Gelsolin a new biomarker of disease activity in SLE patients associated with HDL-c

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ABSTRACT

Objectives: To identify potential biomarkers of disease activity analyzing the proteome of HDL particles from SLE patients in clinical remission and when they develop a flare compared to a healthy control group.

Methods: Quantitative proteomic analyses of purified HDL were performed using Tandem Mass Tag (TMT) isobaric tag-labeling and nanoLC-Orbitrap (nLC-MS/MS) from 9 SLE patients in clinical remission when they developed a flare and from 9 healthy controls (9-9-9). We verified the identified proteins by Western blot and ELISA in a cohort of 104 SLE women patients, 46 healthy women and 14 SLE patients when developed a flare.

Results: We found 17 proteins with a significant fold-change (>1.1) compared with the control group. In lupus patients experiencing a flare compared with those in remission, we identified 4 proteins with a significant fold-change (C4, Indian Hedgehog protein, S100A8 and gelsolin). Plasma Gelsolin (pGSN) levels were decreased in the 104 SLE patients (176.02(74.9) mcg/l) compared with the control group (217.13(86.7)mcg/l); p=0.005 and when they developed a clinical flare (104.84(41.7)mcg/l);p=0002). pGSN levels were associated with HDL-c levels (r=0.316,p< 0.001). Antimalarial treated patients showed significant higher levels of pGSN (214.56(88.94)mcg/l respect 170.35(66.36) mcg/l);p=0.017.

Conclusions: Decreased pGSN are associated with clinical disease activity in SLE patients. Antimalarial treatment and HDL-c are associated with higher levels of pGSN

Keywords: Systemic Lupus Erythematosus, HDL, proteome, biomarker, gelsolin, antimalarial

Key messages:

- Proteome cargo from HDL can be used to distinguish SLE patients from healthy controls.
- pGSN is a potential biomarker of disease activity in SLE patients that develop a flare.
- Antimalarial treatment and HDL-c are associated with higher levels of pGSN in SLE patients.

1. INTRODUCTION

Systemic lupus erythematous (SLE) is a chronic autoimmune disease with multisystem involvement that occurs in the form of outbreaks and promotes a chronic inflammatory state. There is no a specific biomarker to predict an outbreak or to determine the degree of residual inflammation and the increase in cardiovascular risk and organ damage demonstrated in these patients [1]. Plasma high-density lipoprotein cholesterol (HDL-c) metabolism is affected by the presence of lupus and other autoimmune diseases [2][3][4]. The "lupus pattern" of dyslipidemia has been defined by elevated levels of very-low-density lipoprotein cholesterol (VLDL), triglycerides (TG) and lower HDL-c levels [5]. A notable finding has been that disease activity aggravates these alterations. Increases in VLDL and TG levels and decreased HDL-c levels have been directly correlated with SLEDAI scores [2].

An animal model of apoA-I−/−LDLr−/− mice displayed an autoimmune-like phenotype similar to SLE, including increased plasma antibodies against double-stranded DNA, β2-glycoprotein I, and oxidized LDL. Treatment of these mice with lipid-free apoA-1 reversed the autoimmune phenotype and lowered the number of lymphatic nodules Therapeutic strategies using apoliporotein-A1 (Apo-A1) and ApoA-1-mimetic peptides have been initiated in animal models of SLE. [6][7]

In human, titers of antibodies against HDL and ApoA1 were associated in SLE patients with persistent inflammatory disease (measured with the SLEDAI index) [3] [8].

HDL lipoprotein particles show qualitative changes in SLE patients regarding its composition and protein cargo evolving into pro-inflammatory particles [9][10][11]. Shotgun proteomics analyses of HDL particles have highlighted the complex protein composition of HDL. Novel proteins associated with HDL are associated with diverse biological functions beyond lipid metabolism, such as the acute phase response and the activation of the immune and the complement system [12][13] [14][15].

In a previous work, our group showed that in a cohort of patients affected with SLE, the small dense HDL particles were associated with the presence of subclinical atherosclerosis, complement system and inflammatory serum markers such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) [16]. These data highlight that in SLE patients, different HDL subpopulations with different compositions and protein cargo may be implicated in the clinical manifestations of systemic autoimmune diseases [14][17].

The use of mass spectrometry-based proteomics techniques in SLE research is a promising approach for the identification of more specific therapeutic targets in SLE patients by defining novel cellular mechanisms of disease and detecting the early onset of specific complications [18]. The identification of a specific biomarker of clinical flares in SLE patients would enable the administration of treatment in the most effective and timely manner to best manage the adverse side effects of therapies[19][20][21][22]..

The aim of this study was to investigate whether the protein composition of the HDL particles in SLE patients could be used to identify new potential biomarkers implicated in the pathogenesis of SLE and the presence of a clinical outbreak.

2. METHODS

2.1. Study subjects and sample collection

2.1.1 Non-directed proteomic approach analyses.

We included nine female SLE patients with stable clinical situations from our cohort of SLE patients (16). None of the subjects presented with active disease as defined by an SLE disease activity index (SLEDAI) >4. During the follow-up period when these patients presented with a flare (SLEDAI >6), a sample was collected before initiating treatment. The control group comprised nine healthy women matched by age and smoking status. In total, 27 serum

samples were collected during the follow-up of the patients (9 SLE patients under clinical remission; the same 9 SLE under a flare; 9 controls).

2.1.2 Validation of proteomic analyses in a cohort of 104 SLE women patients under remission and 46 healthy women.

One hundred and four women with SLE attending the autoimmune diseases outpatient program at Sant Joan University Hospital (Reus, Spain) were recruited. Patients fulfilled at least four classification criteria for SLE as defined by the American College of Rheumatology, as revised in 1997 [23] we recollected a total number of fourteen serum samples of the same SLE patients when they developed a flare before to start treatment.

Fourty-six apparently healthy women adjusted for age were recruited as controls from the same region.

All samples were stored at -80°C until processing. All patients provided informed consent prior to participation, and the ethics committee of the hospital approved the study.

2.2. Human plasma HDL isolation

Protease Inhibitor Cocktail (PIC; Sigma-Aldrich, Tres Cantos, Spain) was added to the plasma at a concentration ratio of 1:100 (1µL of PIC for 100µL of plasma) to avoid protein degradation.

We used 2.5 mL of plasma to precipitate the Apo B protein with phosphotungstic acid/magnesium chloride (250µL). The samples were incubated for 10 min at room temperature and centrifuged at 4,000 rpm for 20 min. The HDL fraction was obtained by adjusting the supernatant ApoB-depleted plasma density to 1.21 mg/mL with NaBr prior to ultracentrifugation at 40,000 rpm for 40 h at 8°C. The HDL fractions were recovered, dialyzed, and delipidated by chloroform/methanol extraction followed by trichloroacetic

acid/acetone protein precipitation and suspension in tris-borate-EDTA (TBE)/urea buffer before proteomic analysis.

2.3. Proteomic analyses

One hundred micrograms of protein (quantified by Bradford's method) were reduced by the addition of 10 mM Tris(2-carboxyethyl)phosphine (TCEP) in 500mM triethylammonium bicarbonate (TEAB) buffer, pH 7.9, for 1h at 55°C and alkylated with 17 mM iodoacetamide for 30 min at room temperature in the dark. Sample digestion was performed overnight at 37 ºC with sequencing-grade trypsin (Promega,Madrid,Spain) at an enzyme-protein ratio of 1:50. After digestion, the resulting peptides were vacuum dried (Speed-Vac, Thermo, Fisher Scientific, San José, CA, USA) and desalted on a C18 Sep-Pak column (30 mg, 1 cc) from Waters (Bedford,MA,USA) before TMT 10-plex labelling (Thermo Fisher). To normalize all samples in the study along the different TMT-multiplexed batches used, the same sample labeled with TMT-126 tag was included in each TMT batch. The TMT-labeled samples were equally combined according to groups A, B and C and fractionated by iso-electrofocusing on an Agilent 3100 OFFGEL Fractionator (Agilent Technologies,Santa Clara,CA) using 24-well IPG strips (linear gradient from pH 3 to 10) according to the manufacture's protocol. After iso-electrofocusing fractionation, the 24 fractions for each TMT pool were reduced to 12 end fractions based on their isoelectric point and analyzed by nanoLC-MS. The different Off-gel fractions were desalted on C18 SPE and Nano LC-MS analyses were performed on an EASY-II nanoLC couplet to an LTQ-Orbitrap Velos Pro, both from Thermo. Peptides were separated onto a C_{18} nano-column (0.075 x 150 mm; 3µm, Nikkyo Technos Co. LTD,Japan) coupled to a trap nano-column (0.1 x 20 mm; 5µm, Thermo) by a 18 min acetonitrile gradient at a flow rate of 300 nL/min.

Mass spectrometry acquisition was based on two scan segments; an enhanced FT-MS full scan (R=30,000 FHMW) followed by a data dependent FT-MS/MS scan (R=15,000 FHMW) from the ten most intense parent ions with a charge state rejection of one using a HCD collision cell with a normalized collision energy of 45% and dynamic exclusion of 0.5 min.

Protein identification/quantification was performed on Proteome Discoverer software v.1.4.0.288 (Thermo Fisher) by Multidimensional Protein Identification Technology (MudPIT). For protein identification, all MS and MS/MS spectra were analyzed using Mascot search engine (v. 2.4.1.0). Mascot was set up to search SwissProt_2012_03.fasta database (535248 entries), with restrictions for human taxonomy (26944 sequences) and assuming trypsin digestion. Two missed cleavages were allowed and an error of 0.02 Da for FT-MS/MS fragmention mass and 10.0 ppm for a FT-MS parent ion mass were allowed. TMT-10plex was set as quantification modification and oxidation of methionine and acetylation of N-termini were set as dynamic modifications, whereas carbamidomethylation of cysteine was set as static modifications. The FDR were calculated with Target Decoy PSM Validator working between 0.01 and 0.05 for strict and relaxed conditions respectively.

For protein quantification, the ratios between each TMT-label against 126-TMT labels were used and quantification results were normalized based on protein median and Log2 transformed for statistical analysis (Mass Profiler Professional software v. 14.5 from Agilent Technologies).

2.4. Validation of protein associated with HDL by western blot.

Protein concentrations were determined using a Bradford Assay Kit (Bio-Rad;USA). HDL protein were separated on 10% sodium dodecyl sulfate-plyacrylamide gel electrophoresis (SDS-PAGE) and transferred to Immobilon polyvinylidene difluoride (PVDF) membranes using an iBlot® Dry Blotting System (Life Technologies,Madrid,Spain).

Western blot analyses were performed using antibodies against Gelsolin, Indian Hedgehog and S100A8. Antibodies were obtained from Millipore (Temecula,CA;USA) and for Indian Hedgehog from Abcam (Cambridge,UK). Membranes were incubated with the appropriate HRP-conjugated secondary antibody (Dako©,Glostrup,Denmark).

The bands were visualized using ECL reagents (Amersham Pharmacia©,USA) with the Amersham Imager 6000 and quantified with the ImageQuant TL software, version 8.1 (GE Healthcare;Barcelona,Spain).

2.5. Verification with immunoassays of gelsolin

Gelsolin serum concentration (pGSN) was performed in a cohort of 104 SLE patients under clinical remission, 46 healthy control subjects and 14 SLE patients with a clinical flare. We used an ELISA commercial kit (Cusabio Biotech Co.,Newark,DE,USA).

2.6. Statistical analysis

The sample and statistical analysis were performed using Mass Profile Professional software v. 12.6 from Agilent Technologies and SPSS (version 24.0,SPSS Inc.,Chicago,IL,USA).

For proteomic experiments unsupervised multivariate analysis such as Principal Component Analysis (PCA) and Hierarchical Clustering Analysis (HCA) were performed to evaluate the classification of the samples before and after the statistical analysis. The TMT relative quantification results obtained were normalized to log2 and mean centered before data analysis. To analyze changes in protein expression identified by spectrometric analyses in lupus patients respect control subjects and SLE patients during a flare respect the same patients under remission state, a Mann-Whitney paired non-parametric test was performed ($p<0.05$) with Benjamin-Hochberg FDR to avoid false positive findings.

Normality distribution of pGSN levels in the validation cohort of SLE patients (n = 107) and the control group (n = 46) was assessed with the Kolmogorov-Smirnov test. Differences between means from the control subjects and the SLE patients were assessed by ANOVA . Differences between SLE patients under remission respect the same patients when developed a flare (n = 14) was assessed by the Wilcoxon non-parametric test.

We performed Pearson's correlation tests to analyse any association between pGSN levels and other continuous variables. Multiple linear stepwise regression analyses were performed to find the variables that predicted pGSN in SLE patients.

3. RESULTS

3.1. General characteristics of SLE patients and the control group for proteomic studies.

Nine female SLE patients were included in the study. When they developed manifestations of a clinical flare, we collected serum samples before initiating treatment. Five of these patients presented with kidney involvement, five with arthritis and three with several cutaneous manifestations. **Table 1** provides a description of the characteristics of the SLE patients. The SLE patients when developed a flare displayed a significant increase in the SLEDAI index, as expected, and they were positive for anti-DNA antibodies. There were no significant differences between the flare and non-flare data respect the ESR, lymphocytes, creatinine, complement or CRP levels.

Respect the lipid profile we found that SLE patients showed differences respect the control group with significant lower levels of HDL-c, apoA1 and higher levels of triglycerides and ApoB. We did not found differences respect the lipid profile between SLE patients under remission and with a flare. The results are described in **Table 1.**

3.2. Characterization of HDL-associated proteome

In the present study, we identified a total number of 140 different proteins associated with HDL**.** To avoid false-positive results and ensure high protein identification accuracy, the 140 proteins were filtered by frequency with a cut-off percentage of 70% in any 1 out of the 3 groups (controls, SLE in clinical remission and flare). A total of 83 HDL-associated proteins were identified after this selection. **(Table 2)**

3.3. Differential HDL proteome in SLE patients under clinical remission compared with the control group.

In **figure 1A** it is shown a PCA (scores and loadings) constructed from the proteins which already differentiates both groups along component 1 (26% of explained variability).

In the **figure 1B** we show the list of the 17 proteins associated with HDL that enables to differentiate SLE patients respect the control subjects with significant fold-change (>1.1) and a $p<0.05$.

3.4. Differential HDL proteome in SLE patients experiencing a flare compared with those in clinical remission.

In lupus patients experiencing a flare compared with those under remission, we identified 4 significantly altered proteins (FC>1.1, $p<0.05$). HDL from patients under a clinical outbreak revealed a decrease in the complement protein C4 and the Indian Hedgehog protein (IHH) and an increase in Gelsolin (GSN) and S100 calcium binding protein A8 (S100A8).

As we known, is the first time that it has been reported the presence of IHH and S100A8 from HDL in SLE patients. (**Table 2**)

3.5. Western blot analysis of IHH, S100A8 and GSN from purified HDL

Using western blot analysis we confirmed the presence of the three proteins in the plasma from SLE patients, but from isolated HDL we only could confirm the presence of GSN. GSN could be identified from purified HDL from the same sample from the proteomic studies but in order to confirm these results, again we identified GSN from another sample from the same patients.

3.6. Verification if serum pGSN level measured by ELISA is a potential biomarker of disease activity.

We selected gelsolin as a protein that could be a potential biomarker of disease activity. For this purpose we determined pGSN levels in a validation cohort (104 SLE women patients under clinical remission, 46 healthy control women and 14 serum samples of the same SLE patients when they developed a flare).

General characteristics of SLE patients and the control group are shown in **table 3**.

We found that SLE patients showed significant lower levels of pGSN (176.02(74.9)) µg/ml respect the control group (217.13(86.7)) µg/ml; p = 0.004 **(Figure 2A).** From the 14 SLE patients that developed a flare we also found a decrease of pGSN (104.84(41.7.7)) µg/ml respect the SLE under clinical remission (160.21(56.3)); p = 0.002. **(Figure 2B).** In **figure 2,D** we show the decrease of pGSN for each patient before and when they developed a flare.

In SLE patients we did not found any association between the classic immunological variables of disease activity and pGSN (anti-DNA antibodies, lymphocytes, ESR, complement levels or antiphospholipid antibodies). We found that pGSN were negatively correlated with hs-CRP ($r = -0.227$, p = 0.005).

Respect the SLE associated therapies (glucocorticoids, immunosuppressive and antimalarial) we found that patients with hydroxychloroquine (HCQ) treatment showed significant

increased levels of pGSN ((217.13(86.7) µg/ml from SLE patients with HCQ, respect (176.02(74.9) µg/ml without HCQ; p =0.017) **(Figure 2C)**

Respect the lipid and metabolic variables we found differences respect the lipid profile in SLE patients and the control group. SLE patients presented significant lower levels of HDL-c, lower levels of ApoA1, and increased levels of triglycerides.

We could confirm the association between HDL-c and pGSN that we found in the proteomic results. We found a positive correlation between pGSN levels and HDL-c (r = 0.316, p = 0.001) and apoA1 (r = 0.255, p 0 0.01) in SLE patients and also in the control group (**Figure 3A,3B**). In the control group we found an inverse association between pGSN and the BMI (r = −0.409, p = 0.016) but not with CRPhs as in SLE patients.

In multivariate analyses we included those variables associated with disease activity in SLE such as lymphocytes, erythrocyte sedimentation rate, hs-CRP, complement levels (C3 and C4), anti-DNA antibodies, antimalarial treatment and the levels of HDL-c, Apo-A1 and triglycerides. Between those variables only HDL-c predicted the levels of pGSN; ß=0.342(19.12-126.1), p = 0.009.

4. DISCUSSION

The proteomic approach to autoimmune diseases such as SLE is a promising area for the evaluation of more specific biomarkers. Recent studies investigating HDL have linked its functions to the acute phase response and the activation of the innate immune system such as the complement system [24][25].

From this perspective, and based on the relationship between HDL and lupus, we studied the HDL proteome to identify new biomarkers associated with SLE pathogenesis and disease activity.

4.1 HDL proteome in SLE disease

Previous proteomic studies of HDL have highlighted the complexity of the HDL proteome [15]. Since more sophisticated spectrometry technology has evolved and depending on the method used to isolate HDL, approximately two hundred HDL proteins have been reported in different studies with a wide range of diverse and striking functions [26]. We have found that the total number of HDL-associated proteins were similar to that reported in previous studies [27] and we found 12 novels proteins associated with HDL that are involved in the activation of the immune system, the acute phase response, cell adhesion and cartilage development **(table 2).**

We found differences in the expression of 17 proteins that could distinguish SLE patients under remission from the control group. These changes in the composition of these proteins could explain the changes in HDL function in SLE patients. [27] [12]

4.2 Identification of potential biomarkers of disease activity

Another objective of the study was to detect potential biomarkers to predict a clinical flare. In this study with a non-directed proteomic approach, we could detect four proteins associated to HDL with different expression that could be affected by disease activity. We observed in SLE patients with a clinical outbreak an increase of S100A8 and gelsolin and a decrease of C4 and IHH from HDL. The presence of S100A8 and IHH protein has not been reported previously to be associated with HDL. These proteins show interesting physiological functions that could be related to SLE pathogenesis, such as the acute phase response (S100A8)[28] and the presence of arthritis (IHH)[29].

4.3 Validation of pGSN as a potential biomarker of disease activity in SLE patients

GSN is a multifunctional protein with a very well known actin-binding activity with intracellular (cytoplasmic cGSN) and extracellular (secreted, pGSN) isoforms [30]. The

actin-scavenger and clearance activity of GSN avoids the secondary tissue injury due to the high toxicity of actin released to the bloodstream from damaged tissues and attenuating the inflammatory reaction of the host immune [31] [32][33].

Decreased GSN plasma and serum levels (pGSN) have been related with a worse prognostic in diverse clinical outcomes reported in several studies [34][31][35][36][37]. The potential usefulness of pGSN as a prognostic biomarker of sepsis, major trauma, cancers and neurological diseases is increasing of interest in recent years [35] [36]

Respect the role of GSN in autoimmune diseases, there are few studies in patients with rheumatoid arthritis and SLE that have found a decrease of the plasma levels of pGSN respect the healthy group [38] and using proteomic techniques it has been found the presence of GSN in urine and in the synovial fluid from patients with arthritis rheumatoid [34] [21].

We could confirm significant decreased levels of pGSN in SLE patients respect the healthy control group. When we compared levels of pGSN in those patients that developed a clinical flare we observed another significant decrease respect the same patients under remission reflecting the activation of the disease. In **figure 2,D** we show from each patient the decrease of pGSN before and when they developed a flare. We found that the decrease of pGSN is greater from those patients with higher levels in the clinical remission state. Those patients with the lowest pGSN levels did not showed such a clear decrease of pGSN maybe because they were already with some degree of disease.

Moreover we observed that patients under antimalarial treatment presented higher levels of pGSN that is according with the demonstrated protective effect of antimalarial treatment with a better clinical course and lower risk to present a flare.[39][40]

Until known it has not been published a relationship between HDL and pGSN. We observed a positive correlation between HDL-c and pGSN in SLE patients and also in the control group. We hypothesized that the increase of GSN in HDL particles could be associated with

its affinity to bind with complex lipids. The ability of lipid molecules to function as cell agonists to activate the inflammatory response can be compromised or augmented depending the gelsolin-to-lipid ratio [41][42]. It is possible that pGSN has the ability to bind to "pro-inflammatory" HDL in SLE patients with a clinical outbreak [33].

The main limitation of our study is the low number of recruited patients that developed a flare. The discovery, development and validation of novel biomarkers that can predict clinical outcomes is a significant challenge specially in SLE patients because they develop heterogeneous clinical manifestations and they need to start corticoids and immunosuppressive therapies with important secondary adverse effects. We could not include patients with all the possible manifestations of SLE and is needed to validate these results in a broader prospective cohort of SLE patients with diverse clinical manifestations and response to therapies. But, the physiological functions of pGSN support its role as a non-specific biomarker of inflammatory response and organ damage in SLE patients although maybe is not usefulness to distinguish a concrete clinical manifestation related with a specific antigen-antibody interaction.

CONCLUSION:

In conclusion, the proteome cargo from HDL differentiates SLE patients from healthy controls. pGSN is a potential biomarker of disease activity in SLE patients that develop a flare.

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AUTHOR CONTRIBUTION

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us

SP: Designing of the study, analyzing data, writing and revision of the manuscript
AC: Designing of the study, analyzing data, writing and revision of the manuscript
MH: acquiring data, isolament and purification of HDL lipoproteins, biochemical characterization of the lipoproteins.
PH: Acquiring and analyzing data of the proteomic techniques, writing methods section, revision of the final manuscript
NA: Acquiring data, isolament and purification of HDL lipoproteins, biochemical characterization of the lipoproteins revision of the manuscript.
XC: Analyzing data, writing and revision of the manuscript
NC: Acquiring and analyzing data of the proteomic techniques, writing methods section, revision of the final manuscript

Table 1. General characteristics of patients (n=9) and the control group (n =9) from the proteomic analyses.

	SLE PATIENTS "under remission" N=9	SLE PATIENTS "in a flare" N=9	CONTROL GROUP N=9	*P*
• **Age, years**	37(21-57)	-	33(24-45)	NS
• **Women, n(%)**	9(100)	9(100)	9(100)	NS
• **Smoking, n(%)**	3(33.3)	-	3(33.3)	NS
• **BMI**	24.42(20.3-32.9)	-	22.23(19.8-24.7)	NS
• **SLICC**	0.778(0-4)	-	-	-
• **Years since diagnosis**	6.3(7-25)	-	-	-
IMMUNOLOGIC VARIABLES OF DISEASE ACTIVITY				
• **Anti-DNA antibodies, +(%)**	2(22.2)	6(66.7)	-	<0.001
• **SLEDAI**	0.89 (0-2)	7.4	-	<0.001
• **Hb, g/dl**	13.1	12.37	-	NS
• **ESR, mm/h**	13.89	35.89	-	NS
• **Lymphocytes,x10E9/L**	1.23	1.7	-	NS
• **Creat,mmol/L**	0.76	0.61	-	NS
• **CRP, mg/L**	2.77	1.96	-	NS
• **C3g/l,**	82.2	73.3	-	NS
• **C4g/l**	8.9	12.55	-	NS
MANIFESTATIONS OF THE CLINICAL FLARE				
• **Nephritis,n(%)**	-	5(55.6)	-	-
• **Arthritis, n(%)**	-	5(55.6)	-	-
• **Cutaneous, n(%)**	-	3(33.3)	-	-
• **Asthenia,n(%)**	-	1(11.1)	-	-
LIPID PROFILE				
• **Total Chol, mmol/L**	4.88(6.76)	4.96(1.18)	4.49(0.77)	NS
• **TG, mmol/L**	1.07(0.52)	0.89(0.36)	0.59(0.23)[a]	0.037*
• **LDL-c, mmol/L**	2.84(0.59)	3.005(1.007)	2.35(0.53)[a,b]	NS
• **HDL-c, mmol/L**	1.55(0.33)	1.53(0.28)	1.9(0.49)[a,b]	0.039*
• **Apo-A1, g/L**	139.28(24.8)	134.69(19.92)	162.5(24.27)[a,b]	0.022*
• **Apo-B100, g/L**	86.92(15.4)	84.92(18.26)	68.77(18.57)	0.046*
TREATMENT				
• **Hydroxychloroquine, n(%)**	4(44.4)			
• **Prednisone, n(%)**	2(22.2)			
• **Immunosupressive, n(%)**	3(33.3)			
• **Statins, n(%)**	1(11.1)			

a p<0.05 between SLE under remission and the control group
b p<0.05 between SLE in a flare and the control group
c p<0.05 between SEL under remission and SLE in a flare

Table 2. Total number of 83 proteins identified in at least 70% of the samples

ACCESION NUMBER (SWISS PROT)	PROTEIN NAME	Identified in previous studies (number of studies)
LIPID METABOLISM		
P02647	Apolipoprotein A-I [APOA1]	17
P06727	Apolipoprotein A-IV [APOA4]	17
P02656	Apolipoprotein C-III [APOAc3]	17
O14791	Apolipoprotein L1 [APOL1]	17
P02649	Apolipoprotein E [APOE]	16
P02652	Apolipoprotein A-II [APOA2]	16
P02654	Apolipoprotein C-I [APOAC1]	15
P02655	Apolipoprotein C-II [APOAC2]	15
O95445	Apolipoprotein M [APOM]	15
P05090	Apolipoprotein D [APOD]	14
P04114	Apolipoprotein B-100 [APOB100]	13
Q13790	Apolipoprotein F [APOF]	11
P55056	Apolipoprotein C-IV [APOAC4]	9
P55058	Phospholipid transfer protein [PLTP]	7
P04180	Phosphatidylcholine-sterol acyltransferase (LCAT)	6
P11597	Cholesteryl ester transfer protein [CETP]	5
P80108	Phosphatidylinositol-glycan-specific phospholipase D [PHLD]	3
Q6Q788	Apolipoprotein A-V [APOA5]	2
IMMUNE SYSTEM		
P01834	Ig kappa chain C region [IGKC]	5
P01857	Ig gamma-1 chain C region [IGHG1]	5
P01876	Ig alpha-1 chain C region [IGHA1]	4
P49913	Cathelicidin antimicrobial peptide [CAMP]	3
P80748	Ig lambda chain V-III region LOI [LV302]	2
P01859	Ig gamma-2 chain C region [IGHG2]	2
P0CG05	Ig lambda-2 chain C regions [LAC2]	1
Q8TDL5	BPI fold-containing family B member 1 [BPIB1]	1
P30501	HLA class I histocompatibility antigen, Cw-2 alpha chain [1C02]	0
P14209	CD99 antigen [CD99]	0
P01707	Ig lambda chain V-II region TRO [LV204]	0
TRANSPORT PROTEINS		
P02766	Transthyretin [TTHY]	13
P02753	Retinol-binding protein [RET4]	8
P02787	Serotransferrin [TRFE]	12
P02768	Serum albumin [ALB]	13
Q12907	Vesicular integral-membrane protein [LMAN2; VIP36]	7
P05543	Thyroxine-binding globulin [THBG SERPINA7]	1
P02786	Transferrin receptor protein [TFR1]	0
COMPLEMENT SYSTEM		
Q9BUN1	Uncharacterized protein [CA056; C1orf56]	13
P04004	Vitronectin [VTNC, VT]	12
P01024	Complement C3 [CO3; C3]	11
P0C0L4	Complement C4-A [CO4A;C4]	8
P05155	Plasma protease C1 inhibitor [IC1;SERPING1]	7
P68871	Hemoglobin subunit beta [HBB]	6
P04264	Keratin, type II cytoskeletal 1 [K2C1; KRT1]	1
ACUTE PHASE RESPONSE		
P35542	Serum amyloid A-4 protein (SAA4)	13
P02735	Serum amyloid A protein (SAA1)	13
P02671	Fibrinogen alpha chain [FIBA]	11
P19652	Alpha-1-acid glycoprotein 2 [A1AG2]	8
P06396	Gelsolin [GELS]	4
P61769	Beta-2-microglobulin [B2MG]	3
P05109	Protein S100-A8 [S100A8]	0
	PROTEOLISIS	
P15144	Aminopeptidase N [AMPN]	2
Q96KN2	Beta-Ala-His dipeptidase [CNDP1	0

Q04756	Hepatocyte growth factor activator [HGFA]	0
ENZYMES		
P27169	Serum paraoxonase/arylesterase 1[PON1]	15
Q13093	Platelet-activating factor acetylhydrolase [PAFA; PLA2G7]	9
Q15166	Serum paraoxonase/lactonase 3 [PON3]	6
Q9HDC9	Adipocyte plasma membrane-associated protein [APMAP]	3
	PROTEASE INHIBITOR	
P01009	Alpha-1-antitrypsin [A1AT;SERPINA1]	14
P02760	Protein AMBP [AMBP]	9
P01019	Angiotensinogen (AGT;SERPIN)	7
P29622	Kallistatin [KAIN;SERPINA4]	3
Q9UK55	Protein Z-dependent protease inhibitor [ZPI; SERPINA 10]	1
	EPIDERMIS DEVELOPMENT	
P13645	Keratin, type I cytoskeletal 10 [K1C10; KRT10]	1
P35527	Keratin, type I cytoskeletal 9 [K1C9;KRT9]	1
P35908	Keratin, type II cytoskeletal 2 epidermal [K22E]	1
Q86YZ3	Hornerin [HORN]	1
O43790	Keratin, type II cuticular Hb6 [KRT86]	0
	CELL ADHESION	
P58335	Anthrax toxin receptor 2 [ANTR2;ANTXR2]	1
P05556	Integrin beta-1 [ITB1]	1
P17301	Integrin alpha-2 [ITA2]	1
P35858	Insulin-like growth factor-binding protein complex acid labile subunit [ALS; IGFALS]	1
P61224	Ras-related protein Rap-1b [RAP1B](cellular assembly, blood coagulation, endotelial	0
P08514	Integrin alpha-IIb [ITA2B]*	0
	HEME BINDING	
P00739	Haptoglobin-related protein (HPR)	12
P00738	Haptoglobin [HPT]	8
P69905	Hemoglobin subunit alpha [HBA]	7
	APOPTOSIS	
P10909	Clusterin [CLUS]	13
	CARTILAGE OR BONE FORMATION	
P02765	Alpha-2-HS-glycoprotein (AHSG)	12
Q14623	Indian hedgehog protein [IHH]	0
Q9NQ79	Cartilage acidic protein 1 [CRAC1]	0
	ANTIOXIDANT	
Q9UHG3	Prenylcysteine oxidase 1 [PCYOX]	5
	COAGULATION	
P01023	Alpha-2-macroglobulin [A2MG]	6
P02775	Platelet basic protein [CXCL7]	6

Table 3. General characteristics of the SLE women patients (n= 104) and the women control group (n = 46)

Variable	SLE women patients N = 104		Control women group N = 46		*P*
	Mean±SD	***r***	**Mean±SD**	***r***	
• **GELSOLIN, µg/ml**	**176.02(74.9)**		**217.13(86.7)**		**0.004***
ANTHROPOMETRIC					
• **Age**	48.8±16.3	NS	48.7±13.2	NS	NS
• **BMI, kg/m²**	26.3±5.8	NS	24.5±3.2	−0.409*	NS
• **SBP, mmHg**	118.5±19.3	NS	111.26±14.7	NS	NS
• **DBP, mmHg**	75.7±11	NS	72.2±7.7	NS	NS
METABOLISM					
• **Gluc, mmol/L**	4.99±0.7	NS	4.8±0.46	NS	NS
• **Apo-A1, g/L**	1.39±0.22	0.255*	1.48±0.19	0.291*	0.029*
• **Apo-B100, g/L**	0.85±0.21	NS	0.85±0.2	NS	NS
• **Tot Chol, mmol/L**	4.78±1.03	NS	4.86±0.7	NS	NS
• **TG, mmol/L**	1.03±0.6	NS	0.80±0.44	NS	0.021*
• **LDL-c, mmol/L**	2.72±0.76	NS	2.71±0.75	NS	NS
• **HDL-c, mmol/L**	1.59±0.41	0.316**	1.82±0.41	0.297*	0.002*
• **Creatinine, µmol/L**	67.5±15.4	NS	60.0±10.5	NS	0.039*
FACTORS OF DISEASE ACTIVITY AND INFLAMMATION					
• **Anti-DNA antibodies, +(%)**	23.9±55.7	NS	-	-	-
• **Lymphocytes, 10^3/uL**	1659(880,2)	NS			
• **C3, g/L**	1.049±0.3	NS	-	-	-
• **C4, g/L**	0.174±0.1	NS	-	-	-
• **CH50, U arb CH50**	49.55±16.1	NS	-	-	-
• **IgM-anticardiolpin,MPL-U/mL**	8.87±12.84	NS	-	-	-
• **IgG-anticardiolipin,GPL-U/mL**	17.69±32.0	NS	-	-	-
• **IgG-β2-glicoprotein, U/mL**	7.21±12.7	NS	-	-	-
• **IgM-β2-glicoprotein, U/mL**	6.42±14.8	NS	-	-	-
• **ESR, mm/h**	17.98±12.5	NS			
• **Hs-CRP, mg/L**	2.75±3.27	-0.277*	1.84±1.71	NS	NS
TREATMENT					
• **Hydroxychloroquine, n(%)**	39(36.4)		-		
• **Immunossupresive, n(%)**	16(14.9)				
• **Prednisone, n(%)**	27(25.2)		-		
• **Statins, n(%)**	24(22.4)		-		

r : Bivariate correlationship between the Gelsolin and the varibable with a statistically significance of **P* <0.05 or ***P* < 0.001
P: significant differences between the mean of the control group and the SLE-women patients
NS: no significant difference
SBP: systolic blood pressure; DBP: Diastolic blood pressure; ESR erythrocyte sedimentation rate; Hs-CRP: high sensitive C-reactive protein.

FIGURE LEGENDS

Figure 1. (A): PCA shows controls (grey) and inactive SLE-patients (black). **(B):** Proteins identified with differences between groups.

Figure 2. Differences regarding pGSN. **(A):**Controls *vs* inactive SLE-patients; **(B)**:Inactive *vs* active SLE-patients; **(C)**:hydroxychloroquine; **(D)**:pGSN for each patient.

Figure 3. Bivariate correlations between pGSN and HDL-c, ApoA1, CRP-hs and BMI in controls **(A)** and SLE **(B).**