PLASMA CYTOKINES AS POTENTIAL BIOMARKERS OF KIDNEY DAMAGE IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

Pacheco-Lugo Lisandro[1*], Navarro Quiroz Elkin[1], Sáenz-García José[3,4], González Torres Henry[1], Fang Luis[5], Díaz-Olmos Yirys[5], Garavito de Egea Gloria[5], Egea Bermejo Eduardo[5], Aroca Martínez Gustavo[1,2].

[1]Grupo de Nefrología, Universidad Simón Bolívar, Barranquilla, Colombia.

[2]Clínica de la Costa, Barranquilla, Colombia.

[3]Grupo de Genómica Funcional de Parásitos, Universidad Federal de Paraná. Curitiba, Brasil.

[4]Departamento de Ciencias Fisiológicas, Facultad de Ciencias Médicas. UNAN, Managua, Nicaragua.

[5]Universidad del Norte, Barranquilla, Colombia.

*Corresponding author; E-mail: lpacheco28@unisimonbolivar.edu.co

Universidad Simón Bolívar. Barranquilla, Atlántico. Postal code: 080003.

ABSTRACT

Background: Systemic lupus erythematosus (SLE) is a heterogeneous chronic inflammatory autoimmune disorder characterized by an exacerbated expression of cytokines and chemokines in different tissues and organs. Renal involvement is a significant contributor to the morbidity and mortality of SLE, and their diagnosis is based on renal biopsy, an invasive procedure with high risk of complications. Therefore, the development of alternative, non-invasive diagnostic tests for kidney disease in patients with SLE is a priority. *Aim:* To evaluate the plasma levels of a panel of cytokines and chemokines using multiplex xMAP technology in a cohort of Colombian patients with active and inactive SLE, and to evaluate their potential as biomarkers of renal involvement.

Results: Plasma from 40 SLE non-nephritis (LNN) patients and 80 lupus nephritis (LN) patients with different levels of renal involvement were analyzed for 39 cytokines using Luminex xMAP technology. Lupus nephritis patients had significantly increased plasma eotaxin, tumor necrosis factor (TNF)-α, interleukin (IL)-17-α, IL-10 and IL-15 as compared to the LNN group. Macrophage-derived chemokine (MDC), growth regulated oncogene alpha(GRO), and epidermal growth factor (EGF) were significantly elevated in LNN patients when compared to LN individuals. Plasma eotaxin levels allowed a discrimination between LNN and LN patients, which we performed a receiver operating characteristic (ROC) curve to confirm. We observed a correlation of eotaxin levels with active nephritis (SLEDAI). Our

data indicate that circulating cytokines and chemokines could be considered good predictors of renal involvement in individuals with SLE.

1. INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by an intense inflammatory state and loss of self-tolerance to its own antigens by the production of self-reactive antibodies, principally against nuclear antigens. Its physiopathology is not completely understood. Renal involvement in SLE is estimated to affect half of patients and is one of the leading causes of morbidity and a significant contributor to mortality [1]. Renal disease activity is one of the most important prognostic factors of patients with SLE. Thus, the identification of lupus nephritis (LN) in SLE patients is an important clinical implication guiding the treatment of SLE, which may contribute to an early diagnosis and monitoring of the activity of the disease, which could to avoid an immunosuppressive overtreatment in clinical settings, improving the quality of life of these patients, due to the multiple side effects of these medications [2]. However, renal injury in LN does not manifest as one uniform entity. Based on histologic analysis of renal core biopsies, the International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification system has categorized the spectrum of glomerular pathology in one classification ranging from classes I to V. This

classification combines considerate clinical, histologic and laboratory parameters to evaluate the patients [3].

Conventional laboratory markers for follow-up of kidney disease in SLE patients, such as urine protein-creatinine ratio, proteinuria, creatinine clearance, complement, and anti-dsDNA levels are considered inefficient to classify LN stages and long-term outcomes of patients [4]. They are neither sensitive nor specific for differentiating renal activity and damage in LN [5]. Renal biopsy remains the standard of care for the evaluation of suspected flares in LN and helps to indicate the treatment and management of the patients [6]. It is indicated when proteinuria, active urine sediment, or elevated serum creatinine is present. However, renal biopsy carries a small but significant risk, primarily of bleeding resulting in perirenal hematoma, blood transfusion, and in patients with severe (although rare) cases, need for an angiogram [7]. Differences also exist in diagnosis due to the difficulty of indicating a number of active or chronic lesions in a specific class of LN [8]. Thus, laboratory biomarkers are necessary to enhance the diagnostic accuracy and sensitivity of LN, monitoring of treatment response, and early detection of renal flares.

Organ involvement in SLE cannot be accurately predicted, and it is interesting to speculate whether newer tests can help predict disease course. Thus, cytokine measures have been studied for associations with organ involvement as well as their potential ability to monitor disease. For suspected kidney involvement, renal biopsy is the gold standard for diagnosis.

However, as it is invasive and has risks of hemorrhage and infection, it presents a less satisfactory method for monitoring renal involvement. LN requires long-term monitoring over several years, as flares may occur, as well as progressive deterioration of function. Surrogate markers include serum creatinine, serum albumin and urine protein excretion (spot urine protein:creatinine ratio, timed urine protein collection). However, these measures cannot always accurately distinguish between active inflammation and chronic damage.

New technologies for cytokine quantification have recently been developed. Luminex multi-analyte profiling (xMAP) technology from Luminex (www.luminexcorp.com) use proprietary bead sets that are distinguishable under flow cytometry. Each bead set is coated with a specific capture antibody, and fluorescence or streptavidin-labeled detection antibodies bind to the specific cytokine-capture antibody complex on the bead set. Multiple cytokines in a biological liquid sample can thus be recognized and measured by the differences in both bead sets, with chromogenic or fluorogenic emissions detected using flow cytometric analysis. Relatively small volumes (25–50µl) of serum, plasma, urine, or cell culture supernatants can be assayed for cytokines and chemokines. Extensive data have been published validating the Luminex platform for detection of multiple analytes, by comparing this technique with enzyme-linked immunosorbent assay (ELISA) [9,10]. Compared with traditional ELISA, multiplex arrays have a number of advantages including: (a) high throughput multiplex analysis, (b) less sample volume needed, (c) efficiency in terms of time and cost, (d) ability to evaluate the levels of one given inflammatory molecule in the context

of multiple others, (e) ability to perform repeated measures of the same cytokine panels in the same participants under the same experimental assay condition, and (f) ability to reliably detect different proteins across a broad dynamic range of concentrations [11].

In this work, we have evaluated the differential expression profile of 39 cytokines using x-MAP technology in plasma samples of a cohort of Colombian patients with SLE. This multiplex assay aimed to find cytokines that allow discrimination between SLE patients with or without renal involvement (LN).

2. MATERIALS AND METHODS

2.1 Sample

The present study is based on a cohort of Colombian patients with LN (www. nefrored.org). Renal histopathology was classified according to the 2003 revised criteria for glomerulonephritis of SLE, which was published by the International Society of Nephrology/Renal Pathology Society [3]. The study protocol was reviewed and approved by the ethics review board at Simon Bolivar University. Written informed consent was obtained from all patients after explanation of the purpose and procedures of the study.

LN activity was evaluated based on the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [12], which is a weighted, cumulative index of lupus disease activity with a

total score between 0 and 105. A higher score represents increased disease activity. Renal SLEDAI consists of the 4 kidney-related criteria of the SLEDAI (i.e., hematuria, pyuria, proteinuria, and urinary casts). The presence of each 1 of these 4 parameters yields a score of 4 points; thus, the renal SLEDAI score can range from 0 to a maximal score of 16.

2.2. Laboratory evaluation

The next clinical parameters were evaluated for each of the patients enrolled in the study: complete blood count test (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), 24-h urine protein, and anti-double stranded DNA antibody (anti-dsDNA) titer done by indirect immunofluorescence. Serum C3 and C4 concentrations were measured by the immunoturbidimetric method on Roche/Hitachi cobas c systems with a detection limit of 0.04 and 0.02 g/L, respectively.

2.3 Sample processing

After informed consent, whole blood (10 mL) from subjects was collected via a direct venous puncture into tubes with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Blood was processed to isolate plasma within 4 h after the collection, and processed by spinning at 2,000 x g for 10 min at room temperature. Then, the plasma was transferred to microcentrifuge tubes and stored at –80˚C.

2.3 Cytokine analysis

The samples were analyzed in duplicate using the MILLIPLEX MAP Human Cytokine/Chemokine-Premixed 39 Plex kit (Millipore Corp, Missouri, USA) on the Luminex 200 system (Luminex Corporation, Austin, TX, USA). The assay procedure was performed according to the manufacturer's instructions. Table S1 lists the cytokines and chemokines analyzed. Data were collected using Luminex-100 software version 1.7 (Luminex, Austin, TX, USA), and analysis was performed with the MasterPlex QT 1.0 system (MiraiBio, Alameda, CA, USA). Cytokine standards were run on each plate and used to determine an 8-point 5-parameter logistic standard curves. Data were analyzed using either a 5- or 4-parameter logistic or spline curve-fitting method as recommended by the manufacturer. The type of curve-fitting method was chosen for each cytokine with respect to the lowest residual variance (< 5%).

2.4 Data Analysis

Data were analyzed using GraphPad Prism 7 and SPSS v20 and are expressed as mean ± SD. The analysis between LNN and LN groups were made by the Mann–Whitney U test. The analysis between LN groups (NLII, NLIII, and NLIV) was made by Kruskal-Wallis test with Dunnett's post hoc test. Differences were considered to be statistically significant when $p < 0.05$. LNN: Lupus non-nephritis.

3. RESULTS

3.1 Patients

A total of 80 plasma samples, including 10 LN class II (LNII) patients, 10 LN class III (LNIII) patients, 30 LN class IV (LNIV) patients, and 30 SLE non-LN (LNN) patients (Table 1) were included in this study. LN activity was evaluated based on the SLEDAI (see in the methods section). All the information is available in the database www.nefrored.org. SLE is a prototype systemic autoimmune disease that is characterized by a disease incidence of 9:1 in females versus males. Consistent with this, we observed a high proportion of female:male individuals independently of grade of renal involvement (Table 1). Age of patients ranged between 28 to 35 years, and the NLIV group had the older median age. Creatinine and proteinuria values correlated to the progression of the disease, and SLDEAI INDEX.

3.2 Cytokine expression pattern

We used the Luminex® xMAP® technology to simultaneously evaluate a panel of 39 cytokines and chemokines in plasma samples of SLE individuals with and without renal involvement. The correlation matrix showed positive associations with some of the evaluated cytokines in this study. In individuals without renal damage (LNN group), a lower number of correlations were observed. For example, pro-inflammatory cytokines, such as interleukin

(IL)-2, were positively correlated (Rho> 6) with fibroblast growth factor (FGF-2). Tumor necrosis factor (TNF)-α correlated with interferon (INF)-γ, IL-6, IL-12p40, and interferon gamma-induced protein 10 (IP-10). In patients with renal involvement (LN), a higher number of correlations were observed between cytokines. Due to the number of cytokines evaluated and the amount of data obtained, principal component analysis (PCA) was used to identify the expression patterns of these cytokines. The PCA determined from all participants generated 8 components with eigenvalues greater than 1, representing 84.7% of the total of the variance. The first three components capture 65.5% of the variance. The loading plot shows 3 clusters of cytokines due to the degree of correlation between them. The cytokines with correlation coefficients greater than 0.8 represent the greatest contribution to the variance between the data into cytokine-principal component. We identified 3 patterns of cytokines represented in groups A, B, and C; Group A shows the correlation of IL-2, IFN-α2, TNF-β, IL-1Rα, IL-1β, IL-9, IL-4, IL-12p40, IL-12p70, eotaxin, monocyte chemotactic protein-3 (MCP-3), macrophage inflammatory protein-1β (MIP-1β), fractalkine, IL-15, granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte-colony stimulating factor (G-CSF) and granulocyte-colony stimulating factor (FGF-2,Figure 1, group A). Group B was represented by clustered cytokines with cytokine-principal component correlation coefficients between 0.4 and 0.7.IFN-γ, IL-17α, IL-13, IL-5, soluble CD40-ligand (sCD40L), IL-3, TGF-α, and IL-7 were grouped in this pattern (Figure 1, Group B). The third group was represented by cytokines with cytokine-principal component correlation coefficients between

0.05 and 0.4. The expression of TNF-α, IL-6, IL-1α, IL-10, MIP-1α, IP-10, and MCP-1was observed in this pattern (Figure 1, Group C).

3.3 Differentially expressed cytokines between the study groups.

LN patients had significantly increased plasma eotaxin, TNF-α, IL-17-α, IL-10, and IL-15 as compared to the LNN group (Figure 2). Conversely, MDC, GRO, and EGF cytokines were significantly increased in the LNN group when compared with the LN group. We next analyzed cytokine levels in the NLII, NLIII, and NLIV subgroups compared with the LNN group, and we found some differences. Eotaxin ($p=0.0086$), IL-10 ($p=0.0156$), IFN-γ ($p=0.0312$), TNF-α ($p<0.001$), and IL-15 ($p=0.0084$) were significantly higher in the LNIV group than in the LNN group (Figure 3). No statistically significant differences were found when we compared the LNN and the LN groups.

3.4 Heatmap depicting relative expression of cytokines between groups.

To observe possible different cytokine profiles in LNN and LN patients, we performed cluster analysis on cytokines of plasma origin. The result is represented as a heat map, where red indicates low, and purple indicates high relative expression levels (Figure 4). Heatmap of 37 analyzed cytokines show the median of each cytokine and their differential expression profile

between the study groups. Interestingly, some cytokines, such as IL-8, GM-CSF, G-CSF, and MIP-1b showed a differential expression pattern between groups. Although the difference was not significant, these small changes could have an impact on the SLE pathogenesis.

In order to identify which cytokines could be a good predictor of renal involvement, we analyzed the ROC curves of the cytokines found differentially expressed between the study groups. The area under the ROC curve (AUC) is a measure of discrimination; a model with a high area under the ROC curve suggests that the model is able to accurately predict the value of an observation's response. Of the 39 cytokines studied, only six exhibited AUC values higher than 0.6 when compared with the LNN and LN groups, and discrimination was considered adequate (AUC>0.7) only for eotaxin (95% confidence interval, 0.6808 to 0.8738) (Table 2). In addition, we observed a positive correlation between plasma eotaxin and SLEDAI ($r = 0.743$) in individuals with LNIV (Figure 5). These results indicate that plasma eotaxin could be a good predictor of LN in patients with SLE.

Discussion

SLE is a heterogeneous disease regarding presentation, disease severity, response to treatment, and organ injury. Different cytokine profiles may account for these variations observed in clinical practice [13]. Cytokines play an important role in LN, so the use of cytokines as biomarkers of disease activity in SLE and LN is of particular interest [14].

Renal biopsy is the gold standard for diagnosis and follow-up of LN patients. Although considered to be a benign procedure, renal biopsy can have severe complications. In addition, this procedure has been considered highly subjective, highlighting the need for better biomarkers in the management of LN, which are non-invasive and more objective.

The PCA plot shown a PCA1 explaining 49% of the variance. The pathogenesis of LN is complex, involving multiple mediators. In the PCA plot we observed three subsets of cytokines, some of them previously reported as potential biomarkers [15,16]. The C and B clusters are represented by proinflammatory cytokines such as IP-10, MCP-1, TNF-α, MIP-α, IL-4, IL-6 and IFN- α, cytokines typically secreted by activated macrophages, and by mesangial cells in the kidney, podocytes and endothelial cells [17]. These activated macrophages participate in the pathogenesis of LN, presenting self-antigens to CD4+ T cells [18]. The recruitment and activation of macrophages to the kidney is a biomarker of LN flares [18,19]. Also, IFN-γ is involved in the production of long-lived plasma cells (PCs), which have important role in LN pathogenesis [20]. In LNN patients we observe less correlations, with a

low number of cytokines represented mainly by pro-inflammatory cytokines such as TNF-α, a cytokine that we found differentially expressed in our analysis. The new subset of cytokines identified in LN patients (IL-17, MCP-1, MIP- α) could be used with other tests to classify lupus patients with renal involvement. It is important to highlight almost all identified subset of cytokines have been previously reported as potential biomarkers. These cytokines represent most of the variance which is a validation of our data.

Previously, we identified a group of circulating differentially expressed miRNAs in plasma in patients with active SLE. Some of these miRNAs as a group were able to discriminate between LN and LNN/CTL samples with very good sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic efficiency [21]. In this study, we identified some cytokines previously associated with active SLE, such as IL-17, TNF-α, IFN-α, IL-10, and IFN-γ. IL-17 is mainly produced by activated Th17 cells, and recent data has indicated that IL-17-driven inflammation amplifies SLE-induced tissue damage and contributes to tolerance breakdown in SLE patients [22]. Previous studies have shown that elevated IL-17 levels are correlated with active SLE[23–25], and high IL-17 levels have been associated with poor prognosis, rapid progression, and lack of response to immunosuppressive treatment of LN [26]. The role of TNF-α in the pathogenesis of SLE has been previously investigated [27]. TNF-α was found to be markedly increased in active SLE compared to healthy controls [28–30]. However, in one previous study, the TNF-α levels were higher in patients with inactive

disease compared with patients with very active disease and healthy controls, suggesting that TNF-α overexpression could be a protective factor in SLE patients [31]. In our study, circulating TNF-α was significantly elevated in patients with active SLE.

In our analysis, the IFN-a was statistically augmented in the LN group in relation to the LNN group. A role of type I interferon (IFN), predominantly IFN-α, in the pathogenesis of SLE was first suggested based on the observation that serum from patients with active SLE disease had augmented capacity to inhibit the death of virus-infected cells[32].Analysis of transcriptional profiles of pediatric patients with kidney disease show patterns of IFN gene activation, mainly in genes involved in neutrophil recruitment. However, upregulated interferon genes were observed in SLE patients with other clinical manifestations [33,34], hindering its use as a biomarker to diagnosis kidney disease.

To our knowledge this is the first report involving eotaxin in SLE. We found significantly elevated levels of eotaxin in LN patients when compared with SLE patients without renal involvement. Also, we observed a positive correlation between eotaxin and SLEDAI score in LNIV individuals. In addition, ROC curve analysis proved that eotaxin can act as a sensitive biomarker of disease activity. Taken together, our data suggests that eotaxin could be considered a biomarker of renal involvement in SLE.

Eotaxins are C-C motif chemokines first identified as potent eosinophil chemoattractants. They facilitate eosinophil recruitment to sites of inflammation in response to parasitic

infections as well as allergic and autoimmune diseases such as asthma, atopic dermatitis, and inflammatory bowel disease. The eotaxin family currently includes three members: eotaxin-1 (CCL11), eotaxin-2 (CCL24), and eotaxin-3 (CCL26). Despite having only ~ 30% sequence homology to one another, each was identified based on its ability to bind the chemokine receptor, CCR3 [35].

A role for eotaxin in autoimmunity has been shown. High levels of Eotaxin (CCL11) have been described in several chronic inflammatory diseases, such as allergic rhinitis [36], atopic dermatitis [37], asthma [38], gastrointestinal disease [39] and rheumatoid arthritis [40]. Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease of undetermined etiology involving primarily the synovial membranes and articular structures of multiple joints. Chae et al analyzed the genotype and allele frequencies for four SNPs. They suggested that polymorphisms of eotaxin-3 might be associated with susceptibility to RA [41].

Recently, Banchereau *et al*. profiled the blood transcriptome of a longitudinal cohort of pediatric patients and they identified a plasmablast signature as the most robust biomarker of disease activity (DA) [42]. In that work, a link between neutrophils and lupus nephritis was proposed. Eotaxin (CCL11) and/or Eotaxin-2 (CCL24) were shown to induce the recruitment of neutrophils in different tissues [43–45].

Despite the fact that eotaxin has not been previously reported to be involved in the pathogenesis of SLE, the role of chemokines in SLE is known (for a recent review, see [46]).

Chemokines are a large family of signaling molecules that have a role in the maintenance of the immune system[47]. Through interacting with chemokine receptors that are expressed on the cell surface as 7-transmembrane proteins coupled with G-protein for signaling transduction, chemokines can induce firm adhesion of targeted cells to the endothelium and direct the movement of targeted cells to their destination according to the concentration gradient of a given chemokine [48]. Chemokines and chemokine receptors are important in the recruitment of leukocytes to the kidney in the development of LN, and several works have shown the association with chemokines and active SLE[49,50].

The measurement of circulating chemokines may be a noninvasive method for the assessment of the severity of LN, even if further studies are needed to strongly evaluate the real role of these chemokines for clinical study of the disease activity in SLE patients. Accumulating data from clinical studies and animal models support the notion that chemokines and their cognate receptors play a critical role in the recruitment of T cells, macrophages, and dendritic cells during the development of chronic renal injury.

Conclusion

In conclusion, we identified the cytokine profile in plasma from LNN and LN patients. Eotaxin, TNF-a, IL-17a, IL-10, and IL-15 levels could distinguish patients with LN from LNN subjects. Eotaxin might play a role in the pathogenesis of SLE, could have the potential to become

the biomarkers for kidney disease, and might assist in the diagnosis of LN. Prospective studies analyzing a set of cytokines might be useful to confirm our results.

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Competing Interests

The authors declare that they have no conflict of interest.

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	LNN (n=30)	LNII (n= 10)	LNIII (n=10)	LNIV (n=30)	*p* values
Age	37.5 (22-43.3) [a]	20.5(18-43.8) [a]	31.5 (23-35.3) [a]	29(26.8-43) [a]	0.49[b]
Female:Male	30/0[c]	09/1[c]	10/0[c]	27/3[c]	0.29[c]
Proteinuria in 24 hrs.	1,5(1.2-1.5) [a]	495(310-872.5) [a]	547(259.5-1708) [a]	1110(400-2670) [a]	0.0001* [b]
Creatinine	1 (0.8-1.3)[a]	1.05(0.68-1.86) [a]	0.78(0.58-1.01) [a]	1.11(0.74-1.64) [a]	0.22[b]
ANA (+)/(-)	30/0[c]	09/1[c]	10 /0[c]	25/5[c]	0.072[c]
Anti-ds DNA (+)/(-)	28/2[c]	02/8[c]	02/8[c]	17/13[c]	0.0001*[c]
SLDEAI Index	ND[d]	4(3-5.75) [d]	4.5(3-6.25) [d]	8(7-10)[d]	<0.001*[d]

Table 1. Characteristics of the study groups.

[a] Data are expressed as median with 25% and 75% percentiles.

[b] Two-tailed Kruskal-Wallis test. The p-value was calculated by comparing LNN group with all the other groups. (*) statistical significance.

[c] Fisher's exact test. The p-value was calculated by comparing LNN with all the other groups.

[d] p-*value* based on LNII patients compared with the other groups.

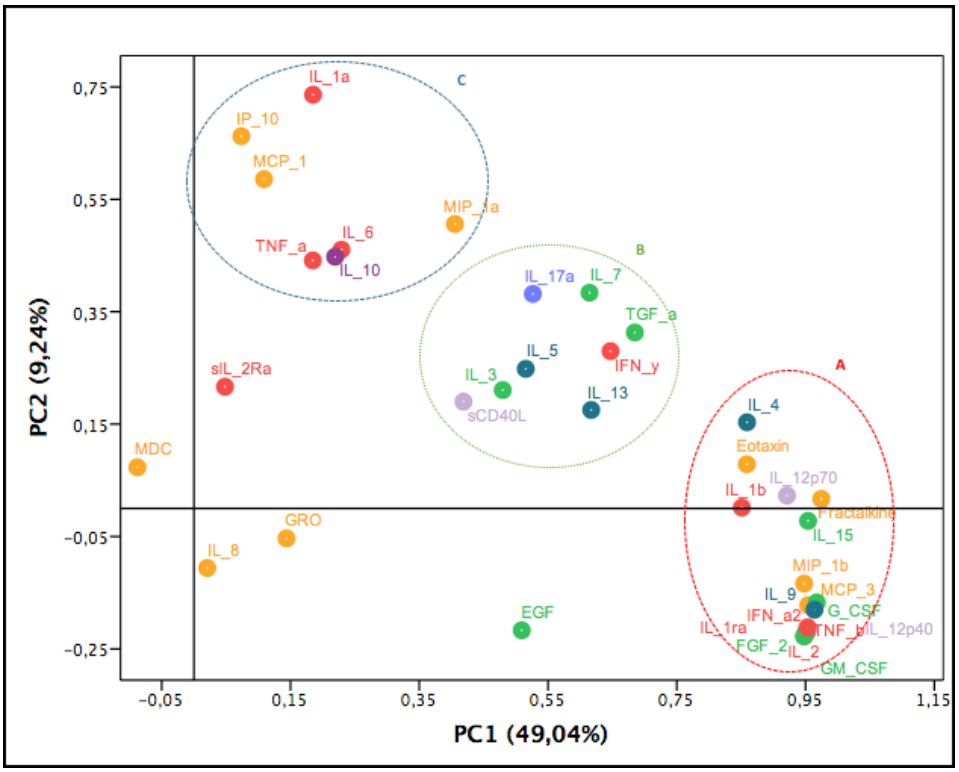
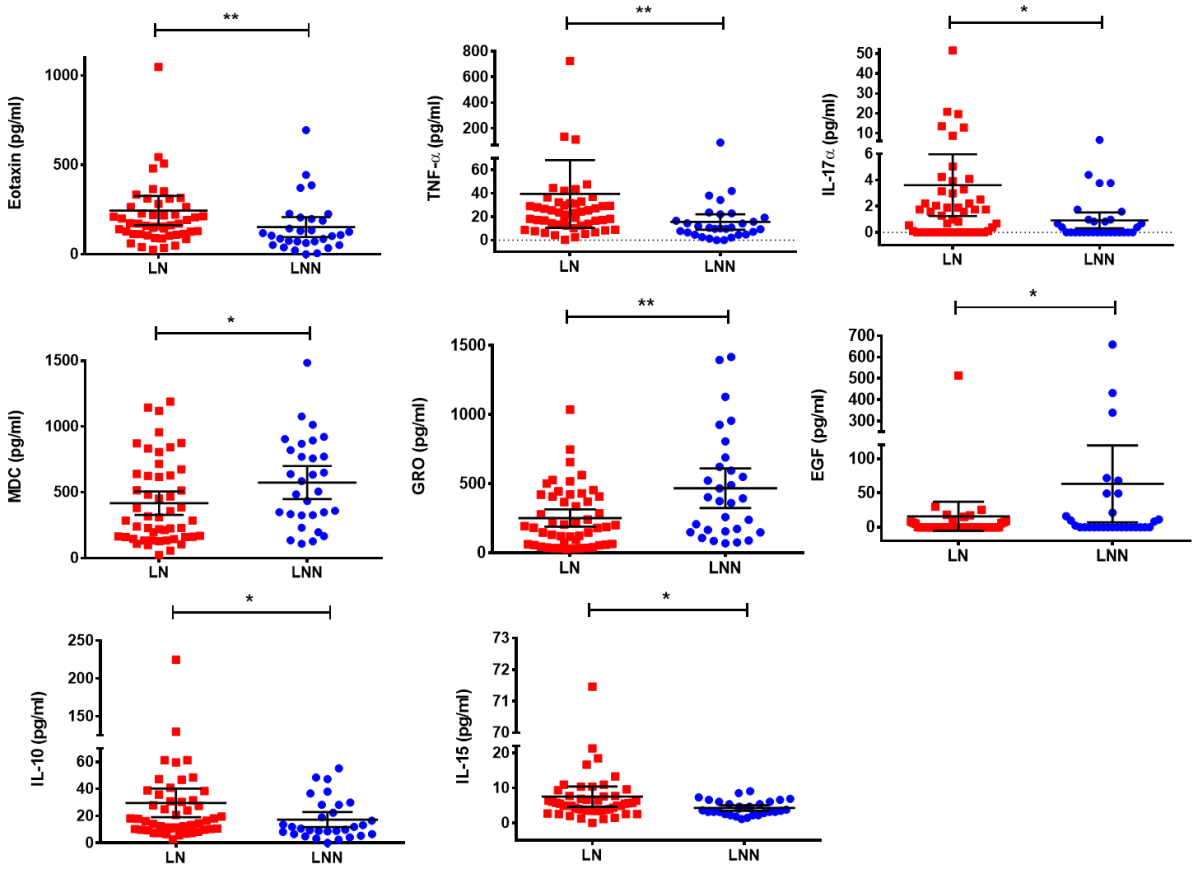


Figure 1. Component principal analysis. This loading plot shows the relative contribution of each cytokine to PC1 and PC2 and identifies three expression patterns. A) shows the expression of IL-2, IFN-α2, TNF-β, IL-1Rα, IL-1β, IL-9, IL-4, IL-12p40, IL-12p70, eotaxin, MCP-3, MIP-1β, fractalkine, IL-15, GM-CSF, G-CSF, and FGF-2. B) shows the expression of IFN-γ, IL-17α, IL-13, IL-5, sCD40L, IL-3, TGF-α, and IL-7C) groups TNF-$\boldsymbol{\alpha}$, IL-6, IL-1$\boldsymbol{\alpha}$, IL-10, MIP-1$\boldsymbol{\alpha}$, IP-10, and MCP-1.



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476 **Figure 2**. **Plasma cytokines with statistical significance between LNN and LN patients**. Plasma
477 concentrations of eotaxin, IL-2, IFN-α2, TNF-α, IL-17α, IL-10, and IL-15 were statistically augmented
478 in the LN group when compared with the LNN group. Conversely, MDC, GRO, and EGF showed a
479 statistical increase in the LNN group when compared with the LN group. Cytokines were measured
480 by Luminex xMAP Technology. All measurements were made in duplicate. The statistical analysis
481 was performed by Mann–Whitney U test, and the results for each group are presented as median
482 with interquartile range.

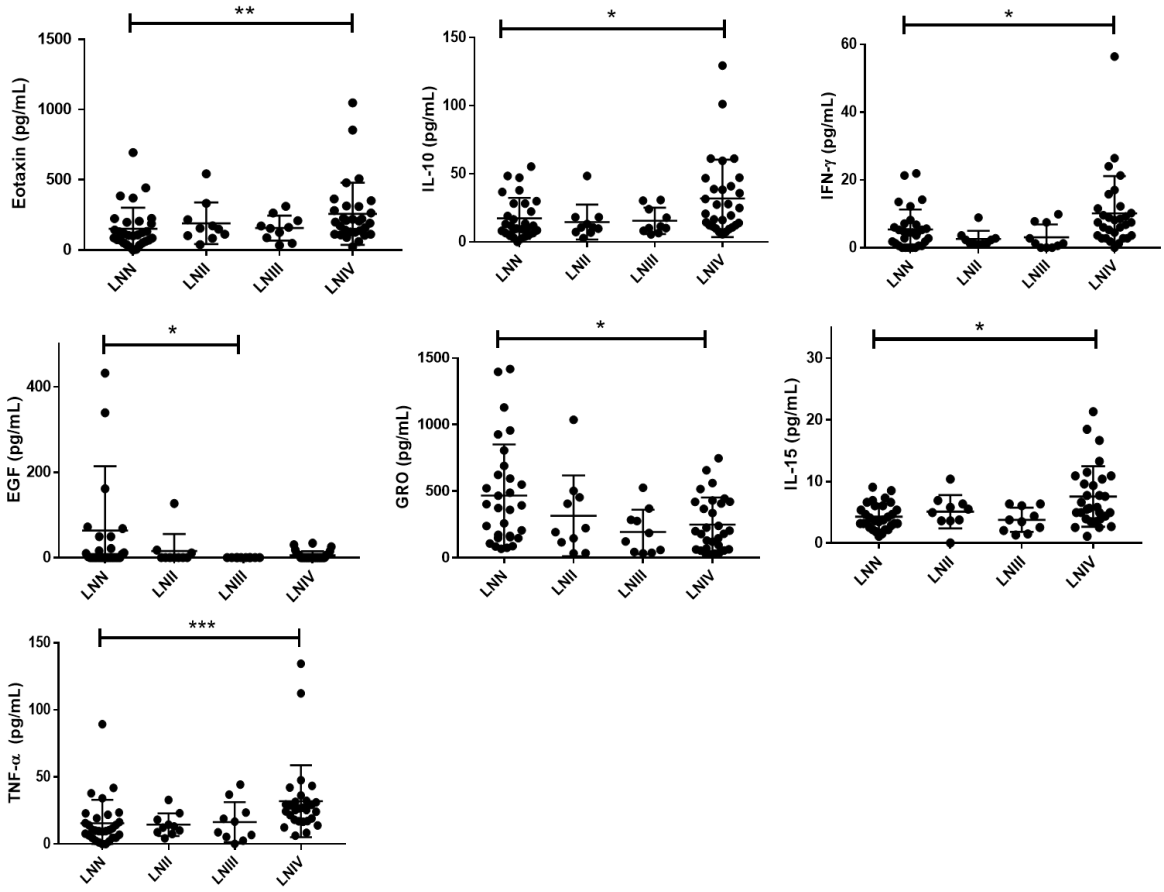
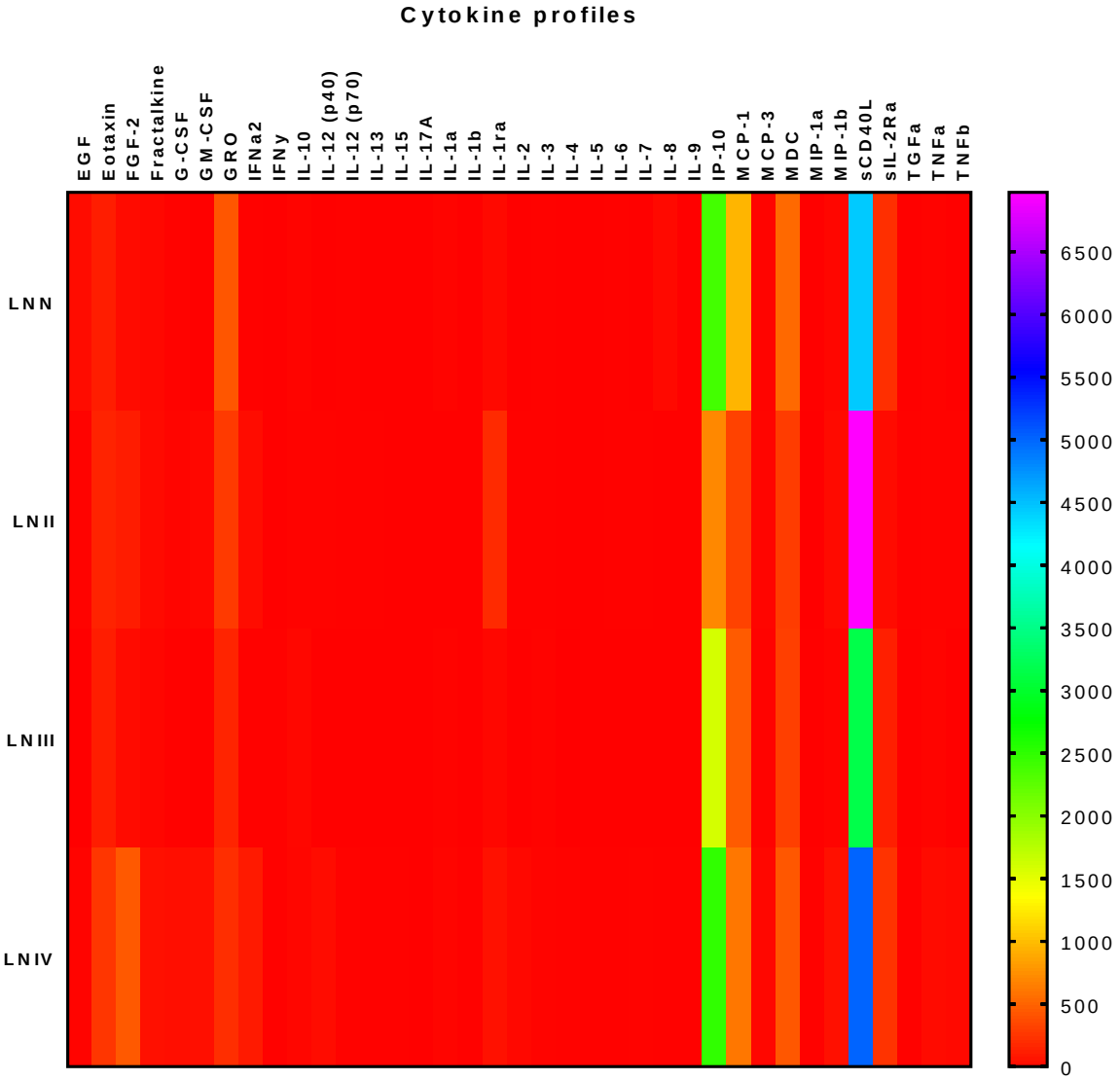


Figure 3. **Differentially expressed cytokines between subgroups**. Plasma concentrations of eotaxin, IL-10, IFN-γ, IL-15, and TNF-α were statistically augmented in the LNIV group when compared with the NLII, NLII, and LNN groups. All measurements were made in duplicate. The statistical analysis was performed by Mann–Whitney U test, and the results for each group are presented as median with interquartile range.


Cytokine profiles
EGF
Eotaxin
FGF-2
Fractalkine
G-CSF
GM-CSF
GRO
IFNa2
IFNy
IL-10
IL-12 (p40)
IL-12 (p70)
IL-13
IL-15
IL-17A
IL-1a
IL-1b
IL-1ra
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-8
IL-9
IP-10
MCP-1
MCP-3
MDC
MIP-1a
MIP-1b
sCD40L
sIL-2Ra
TGFa
TNFa
TNFb
LNN
LNII
LNIII
LNIV
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0

490

491

Figure 4. **Heatmap depicting clustering of plasma cytokine profile**. Circulating plasma levels of thirty-seven cytokines in 50 LN patients and 30 LNN subjects are shown. Each row is a study group. Each column is a cytokine. Heatmap was done with median and standard deviation of each cytokine and LN class.

Table 2. Receiver operating characteristic (ROC) curve of eotaxin, IL-10, TNF-α, IFN-α, and IL-15 for prediction of SLE disease activity.

Cytokine	Area	Standard error	Statistical Significance	95% confidence interval	
				Lower boundary	Upper boundary
Eotaxin	0.777	0.04	<0.0001	0.6808	0.8738
IL-10	0.626	0.06	0.06	0.4967	0.7564
TFN-α	0.685	0.06	0.0068	0.5605	0.8109
IFN-α	0.651	0.06	0.02	0.5288	0.7746
IL-15	0.658	0.06	0.01	0.536	0.78

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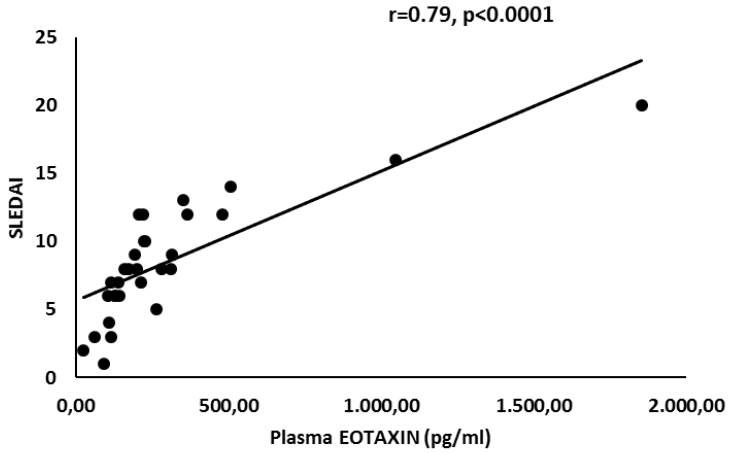

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521 **Fig 5.** Correlation analysis between eotaxin and SLEDAI.

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