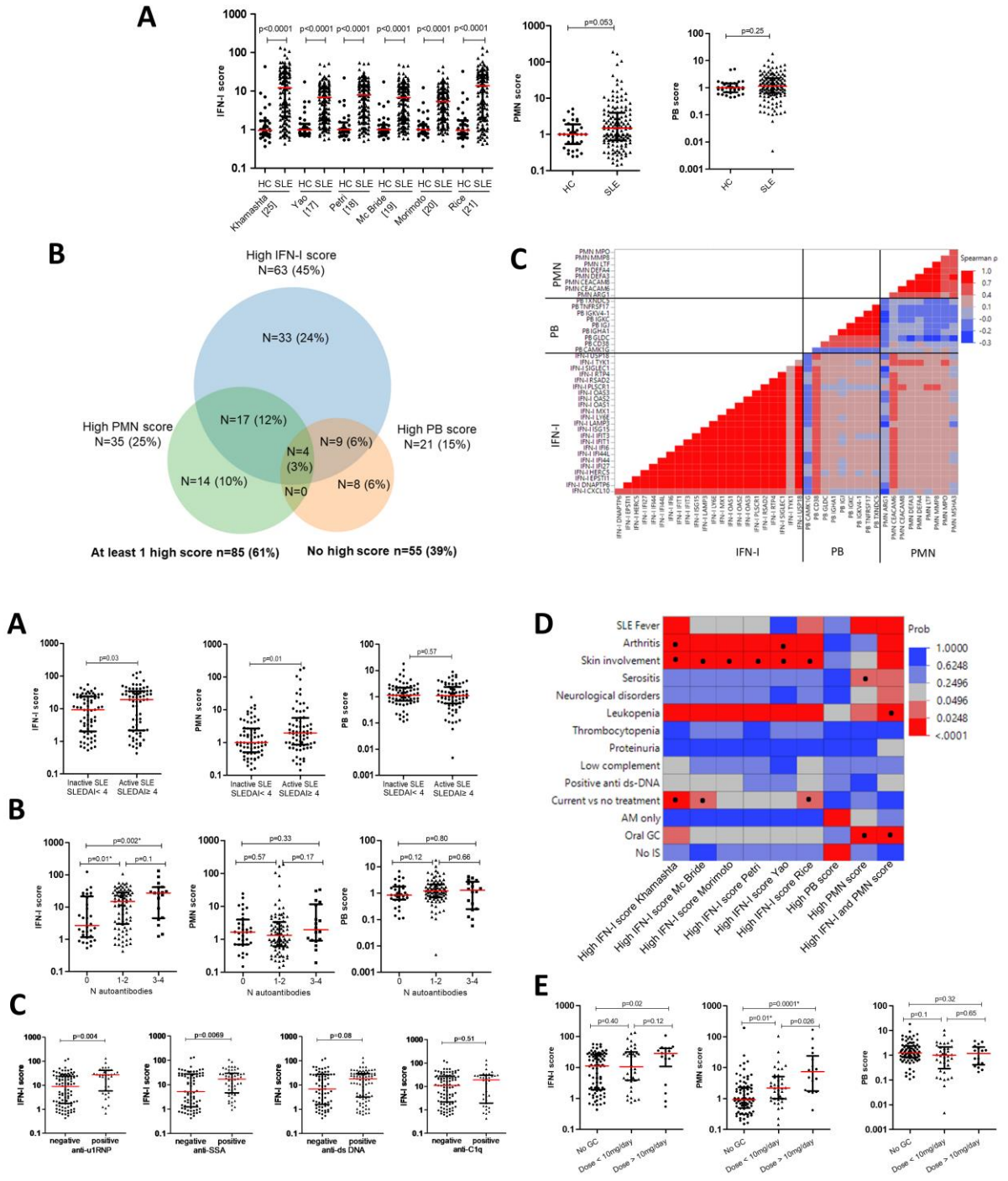
[+] Bootstrap forest model with 10 000 decision trees was used to identify potential contributors and their respective weight expressed as percentage of predicting clinical SLEDAI ≥2.

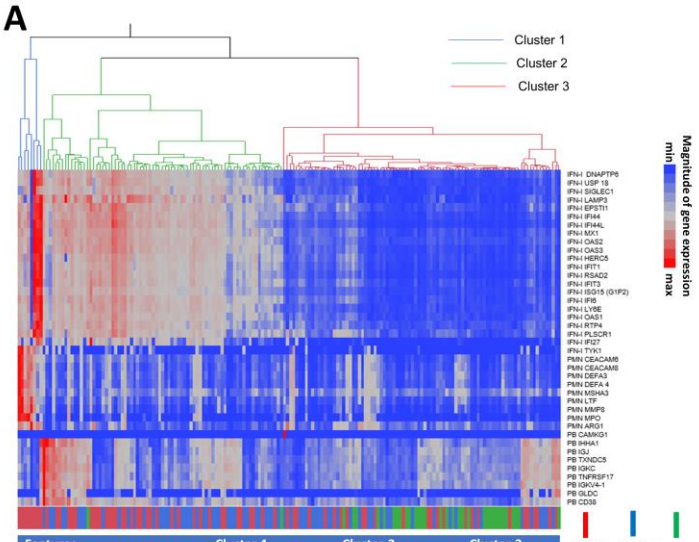
° Logistic regression models to assess the odds of clinical SLEDAI≥2 per unit change in regressor

* Model adjusted for age, sex, ethnicity (Caucasian vs non-Caucasian), current GC use and immunosuppressant agent use.

[#]Composite score: score generated by adding IFN-I genes from (25) to PMN genes

Bold denotes statistically significant values


A
IFN-I score
PMN score
PB score
p<0.0001
p=0.053
p=0.25
HC SLE
Khamashta [25]
Yao [17]
Petri [18]
Mc Bride [19]
Morimoto [20]
Rice [21]
B
High IFN-I score N=63 (45%)
N=33 (24%)
High PMN score N=35 (25%)
N=17 (12%)
High PB score N=21 (15%)
N=9 (6%)
N=4 (3%)
N=14 (10%)
N=0
N=8 (6%)
At least 1 high score n=85 (61%)
No high score n=55 (39%)
C
PMN
PB
IFN-I
Spearman ρ
A
p=0.03
p=0.01
p=0.57
Inactive SLE SLEDAI< 4
Active SLE SLEDAI≥ 4
B
p=0.002*
p=0.01*
p=0.1
p=0.33
p=0.57
p=0.17
p=0.80
p=0.12
p=0.66
N autoantibodies
C
p=0.004
p=0.0069
p=0.08
p=0.51
negative positive
anti-u1RNP
anti-SSA
anti-ds DNA
anti-C1q
D
SLE Fever
Arthritis
Skin involvement
Serositis
Neurological disorders
Leukopenia
Thrombocytopenia
Proteinuria
Low complement
Positive anti ds-DNA
Current vs no treatment
AM only
Oral GC
No IS
Prob
1.0000
0.6248
0.2496
0.0496
0.0248
<.0001
High IFN-I score Khamashta
High IFN-I score Mc Bride
High IFN-I score Morimoto
High IFN-I score Petri
High IFN-I score Yao
High IFN-I score Rice
High PB score
High PMN score
High IFN-I and PMN score
E
p=0.02
p=0.40
p=0.12
p=0.0001*
p=0.01*
p=0.026
p=0.32
p=0.1
p=0.65
No GC
Dose < 10mg/day
Dose > 10mg/day



Features	Cluster 1	Cluster 2	Cluster 3
N patients	8	77	89
HC	0%	3%	36%
Active lupus	100%	52%	29%
High IFN-I score	88%	74%	0%
High PMN score	100%	18%	17%
High PB score	12%	21%	7%
No high score	0%	22%	76%