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Noémie Jourde-Chiche[1], Elizabeth Whalen[2], Bertrand Gondouin[1], Cate Speake[2], Vivian Gersuk[2], Bertrand Dussol[1], Stephane Burtey[1], Virginia Pascual[3], Damien Chaussabel[4] and Laurent Chiche[5]

Abstract

Objective. LN is a severe complication of SLE. Non-invasive biomarkers are needed for identifying patients at risk of a renal flare, for differentiating proliferative from non-proliferative forms and for assessing prognoses for LN.

Methods. We assessed the link between blood transcriptional signatures and LN using blood samples from patients with biopsy-proven LN, extra-renal SLE flares or quiescent SLE. Healthy controls, and control patients with glomerular diseases or bacterial sepsis were included. Modular repertoire analyses from microarray data were confirmed by PCR.

Results. A modular neutrophil signature (upregulation of module M5.15) was present in 65% of SLE patients and was strongly associated with LN. M5.15 activity was stronger in LN than in extra-renal flares (88 *vs* 17%). M5.15 was neither correlated to IFN modules, nor to SLEDAI or anti-dsDNA antibodies, but moderately to CS dose. M5.15 activity was associated with severity of LN, was stronger when proliferative, and decreased in patients responding to treatment. M5.15 activation was not caused by higher CS dose because it correlated only moderately to neutrophil count and was also observed among quiescent patients. Among quiescent patients, those with a past history of LN had higher M5.15 activity (50 *vs* 8%). M5.15 activation was present in patients with bacterial sepsis or ANCA-associated vasculitis, but not in patients with other glomerular diseases. Overall, M5.15 activation was associated with past, present or future flares of LN.

Conclusion. Modular neutrophil signature could be a biomarker for stratifying LN risk and for monitoring its response to treatment.

Trial registration. ClinicalTrials.gov, http://clinicaltrials.gov, NCT00920114

Key words: lupus nephritis, neutrophil, gene expression, systemic lupus erythematosus, biomarkers

- Blood gene expression modular analysis identified a neutrophil signature in 65% of adult SLE patients.
- A modular neutrophil signature was associated with the occurrence, severity and response to treatment of LN in SLE patients.
- A modular neutrophil signature was shared by ANCA-associated vasculitis, but not by primitive glomerular diseases.

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Introduction

LN is a severe and frequent complication of SLE, affecting 20–30% of patients in Europe [1], and with rates as high as 70% in other ethnicities [2]. LN affects patients' survival [3, 4], and severe forms lead to end-stage renal disease (ESRD) in 5–10% of patients after 10 years [5, 6]. LN prognosis is improved by early diagnosis and treatment [7], whereas diagnostic delay is associated with increased risk of ESRD [8]. A kidney biopsy is the gold standard for assessing LN severity and guiding treatment [9, 10].

Classification of LN according to the current International Society of Nephrology/Renal Pathology Society (ISN/RPS) [11] allows stratification of patients between those with proliferative LN, who require immunosuppressive therapy [9, 10], and those with non-proliferative or chronic LN lesions. However, a kidney biopsy remains an invasive procedure [12, 13] that cannot be easily performed repeatedly [14], even less pre-emptively, although SLE immune-mediated renal injury can precede the detection of proteinuria [15]. Moreover, the response to treatment is unpredictable, and clinical remission is not always associated with resolution of histological activity in LN [16]. Non-invasive biomarkers are therefore needed for identifying patients prone to LN, and for determining the severity and a prognosis for renal flares when they occur. To date, although some serological or urinary markers have been associated with LN occurrence or severity, none is sufficiently reliable for replacing a kidney biopsy, guiding treatment and predicting LN outcomes [17–21].

Over the last decade, omics-based techniques have been used successfully to discover biomarkers in renal diseases [22] and in SLE [23, 24]. Modular transcriptional repertoire analysis [25, 26] of whole-blood samples, in particular, has revealed new aspects of the SLE IFN signature [27]. The aim of this study was to use modular repertoire analyses to analyse the links between blood transcriptional signatures and LN.

Methods

Selection of patients and characterization

The study comprised 143 patients and controls overall. The 62 consecutive patients with SLE who fulfilled the 1997 ACR criteria were enrolled and followed-up prospectively at a French reference centre for autoimmune disease (Hôpital de la Conception, Marseille, France). Blood was collected by peripheral venipuncture using Tempus tubes (3 ml) at inclusion and longitudinally at each follow-up visit, and distant from any infectious event. Complete clinical and biological evaluation of disease activity was performed, as well as pathological analysis of renal biopsies (see supplementary Methods, pathological analysis of renal biopsies section, available at *Rheumatology* Online).

Healthy controls ($n = 21$) matched for age, gender and ethnicity, were sampled once. Pathological controls ($n = 40$) comprised patients with crescentic GN caused by ANCA-associated vasculitis (AAV) ($n = 10$); patients with primitive glomerular diseases ($n = 15$) matched for renal parameters with patients with LN, and who were sampled once at the time of a renal biopsy; and patients with bacterial pneumonia who were sampled once at the time of infection ($n = 15$). Healthy controls of these pathological controls ($n = 20$) were also sampled once.

Blood samples from SLE patients were split into three groups, according to disease activity and renal involvement: biopsy-proven LN comprised samples collected at the time of a biopsy-proven LN flare with active lesions, whether they were proliferative (class III or IV ± V) or not (class II or V of the ISN/RPS 2003 classification). Patients showing only chronic lesions (class III-C or IV-C with no activity) were not included in this group. Extra-renal SLE flare comprised samples collected at the time of an extra-renal flare, with no sign of active LN. Quiescent lupus comprised samples from SLE patients at their first clinically quiescent visit, defined by the absence of flare or treatment modification in the 60 days prior to the visit and a SLEDAI of $\leqslant 4$ (immunological activity authorized). In each group, the existence of a past history of LN was recorded and the patients were considered as ever renal if they had a past and/or a current history of LN and/or had developed LN during the follow-up.

RNA preparation and microarray hybridization

RNA was processed as described elsewhere [27] using Illumina beadchips (see supplementary Methods, RNA preparation and microarray hybridization section, available at *Rheumatology* Online). Data are deposited in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO Series accession number GSE49454). PCR analyses were performed on the same samples using a Fluidigm Real-Time PCR platform (see supplementary Methods, PCR analyses section, available at *Rheumatology* Online). Publicly available blood and tissue gene-expression profiles were also used (see supplementary Methods, public domain datasets section, available at *Rheumatology* Online).

Modular transcriptional repertoire analyses

Analyses were performed using the second generation of a modular framework as previously described [23, 26, 27] (see supplementary Methods, modular transcriptional repertoire analyses section, available at *Rheumatology* Online).

The level of regulation of each module was calculated as the per cent difference: % upregulated probes − % downregulated probes. A module was considered active (upregulated) if the percentage difference was $\geqslant 20\%$ and silenced (downregulated) if the percentage difference was -20% or less.

Statistical analyses

Numerical data were processed and analysed using R statistical software. For continuous data, comparisons between groups were conducted using analysis of variance (ANOVA) (assuming normality was appropriate) or the non-parametric Kruskal–Wallis test. Student's *t*-test or

Wilcoxon's test was conducted if further testing was needed to determine which group was different. Linear models were used to test for trend. For categorical variables, Fisher's exact test was used to determine differences in contingency tables, and the Chi-squared test to determine trends in proportions if the categorical variable was ordinal. Correlations were assessed by Pearson's (assuming normality was appropriate) or Spearman's correlation test.

For longitudinal analyses, a mixed model was used to determine the relationship between module activity variation and response to treatment in patients with proliferative LN who had at least three blood samples. This model comprised fixed effects of the group (responder *vs* non-responder), time (as a continuous variable), and an interaction term between time and group, with a random effect for individual. Response was defined by complete or partial remission at M6 [28].

Values of $P < 0.05$ were considered to indicate statistical significance, with adjustment by multiple-testing correction when needed (Benjamini–Hochberg procedure).

Ethics and informed consent

Patients were included in the LUPUCE study: Estimate of the Activity and the Forecast of the Lupus Disease of the Adult by a Transcriptomic Score (NCT00920114): the design details are reported in [27]. This study was conducted according to the principles expressed in the Declaration of Helsinki. The LUPUCE study and this study were approved in France by the Comité de Protection des Personnes Sud Méditerranée 1 (IDRCB 2009-A00257-50) and in the USA by the Institutional Review Boards of the Baylor Institute of Immunology Research (IRB 011-173) and the Benaroya Research Institute (IRB 12085). Informed written consent was obtained from each patient and enrolled control prior to any study-related procedure.

Results

Characteristics of the SLE patients

The characteristics of the 62 enrolled SLE patients are detailed in supplementary Table S1, available at *Rheumatology* Online. The median age was 38 years, 85% of patients were women, and 89% were White. The median duration of SLE was 7.8 years. Data on 157 visits were collected. Twenty-four patients were sampled at the time of a renal biopsy and showed active LN that was either proliferative [$n = 14$, class III or IV-(A) or (A/C) ± class V] or non-proliferative ($n = 10$, class II, class V or interstitial nephritis). It was the first LN flare event for 13 and a relapse for 11 patients. Nephrotic syndrome was present in 14 patients and 9 had acute kidney injury. During a median follow-up of 42 months (range: 6–55), one patient died (sudden death) after the initiation of dialysis, two other patients reached ESRD, and the median serum creatinine at the last visit was 68 μmol/l (range: 43–1178). Detailed renal parameters of patients with active LN are provided in supplementary Table S1, available at *Rheumatology* Online. Eleven patients were sampled at the time of an extra-renal (cutaneous, articular and/or haematological) flare (supplementary Table S1, available at *Rheumatology* Online). Thirty-four samples were collected from SLE patients at the time of their first quiescent visit. Among them, 22 patients had a past history of LN (supplementary Table S1, available at *Rheumatology* Online).

Module repertoire analysis reveals a strong neutrophil signature in SLE

At the group level, in addition to the previously reported IFN signature (upregulation of the three IFN-related modules M1.2, M3.4 and M5.12) [27], modular repertoire analysis revealed strong upregulation of module M5.15 (Fig. 1). A similar upregulation pattern was also observed in three other SLE datasets generated in children and adults from various ethnicities (supplementary Fig. S1, available at *Rheumatology* Online). Module M5.15, annotated neutrophil, comprises 24 probes corresponding to 22 genes (supplementary Table S2, available at *Rheumatology* Online) strongly related to neutrophil functions (supplementary Fig. S2, available at *Rheumatology* Online) [29]. In this cohort of SLE patients, 20/24 (83%) probes belonging to M5.15 were upregulated, whereas no probe was downregulated compared with matched healthy controls (Fig. 1). At the individual level, M5.15 was active (⩾20% difference) in 92 (59%) SLE samples, and 40/62 patients (65%) showed this modular neutrophil signature at least once during the follow-up (characteristics according to the presence of a modular neutrophil signature are detailed in supplementary Tables S3 and S4, available at *Rheumatology* Online, respectively).

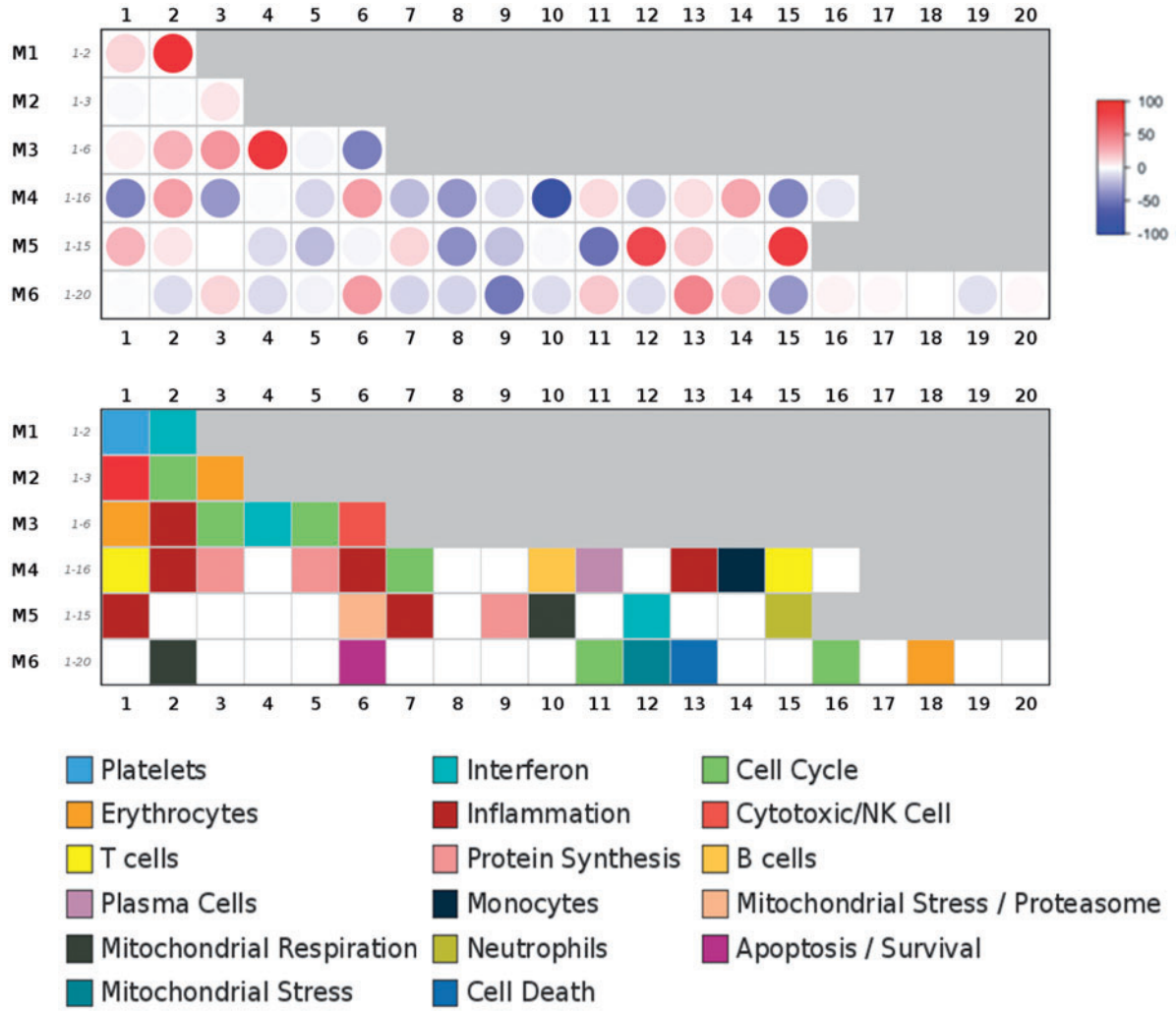
Modular neutrophil signature and clinical or biological activity parameters of SLE

There was no significant correlation between IFN modules activity, either individually or as a combined modular IFN score, and M5.15 activity (Fig. 2). At the individual level, M5.15 activity was not correlated to age or ethnicity, nor with SLEDAI or the titre of anti-dsDNA (Table 1). M5.15 was associated with renal flares, independently of CS dose (Table 1). Samples with M5.15 activity were less likely to be from patients experiencing an isolated cutaneous or articular flare. Importantly, among the 62 modules tested, M5.15 was the module exhibiting the strongest association with active LN (adjusted *P*-values = 0.0014 after Benjamini–Hochberg multiple-testing correction), whereas none of the three IFN modules (M1.2, M3.4, M5.12) showed a significant association (only M5.12 had a *P*-values of 0.007 before multiple-testing correction). M5.15 was moderately correlated with daily CS dose ($r = 0.43$, $P < 0.001$), independently of neutrophil count.

Modular neutrophil signature and severity of LN

M5.15 activity was stronger in patients with active LN than in those with extra-renal flares (88% *vs* 17% probes upregulated, with no probe downregulated, respectively).

FIG. 1 Modular repertoire analyses at the group level in SLE patients



Four modules were strongly upregulated in SLE patients (*vs* healthy controls): three INF-related modules (M1.2: 100% probes upregulated; M3.4: 90% probes upregulated; M5.12: 79% probes upregulated, with 0% probes downregulated) and M5.15 (83% probes upregulated, 0% downregulated). Other active modules (per cent difference $\geqslant$20%) were those related to inflammatory response (M3.2, M4.2, M4.6), cell cycle (M3.3), cell death (M6.13) and apoptosis/survival (M6.6). In addition, silencing of modules related to B cells (M4.10), T cells (M4.1 and M4.15), cytotoxicity/NK cells and protein synthesis (M4.3) was observed.

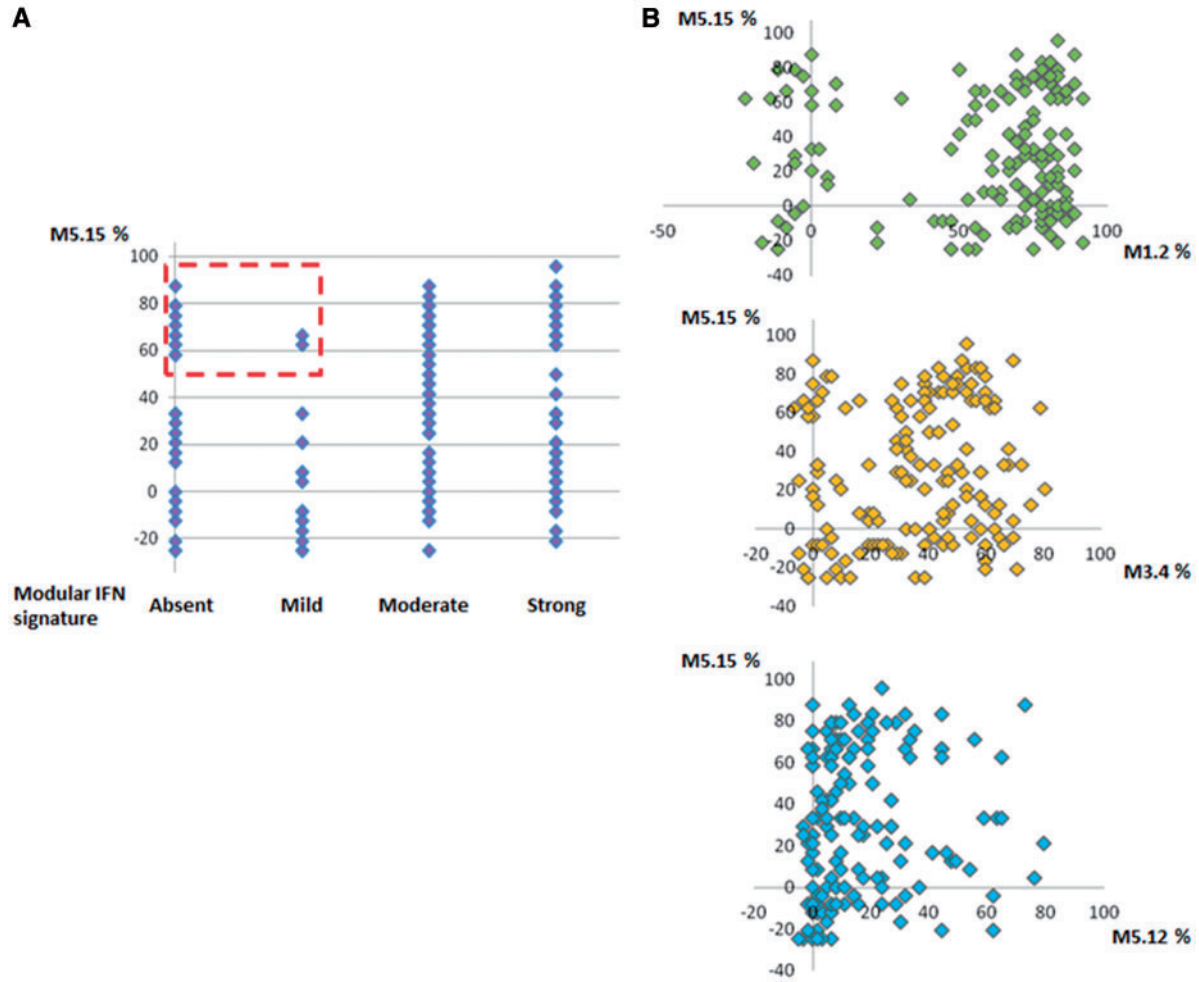
The activation of M5.15 was associated with some indicators of LN severity, such as serum-creatinine level, 24-h proteinuria and lower serum albumin (supplementary Table S3, available at *Rheumatology* Online). M5.15 activity was associated with the presence of acute kidney injury ($P = 0.03$) and inversely correlated with serum albumin ($r = -0.30$, $P = 0.01$), but not with the activity of urinary sediment. M5.15 at inclusion was correlated with serum creatinine at the last follow-up visit ($r = 0.28$, $P < 0.001$).

Among patients with active LN, median M5.15 activity was higher in those with proliferative than in those with non-proliferative LN (respectively, 66.7 *vs* 18.8%, $P = 0.04$). Yet, there was no correlation between M5.15 and pathological parameters such as the National Institutes of Health (NIH) activity and chronicity indices or tubulo-interstitial score, or with the percentage of activity or chronicity according to the ISN/RPS classification.

After the initiation of immunosuppressive therapy in patients with proliferative LN and $\geqslant$3 longitudinal samples ($n = 10$), a significant association was observed between the decrease in M5.15 activity and remission at M6 ($P = 0.046$) (Fig. 3). Conversely, there was no relation between IFN signature and response to treatment in these patients (data not shown).

The analysis of published transcriptomic data [30], generated from glomeruli and tubulo-interstitium of micro-dissected kidneys from SLE patients with active LN, revealed that the expression of the genes *LTF* (lactotransferrin) and

FIG. 2 Neutrophil signature is not correlated to IFN signature in SLE patients



M5.15 activity was not correlated to (**A**) modular IFN signature (absent: 0, mild: 1, moderate: 2, strong: 3 active IFN modules) ($r = 0.1$, $P = 0.2$) or (**B**) activity of each IFN module individually ($r = 0.06$, $P = 0.4$ for M1.2; $r = 0.15$, $P = 0.06$ for M3.4 and $r = 0.13$, $P = 0.09$ for M5.12). Thirteen samples (from seven patients) had a strong modular neutrophil signature (M5.15 >50%) and an absent/mild IFN signature.

MPO (myeloperoxidase), belonging to M5.15, was significantly upregulated compared with healthy renal tissue (supplementary Table S5, available at *Rheumatology* Online).

Modular neutrophil signature and potential confounding factors

SLE patients with active LN, who displayed a strong neutrophil signature, also received higher doses of CSs and had worse renal function than others (supplementary Table S1, available at *Rheumatology* Online). We thus conducted additional analyses to confirm the link observed between the modular neutrophil signature and LN.

M5.15 activity was correlated with blood neutrophil count ($r = 0.38$, $P < 0.001$), but this correlation was not as strong as for different types of lymphocytes with their corresponding modules (supplementary Fig. S3, available at *Rheumatology* Online). M.15 activity could be high in some quiescent patients receiving low doses of CSs, and low in some patients receiving high doses of CSs (Fig. 4), which mirrors what was observed in a paediatric cohort (supplementary Fig. S4, available at *Rheumatology* Online). Among quiescent SLE patients with low and stable doses of CSs, M5.15 activity was higher in those with a past history of LN (50% *vs* 8% in those without, respectively) (Fig. 5A).

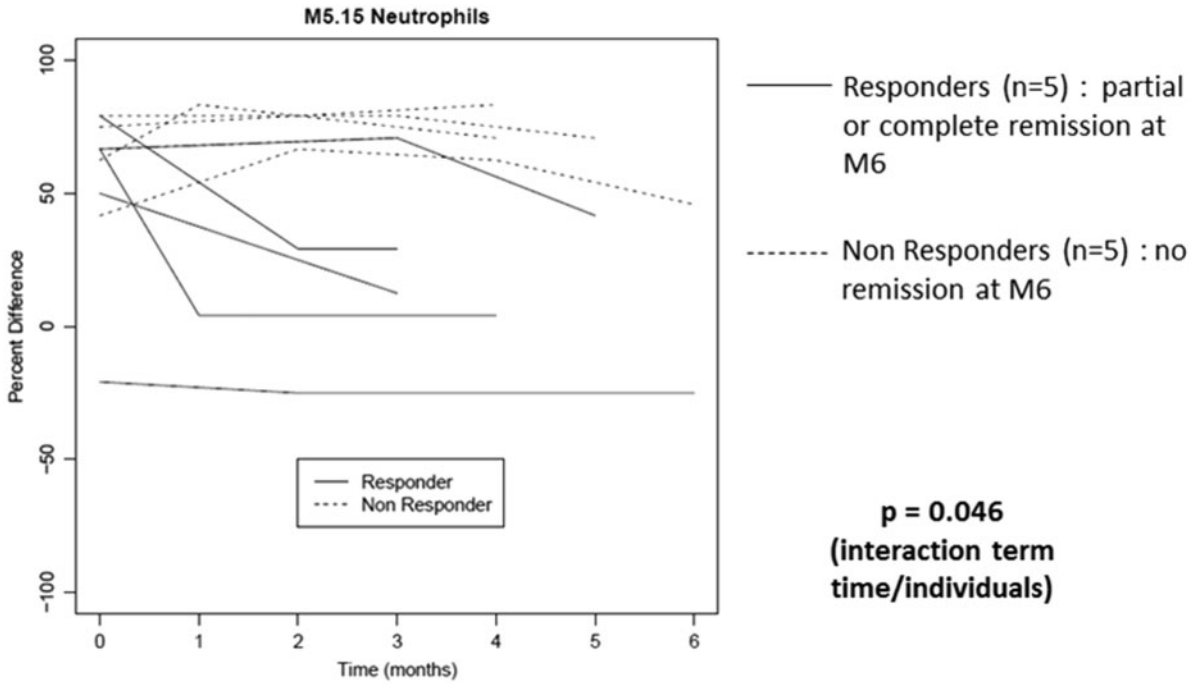
Modular repertoire analyses of SLE patients with active LN were compared with those of control patients from this study or from publicly available datasets (supplementary Table S6, available at *Rheumatology* Online). The modular neutrophil signature was shared by patients with bacterial sepsis and patients with AAV (Fig. 5B). Conversely, patients with primary glomerular diseases, matched for renal

Table 1 Correlation of M5.15 activity with SLE patients' characteristics and disease-activity parameters

Characteristics	Correlation coefficient (Spearman)	*p*-values (Spearman)	*p*-values adjusted for neutrophil count and steroid use
Age	−0.037	0.647	0.314
SELENA-SLEDAI	0.136	0.089	0.862
Anti-dsDNA titre	−0.054	0.514	0.346
Neutrophil count	**0.376**	**2.27×10^{-6}**	**8.01×10^{-5}**
Daily CS dose	**0.427**	**2.38×10^{-8}**	**0.0042**
		Wilcox test *p*-values	*p*-values adjusted for neutrophil count and steroid use
Gender, male		**4.16×10^{-5}**	**8.88×10^{-5}**
Ethnicity		0.866	0.914
Cutaneous flare		**0.029**	**0.0015**
Articular flare		**0.0081**	**0.0007**
Haematological flare		0.897	0.992
Renal flare		**2.33×10^{-5}**	**0.026**

SELENA: Safety of Estrogens in Lupus Erythematosis National Assessment trial. Values highlighted in bold are for adjusted $p < 0.05$.

Fig. 3 M5.15 activity over time in patients with proliferative LN

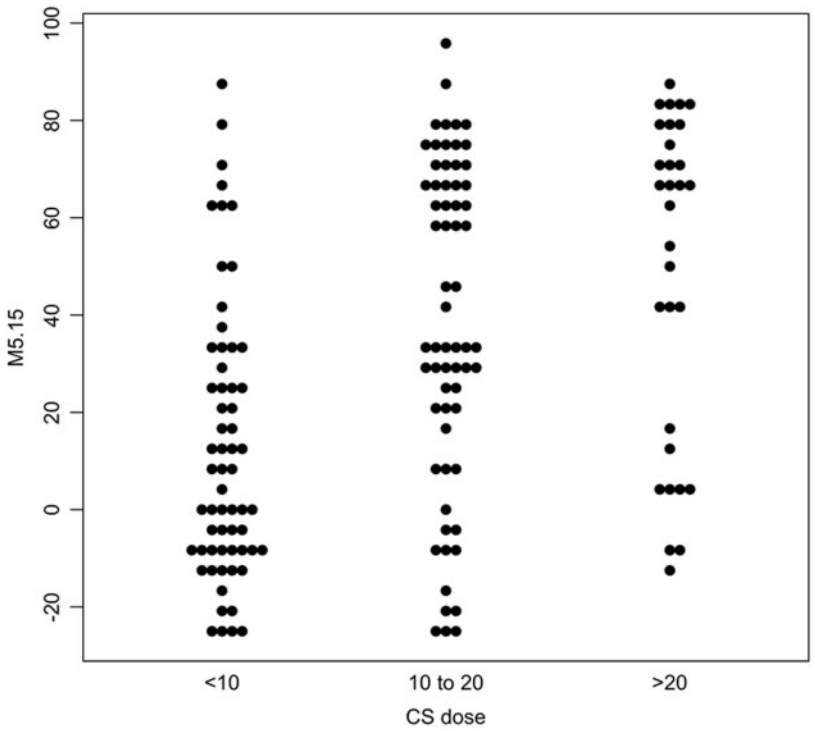


A decrease in M5.15 activity was observed over time in patients responding to treatment ($n = 5$) compared with non-responders ($n = 5$).

function and levels of proteinuria, displayed no activation of M5.15. Of note, active LN and active tuberculosis both displayed an IFN signature (Fig. 5B) and the activation of modules related to inflammation, cell death and apoptosis. Silencing of modules related to B-cells, T cells and cytotoxicity/NK cells were common to patients with SLE, tuberculosis, AAV or bacterial sepsis.

Additionally, we tested the association of smoking status with M5.15 activity. There was no correlation between M5.15 activity and smoking in SLE patients, and

FIG. 4 M5.15 activity according to CS dose in SLE patients ($n = 157$ visits)



smoking status was not significantly different between SLE groups.

Modular neutrophil signature and prognosis of LN

IFN signature could not differentiate between patients with active LN and those with extra-renal flares or quiescent SLE. Conversely, M5.15 activation (M5.15 $\geqslant$20%) was observed, respectively, in 16/24 (67%), 2/11 (18%) and 16/34 (47%) within these three groups (Fisher's exact test: $P = 0.027$). Interestingly, of the two patients with an extra-renal SLE flare and a modular neutrophil signature, one had a past history of LN and the other subsequently developed LN (class III, after 24 months). Among the nine other patients, only two eventually developed LN (class IV, after 12 and 33 months). In quiescent patients, 10/16 with a modular neutrophil signature had a past history of LN, and 4/16 subsequently developed a LN flare (class IV after 18, 21 and 36 months; class V after 13 months). Overall, the presence of a modular neutrophil signature at least once during the follow-up was associated with past, current or future LN (82.5% of ever renal patients *vs* 54.5% of patients who never presented LN, $P = 0.039$) (supplementary Table S4, available at *Rheumatology* Online).

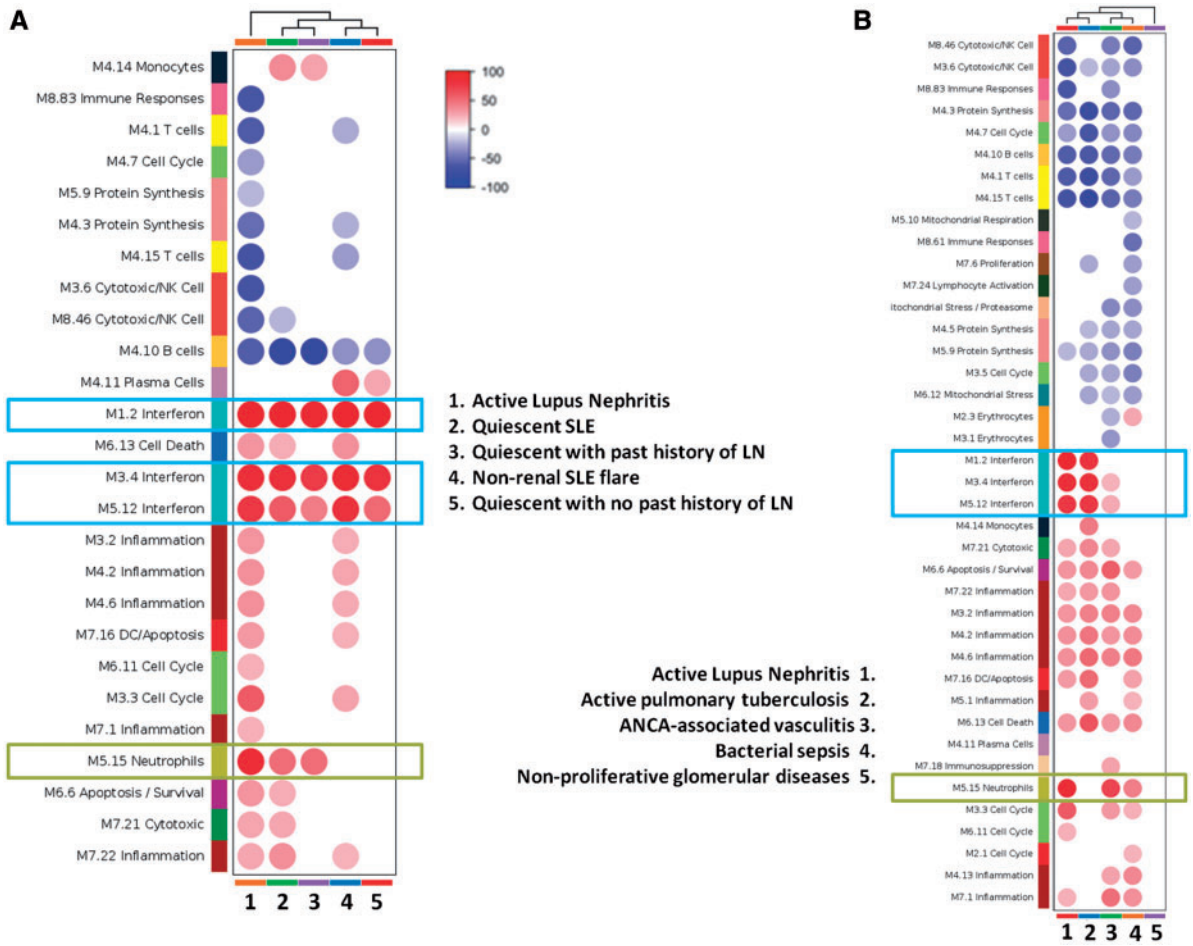
PCR validation and neutrophil score

To validate the microarray data, we confirmed the overexpression of four genes (*DEFA4*, *ELANE*, *CEACAM6*, *CEACAM8*) belonging to M5.15, using whole-blood RT-PCR in the same SLE samples. There were excellent correlations between microarray and TaqMan assays across the samples (supplementary Fig. S5, available at *Rheumatology* Online). A PCR neutrophil score was calculated, corresponding to the mean of the log2 (fold change) values of SLE samples compared with matched healthy controls for these four genes. This score correlated well with global M5.15 activity, with a 93% accuracy in predicting the presence of a modular neutrophil signature (supplementary Fig. S6, available at *Rheumatology* Online).

Discussion

Over the last decade, omics-based techniques (e.g. genomics, transcriptomics and proteomics) have extended our understanding of the molecular basis of SLE and provided candidate biomarkers for disease prognosis and response to treatment [23–25]. In this study, conducted in an adult European cohort of SLE patients recruited in Nephrology and Internal Medicine, two-thirds of patients displayed a modular neutrophil signature at least once during the study period. Importantly, a comparable signature was observed in independent cohorts of paediatric patients or adult patients with different ethnic backgrounds. More importantly, we have shown, for the first time, a strong association between this modular

FIG. 5 Modular repertoire analysis in various groups of SLE patients and in control patients



(**A**) Modular repertoire analysis of SLE patients, stratified according to renal activity/involvement. In contrast to the IFN signature, which was present in all SLE groups, a modular neutrophil signature was present in patients with active LN (1) and in quiescent patients (2), particularly those with a past history of LN (3). (**B**) Modular repertoire analysis of patients with LN and other conditions, with or without renal involvement. Active LN (1) and tuberculosis (2) shared an IFN signature and clustered together (hierarchical clustering), ANCA-associated vasculitis (3) and bacterial sepsis (4) shared the neutrophil signature of SLE and clustered together, while other renal diseases (5) displayed no particular modular signature.

neutrophil signature (i.e. module M5.15 activity) and the occurrence, severity and prognosis of LN.

Strikingly, the modular neutrophil signature was not correlated with markers for global disease activity, such as SLEDAI or anti-dsDNA antibody titres, or to the IFN signature of SLE. However, the association between M15.5 and LN was reinforced by further analyses. First, the proportion of patients with M5.15 activation was higher in patients with LN than in those with extra-renal SLE flares. Second, among patients with biopsy-proven active LN, M5.15 activity was stronger among those with proliferative (*vs* non-proliferative) nephritis, and M5.15 was associated with various indicators of LN severity, including serum-creatinine level (at diagnosis and at last follow-up), 24-h proteinuria, serum albumin and acute kidney injury. Third, an association was observed between the decrease in M5.15 activity and remission at M6 after the initiation of immunosuppressive therapy in patients with proliferative LN and who were followed longitudinally.

Finally, some of the neutrophil-specific transcripts belonging to the M5.15 module were also identified as overexpressed in the kidney tissues of SLE patients [30]. These observations are in accordance with a previous report by Bennett *et al.* [24], where a neutrophil signature had been initially reported in 25/30 (83%) children, of which 18 (60%) had LN. Altogether, these results suggest

that the modular neutrophil signature could be associated with severe manifestations of SLE (i.e. LN) as well as with the severity of such manifestations (i.e. proliferative LN and/or renal severity markers), providing a non-invasive marker for monitoring patients with SLE.

As patients with LN had both increased doses of CSs and worse renal function, complementary analyses were performed to investigate these potential confounding factors. Indeed, although M5.15 was associated with LN independently of CS dose, CSs are known to promote demargination of circulating neutrophils [31], and could increase neutrophil gene expression in whole blood because of mere neutrophilia. Yet, conversely to some cell counts that were strongly correlated to corresponding modules, M5.15 was only moderately correlated with neutrophil count. This suggests that M5.15 reflects a functional activation of neutrophils. Of note, such a neutrophil signature was first reported in SLE and AAV in studies using peripheral blood mononuclear cells, and was attributed to low-density granulocytes, a distinct class of neutrophils migrating with the peripheral blood mononuclear cell fraction [24, 31, 32]. Also, some SLE patients receiving no or low-dose steroids had high M5.15 activity, and others receiving high-dose steroids displayed no neutrophil signature. This supports M5.15 activation being a reflection of an inflammatory response in some SLE patients with an aggressive disease. We did not observe a modular neutrophil signature in patients with various glomerular diseases that were matched for renal function and proteinuria; this excludes the possibility that M5.15 activation could be a consequence of acute kidney injury or nephrotic syndrome. Additionally, although cigarette smoking can induce neutrophilia and neutrophil activation in lungs [33], there was no association of blood M5.15 activity with smoking status in patients with SLE.

Interestingly, we also observed a modular neutrophil signature in quiescent SLE patients, including patients with absent or low modular IFN signatures. In addition, most quiescent patients displaying this signature had a past history of LN or had developed LN during the follow-up period. Overall, this suggests that M5.15 activity could allow the identification/stratification of SLE patients who are at risk for LN. Interestingly, an enrichment for a granulocyte signature in patients developing LN was also observed in a large longitudinal cohort of paediatric patients with SLE [34]. This finding is of the utmost importance, as LN does not occur in all SLE patients and, although genetic, hormonal and ethnic backgrounds are risk factors for LN, no clinical or biological parameter can currently identify patients at high risk of developing LN [35]. Finally, these observations further support a pathogenic role for neutrophils in the severity of immune dysregulation observed in adult SLE patients, in particular those with LN.

The understanding of the potential role of neutrophils in SLE and LN has expanded with the discovery of neutrophil extracellular traps (NETs) [36]. NETs are fibrous networks of DNA and antimicrobial factors that are released by dying neutrophils to trap and kill pathogens, but can also lead to tissue damage [37]. NETs are also involved in the pathogenesis of many autoimmune diseases, including SLE and AAV [37–44], and the impaired degradation of NETs is associated with LN in SLE patients [45]. In our study, we observed a similar modular neutrophil signature in SLE patients with active LN and in patients with AAV or bacterial sepsis. Indeed, 8 of the 11 genes identified in a recent publication as correlated with AAV disease activity are included in M5.15 [32]. Genes belonging to M5.15 include several genes implicated in early granulopoiesis, including *MPO*, neutrophil elastase (*ELANE*), cathepsin G (*CTSG*), defensin A4 (*DEFA4*) and *LTF*. Most of these genes have established roles in neutrophil maturation and NETs formation (including *DEFA4*, *CTSG* and *ELANE*). In particular, *DEFA4* participates in immune-mediated tissue damage consecutive to autoantibody deposition in SLE [37]. Furthermore, both *DEFA4* and *CTSG* (a serine protease that is also released by neutrophils during NETs formation) are expressed at higher levels in SLE patients with an active disease [46].

This study has several strengths. Namely, samples were collected together with careful recording of clinical and laboratory data**.** In addition, active LN was systematically documented by a renal biopsy and classified by an expert pathologist according to the ISN/RPS [11]. Access to various control conditions allowed us to validate our findings across independent datasets.

This study also has limitations. First, the absence of correlation between M5.15 activity and pathological markers of activity implies that blood modular analysis is not meant to replace renal biopsy, and M5.15 activity is to be considered as a risk marker of LN, but not a diagnostic pathological tool. Then, the use of whole-blood samples prevented investigation of the exact cellular source of the neutrophil signature observed in SLE patients, and no functional assay on neutrophil functions was conducted. However, this study was mainly dedicated to biomarker discovery. PCR validation of the microarray results, which showed very good correlation with the four-gene PCR neutrophil score, suggests a possible translation into clinical practice. Finally, the limited number of longitudinal samples warrants further studies for confirming the prognostic value of the modular neutrophil signature in the progression of quiescent patients to LN and in the monitoring of responses to treatment in patients with LN.

After the discovery of an IFN signature in SLE a decade ago, immune dysregulation of SLE has often been compared with a chronic virus-like response, and is also quite close to the IFN signature observed during infection with intracellular bacteria, such as tuberculosis [47]. Alternatively, the strong neutrophil signature observed in SLE patients with LN, as in patients with AAV but also in those with severe bacterial sepsis, demonstrates that a bacterial-like immune response may also be involved in SLE pathogenesis, especially in patients with severe organ involvement. A practical consequence of these observations is that these various biomarker signatures are

not specific to SLE and should be assessed at a distance from infectious events.

Collectively, our results suggest that the presence of a modular neutrophil signature is associated with a more aggressive course of SLE with the occurrence of LN, and could be associated with LN severity and prognosis. Interestingly, simultaneous assessment of both modular IFN and neutrophil signatures could allow a molecular stratification of patients with various inflammatory conditions and identify pathways to be targeted for treatment.

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Supplementary data

Supplementary data are available at *Rheumatology* Online.

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