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ORIGINAL ARTICLE

Serum levels of P-glycoprotein and persistence of disease activity despite treatment in patients with systemic lupus erythematosus

Edsaul Emilio Perez-Guerrero[1] · Jorge Ivan Gamez-Nava[1,2] · Jose Francisco Muñoz-Valle[3] · Ernesto German Cardona-Muñoz[4] · David Bonilla-Lara[1] · Nicte Selene Fajardo-Robledo[5] · Arnulfo Hernan Nava-Zavala[2,6,7] · Teresa Arcelia Garcia-Cobian[4] · Ana Rosa Rincón-Sánchez[1] · Jessica Daniela Murillo-Vazquez[1] · David Cardona-Müller[4] · Maria Luisa Vazquez-Villegas[1,8] · Sylvia Elena Totsuka-Sutto[4] · Laura Gonzalez-Lopez[1,9,10]

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Abstract Around 25% of patients with systemic lupus erythematosus (SLE) could be refractory to conventional therapies. P-glycoprotein expression on cell surface has been implied on drug resistance, however, to date, it is unknown if P-gp serum levels are associated with SLE disease activity. Evaluate the association of serum P-gp levels and SLE with disease activity despite treatment. A cross-sectional study was conducted on 93 female SLE patients, all receiving glucocorticoids at stable doses for the previous 6 months before to baseline. SLE patients were classified into two groups: (a) patients with active disease [SLE disease activity index (SLEDAI) ≥ 3] despite treatment, and (b) patients with inactive disease (SLEDAI < 3) after treatment. Forty-three healthy females comprised the control group. Serum P-gp, anti-DNA, and both anti-nucleosome antibody levels were measured using ELISA. Active-SLE patients despite treatment had higher P-gp levels compared with inactive-SLE after treatment (78.02 ng/mL ± 114.11 vs. 33.75 ng/mL ± 41.11; $p = 0.018$) or versus reference group subjects (30.56 ng/mL ± 28.92; $p = 0.011$). P-gp levels correlated with the scores of SLEDAI ($r = 0.26$; $p = 0.01$), Mexican-SLEDAI (MEX-SLEDAI) ($r = 0.32$; $p = 0.002$), SLICC/ACR damage index ($r = 0.47$; $p < 0.001$), and with prednisone

✉ Laura Gonzalez-Lopez
dralauragonzalez@prodigy.net.mx

[1] Present Address: Programa de Doctorado en Farmacologia, Centro Universitario de Ciencias de la Salud (CUCS), Universidad de Guadalajara, Sierra Mojada 950, Col. Independencia, 44340 Guadalajara, Jalisco, Mexico

[2] Unidad de Investigación Biomédica 02 (UIEC), UMAE Hospital de Especialidades, Centro Médico Nacional de Occidente, Instituto Mexicano del Seguro Social, Avenida Belisario Domínguez 1000, Col. Independencia Oriente, 44340 Guadalajara, Jalisco, Mexico

[3] Instituto de Investigación en Ciencias Biomédicas, Centro Universitario de Ciencias de la Salud (CUCS), Universidad de Guadalajara, Sierra Mojada 950, Col. Independencia, 44340 Guadalajara, Jalisco, Mexico

[4] Departamento de Fisiología, Centro Universitario de Ciencias de la Salud (CUCS), Universidad de Guadalajara, Sierra Mojada 950, Col. Independencia, 44340 Guadalajara, Jalisco, Mexico

[5] Laboratorio de Investigación y Desarrollo Farmacéutico, Centro Universitario de Ciencias Exactas e Ingenierías, Universidad de Guadalajara, Blvd. Marcelino García Barragán 421, 44430 Guadalajara, Jalisco, Mexico

[6] Departamento de Inmunología y Reumatología, Hospital General de Occidente, Secretaria de Salud, Av Zoquipan 1050, Seattle, 45170 Zapopan, Jalisco, Mexico

[7] Programa Internacional de Medicina, Universidad de Autónoma de Guadalajara, Av. Patria 1201, Col. Lomas del Valle, 45129 Zapopan, Jalisco, Mexico

[8] Unidad Médica Familiar 4 y 8, Departamento de Epidemiologia, Instituto Mexicano del Seguro Social (IMSS), Fidel Velázquez Sánchez 1531, Atemajac del Valle, 44218 Guadalajara, Jalisco, Mexico

[9] Departamento de Medicina Interna-Reumatología, Instituto Mexicano del Seguro Social (IMSS), Hospital General Regional 110, Av Circunvalación Oblatos 2208, Colonia Circunvalación Oblatos, 44716 Guadalajara, Jalisco, Mexico

[10] Avenida Salto del Agua 2192, Colonia Jardines del Country, 44210 Guadalajara, Mexico

doses ($r = 0.33$; $p = 0.001$). In the multivariate model, the high P-gp levels were associated with SLICC/ACR score ($p = 0.001$), and SLEDAI score ($p = 0.014$). Our findings support a relationship between serum P-gp levels and SLE with disease activity despite treatment, but it requires further validation in longitudinal studies.

Keywords Drug resistance · P-glycoprotein · Glucocorticoids · Systemic lupus erythematosus · Disease activity

Introduction

In patients with systemic lupus erythematosus (SLE), the improvement in strategies for earlier diagnosis, the constant development of novel therapeutics, and the appropriate surveillance of patients have improved the disease outcomes, achieving better probabilities of therapeutic response, decreasing the development of relapses, leading to a significant increase in survival rates in the last decades [1–3]. However, it has been observed that around 25% of patients with SLE could be refractory to conventional therapies [4]. This lack of response (primary or secondary) to therapies leads to insufficient disease control, with a diverse grade of disease activity and a subsequent increase in the risk damage to organs, worsening the prognosis.

Ineffectiveness of treatment in autoimmune diseases such as SLE is considered attributable to multifactorial causes, some of these related with (a) the patient's individual characteristics, such as genetics, ethnicity, and concomitant comorbidities, but also (b) depending on disease characteristics, such as SLE severity, organs involved, and delay in treatment onset [5]. However, among other important factors for consideration in treatment, for instance, extrusion of drugs from the cells [6]. In this manner, drug resistance is associated with the increase in the expression of P-glycoprotein (P-gp), which is mediated genetically by the multiple drug resistance gene (*MDR-1*). Overexpression of P-gp has been observed as a relevant factor in explaining treatment resistance in some chronic diseases, such as infections by the human immunodeficiency virus (HIV), some types of cancer, or epilepsy [7–9]. In addition, soluble P-gp has been detected in cell culture supernatant of drug-resistant tumor cell lines [10]. Soluble P-gp levels have also been detected in serum and plasma of patients with cancer, and these levels are consistent with P-gp surface expression on cellular membrane [10, 11]. Although the role of P-gp as a factor implied in drug resistance in patients with cancer is known; nevertheless, the evidence of a possible role of soluble P-gp levels as biomarker of drug resistance on other chronic diseases is scarce and requires evaluation.

P-gp plays a role as an adenosine triphosphate (ATP)-dependent pump located in the plasma membrane of epithelial cells of liver, kidney, intestine, and lymphocytes, with the function of actively transporting drugs from the inside of the target cells to outside, extruding these drugs or active metabolites into the circulation [12–15]. Among drugs that are targets of P-gp are glucocorticoids, methotrexate, and other agents employed for the treatment of rheumatic disorders [6, 16, 17]. There is strong evidence supporting the role of P-gp (ABCB1) mediating the ATP-dependent efflux of anticancer drugs transporter on chemo-resistance for many drugs included methotrexate [18]. Therefore, P-gp overexpression in the lymphocytes in SLE patients may result in a decrement in the quantity of the intracellular drug available for exerting their effects, decreasing action on the target cells, leading to treatment failure with the subsequent development of disease activity [19]. Several authors have reported an increase in P-gp expression in SLE patients compared with controls [20, 21]. Overexpression of P-gp in the peripheral blood lymphocytes of patients with SLE has been associated with disease activity, as well as with resistance to glucocorticoids [20–22].

To date, there is a lack of information concerning whether P-gp serum levels in patients with SLE can be markers of permanence of disease activity despite therapy. Thus, studies are required to identify whether serum P-gp levels are increased in active-SLE in patients with uncontrolled disease despite of treatment and whether these are correlated to disease severity. Therefore, the objective of this study was to evaluate the association of serum P-gp levels with disease activity of SLE despite treatment.

Methods

Patients

This cross-sectional study was conducted on 93 female patients with SLE, who met 1982 American College of Rheumatology (ACR) criteria [21]. All patients were attending from a rheumatology outpatient clinic at a secondary-care center in Guadalajara, Mexico [Department of Internal Medicine Rheumatology, Hospital General Regional 110 (HGR 110), Mexican Institute of Social Security (IMSS)]. Patients were included in the study if they were ≥18 years, if they had received glucocorticoids at stable doses for at least the past 6 months, and if they exhibited treatment compliance. Exclusion criteria were overlapping syndrome, pregnancy, or patients with modifications in their therapy schemes during the 3 months prior to study inclusion. Patients were not included if they were receiving any of the following drugs: clarithromycin, cyclosporine, tacrolimus, erythromycin, fluoxetine,

ketoconazole, paroxetine, progesterone, or verapamil, because these drugs may inhibit the P-gp function.

We selected, as control group, 43 clinically healthy females obtained from among healthy blood donors or if they pertained to those in the Preventive Medicine Area of the same hospital, with age similar to that of the patients (≥18 years) and similar exclusion criteria were applied.

Clinical assessments

Patients with SLE were assessed using a structured interview and clinical chart review to identify demographics, disease characteristics, comorbidities, and their history of pharmacological treatment. Regarding to corticosteroid therapy, patients might receive prednisone or deflazacort. For purposes of standardize the dosage all of them are presented in terms of prednisone doses: 6 mg of deflazacort were equivalent to 5 mg of prednisone. Disease damage was assessed with the Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) damage indexes [23]. Disease activity was assessed according to the original systemic lupus erythematosus disease activity index (SLEDAI) and the Mexican systemic lupus erythematosus disease activity index (MEX-SLEDAI) [24, 25]. We used the definition of relapse in SLE published by Petri et al. [26], in order to classify patients with SLE according to the their SLEDAI scores after at least 3 months of treatment into two groups: (a) patients with active disease in SLE (active-SLE) if the SLEDAI score was ≥3 points despite treatment, and (b) as patients with inactive-SLE disease (inactive-SLE) if the SLEDAI was <3 points after treatment.

Serum P-gp-level evaluation

Blood samples were taken from all patients with SLE and from reference group subjects on the same day as that of the clinical evaluation during the morning regardless of their fasting state. The serum was frozen at −20 °C, until determination of P-gp levels. Serum samples were coded prior to quantification of P-gp levels to minimize measurement bias. Serum P-gp levels were measured using ELISA (MyBioSource, Inc., San Diego, CA., USA) with an inter-assay CV of 5.6%. According to the manufacturer, the sensitivity of this assay is 1.0 ng/mL and spike recovery of 94–103%. All measurements for P-gp were performed by the same researcher, who was blinded to the groups from which these sera derived, and to the clinical variables of the SLE patients.

Other laboratory determinations

Other laboratory variables in patients with SLE included urinalysis, complement component test (C3 and C4), C-reactive protein (CRP), antibodies against double-stranded DNA (anti-dsDNA), and autoantibodies against nucleosomes (anti-nucleosome). For measurement of anti-dsDNA and anti-nucleosome antibodies, we utilized a commercial ELISA kit (EUROIMMUN^{TM}, Lübeck, Germany) with inter-assay variability of 3.8 and 4.7%, respectively.

Statistical analysis

Quantitative variables were expressed as means ± standard deviations (SD), and qualitative variables, as frequencies and percentages (%). Comparison of differences between groups was computed using the independent sample *t* test. In the case of comparisons between differences in variance among the three groups (active-SLE despite treatment, inactive-SLE after treatment, and the control group), we employed one-way analysis of variance (ANOVA) with the Scheffé correction. The Chi-square test (or the Fisher exact test) was utilized for comparison between proportions. The Pearson test was computed to identify correlations between serum P-gp levels and the SLEDAI score, MEX-SLEDAI, disease duration, glucocorticoid doses, and other quantitative variables.

In order to identify the adjusted value of P-gp levels and other variables associated with active-SLE despite treatment, we performed a multivariate logistic regression analysis using forward method. The final model was built using disease activity despite treatment (SLEDAI > 3) as dependent variable. Covariates included in the model were those that had a significance <0.20 in the univariate comparison or had clinical meaningful. Additionally, we used a multiple regression analysis with stepwise method to identify variables associated with serum P-gp levels excluding potential confounders. In the final model, we included as covariates those variables that were correlated with the P-gp levels in the univariate analysis or were meaningful such as age, disease duration, SLEDAI score, prednisone doses, and C3 complement. All analyzes were performed using IBM SPSS ver. 23 statistical software (Statistics/IBM Corp., Chicago, IL, USA). *P* value was set at a level of <0.05.

Results

In this study, were included 93 female patients with SLE and 43 volunteers as the control group. In data not shown in the tables, no differences were observed in age, weight, alcohol consumption, and smoke exposure between patients with SLE and the control group.

Table 1 presents the clinical characteristics of patients with SLE. The disease duration of patients with SLE was 9.77 (±7.13) years. According to the SLEDAI score ≥3,

Table 1 Clinical features of patients with systemic lupus erythematosus (SLE)

Variable	SLE $n = 493$
Age, years (mean ± SD)	44.24 ± 12.44
Disease duration, years (mean ± SD)	9.77 ± 7.13
Organs involved any time during disease duration	
Mucocutaneous, *n* (%)	84 (90.3)
Renal, *n* (%)	43 (46.23)
Central nervous system, *n* (%)	18 (19.4)
Other, *n* (%)	15 (16.1)
SLEDAI score (mean ± SD)	4.74 ± 4.89
SLEDAI score ≥3, *n* (%)	49 (52.7)
MEX-SLEDAI score (mean ± SD)	3.09 ± 3.34
MEX-SLEDAI score ≥3, *n* (%)	31 (22.8)
SLICC/ACR score (mean ± SD)	0.59 ± 0.88
Organs involved at time of study inclusion	
Renal, *n* (%)	28 (30.1)
Mucocutaneous, *n* (%)	26 (28.0)
Central nervous system, *n* (%)	10 (10.8)
Other, *n* (%)	9 (9.6)
Immunosuppressive therapy at the time of the study, *n* (%)	69 (74.2)
Azathioprine, *n* (%)	37 (39.8)
Mycophenolate mofetil, *n* (%)	28 (30.1)
Intravenous cyclophosphamide, *n* (%)	4 (4.3)
Other treatments, *n* (%)	58 (62.3)
Methotrexate, *n* (%)	17 (18.3)
Chloroquine, *n* (%)	41 (44.1)
Prednisone dose or equivalent, mg/day (mean ± SD)	14.56 ± 13.93
Serological findings	
Low C3 levels, *n* (%)	10 (10.8)
Low C4 levels, *n* (%)	5 (5.4)
Anti-dsDNA-positive antibodies, *n* (%)	28 (30.1)
Anti-nucleosome-positive antibodies, *n* (%)	26 (28)

SLE systemic lupus erythematosus, *SD* standard deviation, *SLEDAI* systemic lupus erythematosus disease activity index, *MEX-SLEDAI* Mexican systemic lupus erythematosus disease activity index, *SLICC/ACR* Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage indexes, *Anti-dsDNA* antibodies against double-stranded DNA

52.7% of patients were active-SLE and the most common organ involvement was kidney. All patients were taking glucocorticoids, including prednisone, methylprednisolone, or deflazacort-based schemes, while 74.2% out of them were taking immunosuppressive drugs. Mean doses of glucocorticoids taken as equivalent to prednisone were 14.56 (±13.93) mg per day.

Figure 1 illustrates a comparison of P-gp levels between control group, inactive-SLE after patients, and active-SLE patients despite treatment. Active-SLE had higher P-gp levels than inactive-SLE after treatment patients (78.02 ng/mL ± 114.11 vs. 33.75 ng/mL ± 41.11; $p = 0.018$) and higher levels than the control group (30.56 ng/mL ± 28.92; $p = 0.011$). Additionally, we found no differences between the control group and inactive-SLE patients ($p = 0.98$).

Table 2 shows the comparison of clinical features between inactive-SLE after treatment and active-SLE despite treatment patients. In active-SLE patients, were increased frequency of renal and central nervous system manifestations in any time during disease duration (68.0% vs 23.6%, $p = <0.001$) and (34.7% vs 2.3% $p = <0.001$), respectively. Also, patients with active-SLE despite treatment had higher P-gp levels compared with inactive-SLE with the treatment (78.02 ng/mL versus 33.75 ng/mL, respectively; $p = 0.014$).

In data not shown in the tables, we compared the P-gp serum levels of 69 SLE patients receiving immunosuppressive drugs (intravenous cyclophosphamide $n = 4$, mycophenolate mofetil $n = 428$, and azathioprine $n = 437$), versus those with no immunosuppressive treatment ($n = 424$). We did not observe statistical differences

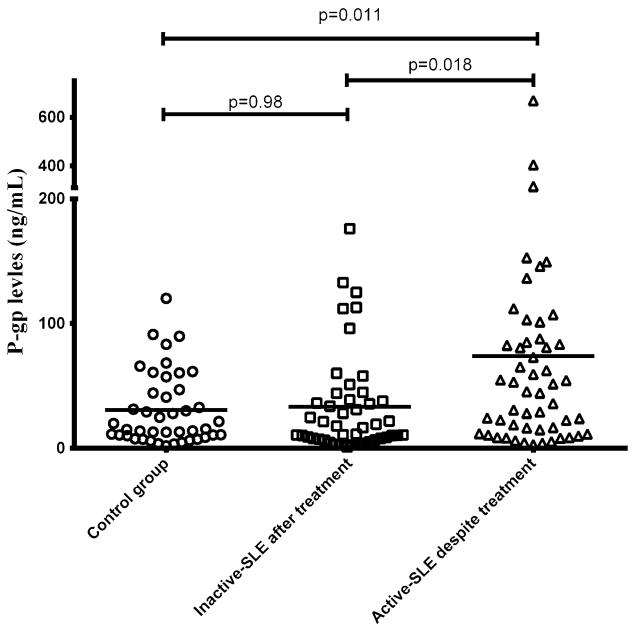

Fig. 1 P-Glycoprotein (P-gp) serum levels comparison between three groups: control group, inactive patients with systemic lupus erythematosus (SLE) after treatment and active-SLE despite treatment. *Horizontal bars* indicate the means of serum P-gp levels (ng/mL). ANOVA *p* values indicate the significance of the overall trend while comparisons between groups were compared by Scheffé's "post hoc" test

in P-gp levels in patients with SLE with different therapeutic schemes (61.91 ng/mL ± 100.93 vs. 43.18 ng/mL ± 43.86, $p = 0.47$). In contrast, P-gp levels were around threefold higher in 79 patients with SLE, who were receiving a prednisone or equivalent dose of ≥30 mg per day in comparison with 14 patients with SLE with a lower dose (133.64 ng/mL ± 194.48 vs. 41.64 ng/mL ± 43.95; $p < 0.001$). In data that are not shown in tables, patients with antecedents of central nervous system (CNS) involvement ($n = 418$) versus patients without antecedents CNS involvement ($n = 475$) observing no statistical differences (96.23 ± 172.23 ng/mL versus 47.68 ± 52.59 ng/mL, $p = 0.25$). We also compared the P-gp levels in patients with CNS involvement at the time of the study ($n = 410$) versus patients without CNS involvement at time of the study ($n = 483$), and we did not observe statistical differences (63.55 ± 121.85 versus 56.30 ± 86.19, respectively, $p = 0.81$).

Table 3 shows correlations between P-gp levels and other clinical variables. Serum P-gp levels correlated with both disease activity indices: SLEDAI score ($r = 0.26$; $p = 0.01$), and MEX-SLEDAI score ($r = 0.32$; $p = 0.002$). In addition, serum P-gp levels correlated with the SLICC/ACR damage index score ($r = 0.47$; $p < 0.001$) and prednisone doses ($r = 0.33$; $p = 0.001$), although we did not observe a correlation between P-gp levels and age, complete blood count, antibody concentration, and CRP.

Table 4 shows the results of logistic regression analysis identifying factors associated with disease activity in SLE. After controlling for age and disease duration, P-gp levels (OR = 1.05, $p = 0.025$) and prednisone doses were associated with active-SLE despite treatment (OR = 1.06, $p = 0.015$). Table 5 shows the variables associated with serum P-gp levels using multiple regression analysis, after adjusting for prednisone dose, age, disease duration, and C3 complement, variables associated with these levels were SLICC/ACR score ($p = 0.001$) and high SLEDAI score ($p = 0.014$).

Discussion

In this study, we observed an association between P-gp serum levels and SLE with disease activity. We found that P-gp serum levels were higher in active-SLE despite treatment compared with inactive-SLE after treatment patients and with the control group. Furthermore, P-gp serum levels correlated with severity of disease activity measured by SLEDAI and MEX-SLEDAI scores. P-gp levels were similar in the control group and inactive-SLE group, although no association was observed between P-gp levels and the specific drug employed for immunosuppressive therapy. Finally, after adjusting for other potential confounders, P-gp levels were significantly associated with SLEDAI.

There is a lack of information regarding the usefulness of serum P-gp-level measurement in SLE. To the best of our knowledge, this is the first study to identify the relationship between serum P-gp levels and disease activity in SLE after adjusting for confounders. These findings are in correspondence with those observed "in vitro" measuring P-gp expression in the lymphocyte membrane of patients with SLE [20–22]. In these studies, it was observed that higher disease activity levels were associated with an increase in P-gp expression in the lymphocyte membrane [20–22]. Previously, Chu et al. [10] detected P-gp soluble in culture media of drug-resistant tumor cell and extracellular fluids of cancer patients. This P-gp soluble has the same molecular weight that membrane P-gp and was hypothesized to be a secreted form of P-gp, rather than an end product of degradation of the P-gp-positive cells [10]. Also, P-gp soluble levels were consistent with a P-gp surface expression on the cellular membrane [11]. Our observation is relevant because our data support that soluble P-gp levels can be a surrogate marker for disease activity in SLE.

In this study, we found that P-gp serum levels were similar between the control group and inactive-SLE after

Table 2 Comparison of clinical features between active-SLE despite treatment and inactive-SLE patients with treatment at the time of the study

Variable	Inactive-SLE with treatment (at the time of the study) $n = 444$	Active-SLE despite treatment (at the time of the study) $n = 449$	p
Age, years (mean ± SD)	46.20 ± 11.54	42.5 ± 13.1	0.15
Disease duration, years (mean ± SD)	10.71 ± 7.22	8.93 ± 7.01	0.23
Organs involved any time during disease duration			
Mucocutaneous, *n* (%)	39 (88.6)	45 (91.8)	0.73
Renal, *n* (%)	10 (23.6)	34 (68.0)	<0.001
Central nervous system, *n (%)*6 (12.2)	1 (2.3)	17 (34.7)	<0.001
Other, *n* (%)	9 (20.5)	6 (12.2)	0.28
Current treatment			
Prednisone, mg/day (mean ± SD)*	10.04 ± 9.6	18.62 ± 15.91	0.002
Immunosuppressive therapy, *n* (%)	32 (72.7)	37 (75.5)	0.75
C3, mg/dL (mean ± SD)	134.25 ± 47.14	132.49 ± 52.22	0.87
C4, mg/dL (mean ± SD)	35.62 ± 19.01	39.83 ± 56.83	0.64
Anti-dsDNA-positive antibodies, *n* (%)	13 (29.5)	15 (30.6)	0.91
Anti-nucleosome-positive antibodies, *n* (%)	11 (25.0)	15 (30.6)	0.55
P-gp, ng/mL (mean ± SD)	33.75 ± 41.11	78.02 ± 114.11	0.014

Serositis included pleurisy and pericarditis. Renal damage included urinary casts, proteinuria (>0.5 g/24 h). Hematological included hemolytic anemia, leukopenia, lymphopenia, and thrombocytopenia

SLE systemic lupus erythematosus, *SD* standard deviation, *Inactive-SLE* systemic lupus erythematosus disease index (SLEDAI) score <3, *Active-SLE* SLEDAI score ≥3, *Anti-dsDNA* against double-stranded DNA, Mucocutaneous included rash, alopecia, malar erythema, photosensitivity, and mucosal ulcers

* Prednisone dose, Dose of corticosteroids calculated as equivalent of prednisone. Comparisons between proportions were compared with Chi-square or Fisher exact test (when required). Comparisons between means were evaluated with Student *t* test for independent samples

treatment patients. Zhang et al., Tsujimura et al., and Kansal et al. described increased expression of P-gp in lymphocytes from SLE patients compared to controls [20–22]. However, as we noted in our results merely having the SLE does not increase the concentration of P-gp probability due to lymphocytes of inactive-SLE patients are not stimulated.

Among the more relevant drugs that stimulate the expression of P-gp, we find the glucocorticoids. Although our study did not include patients without glucocorticoids, we observed a correlation between the doses and scores of the disease activity indices. Kansal et al. and Zhang et al. reported that higher doses of glucocorticoids are associated with higher P-gp expression [20, 22]. These interesting results render it necessary to conduct future studies that longitudinally evaluate whether changes in glucocorticosteroid doses are followed by changes in serum P-gp levels. In our multivariate analysis, after adjusting by confounders, P-gp levels remain associated with SLEDAI score. These results support the hypothesis that P-gp levels are biomarkers for disease activity despite of treatment.

It has been reported that P-gp expression on lymphocytes could be regulated by IL-2 via human Y-box-binding protein-1 (YB-1) [27]. This upregulated P-gp expression on lymphocytes, in vitro, leads to a reduction in glucocorticoids intracellular concentration. [28]. Furthermore, P-gp expression has been associated with a subset of pro-inflammatory Th17 lymphocytes that are unresponsive to glucocorticoids [29]. Others cytokines such as IL-4 and IFN-γ have been associated with P-gp expression [30]. Furthermore, P-gp in lymphocytes can act on the release to circulation of certain cytokines contributing to the development of activity in patients with lupus [31, 32]. So there is a point that remains to be elucidated in future studies regarding whether a relatively specific profile of interactions among SLE related cells and glucocorticoid stimulated cells could led to an overexpression of P-gp.

Several limitations of this study should be considered. First, this study is designed with a clinical objective and it is unable to determine whether the increase observed in P-gp levels is resultant or not of an overexpression on lymphocytes cells in SLE. P-gp is constitutively expressed by a multiplicity of cells including: epithelial cells of small intestine, colon, kidney proximal tubules, pancreatic and bile ductless, canalicular membrane of hepatocytes, cerebrovascular endothelial cells, and adrenal glands [12, 13, 33]. Although, constitutively P-gp is also expressed by lymphocytes (T helper and B cells), monocytes, granulocytes, and natural killer [15], this expression of P-gp in resting lymphocytes is marginal; even though, this expression can be induced by genotoxic stress, interleukin-2, as well as pro-inflammatory cytokines included TNF-α, and

Table 3 Correlations between P-glycoprotein serum levels and clinical features in the clinical features of patients with systemic lupus erythematosus

Variables	*r*	*p*
Age (years)	−0.030	0.740
Disease duration (years)	0.580	0.590
SLEDAI score	0.260	0.012
MEX-SLEDAI score	0.320	0.002
SLICC/ACR score	0.470	<0.001
Prednisone equivalent dose (mg/day)	0.330	0.001
C3 (mg/dL)	−0.140	0.183
C4 (mg/dL)	−0.020	0.849
Anti-dsDNA antibodies (IU/mL)	0.063	0.590
Anti-nucleosome-positive antibodies (IU/mL)	0.099	0.380
C-reactive protein (mg/mL)	0.058	0.624

SLE systemic lupus erythematosus, *SLEDAI* systemic lupus erythematosus disease activity index, *MEX-SLEDAI* Mexican systemic lupus erythematosus disease activity index, *SLICC/ACR* Systemic Lupus International Collaborating Clinics/American College of Rheumatology (damage indexes). *Anti-dsDNA* against double-stranded DNA. Correlations were evaluated by the Pearson correlation coefficient

interleukin-6 [19]. Therefore, future studies performed in SLE patients with the aim of measuring simultaneously serum levels of P-gp and expression of P-gp on lymphocytes cell surface would produce interesting findings. One interesting finding that deserves attention for future studies was the increase in P-gp serum levels only in those patients with active-SLE despite treatment but not in inactive-SLE patients as compared with the levels observed in controls. This finding points out to hypothesize that an overexpression P-gp on some cells of patients with SLE could be associated with the inflammatory response, although future studies should test this hypothesis. Second, this design is also unable to evaluate a temporal relationship observed between disease activity and P-gp levels. Thus, further longitudinal studies should demonstrate whether this increment in P-gp levels is followed by a relapse or lack of therapeutic response in SLE. However, the associations found in our study are relevant for citing P-gp levels as potential biomarkers with clinical usefulness for identifying patients with an active disease associated with a lack of therapeutic response. We were limited by a sub-analysis of subgroups for patients receiving different immunosuppressive drugs, due to the decrease in sample size when patients were grouped according to the specific immunosuppressive drug. Therefore, a type II error due insufficient statistical power cannot be excluded in this part of the analysis.

However, our findings support a relationship between serum levels of P-gp and active-SLE despite treatment that, to our knowledge, has not evaluated previously. These results require support by further longitudinal studies to establish a temporal relationship between an increase in P-gp and disease activity caused by lack of response to treatment. Therefore, we considered that serum levels of P-gp can be considered a biomarker for clinicians in patients with SLE treatment resistance.

Table 4 Factors associated with disease activity in the logistic regression

Variables	Odds Ratio	IC95%	*p*
Prednisone equivalent dose (mg/day)	1.06	1.06 to 1.15	0.015
Serum P-gp levels (ng/mL)	1.05	1.002 to 1.12	0.025
Disease duration (years)	–	Not significant in the model	
Age (years)	–	Not significant in the model	

Multivariate analysis logistic regression analysis. *Dependent variable* disease activity despite treatment according to SLEDAI > 3. This model was adjusted by disease duration and age using forward condition method. Covariates included in this analysis were those variables with statistical significance in the univariate analysis or were considered with biological plausibility to disease activity

Table 5 Factors associated with serum P-gp levels in the multiple regression

Variables	B coefficient (I.C. 95%)	r^2	p
Age (years)	No significant in the model	–	–
Disease duration (years)	No significant in the model	–	–
C3 complement (mg/dL)	No significant in the model	–	–
Prednisone dose (mg/day)	No significant in the model	–	–
SLICC/ACR score	40.50 (18.20–62.80)	0.218	0.001
SLEDAI score	35.32 (10.72–59.91)	0.277	0.014

Multivariate analysis multiple regression analysis, *Dependent variable* serum P-gp levels, This model was adjusted by prednisone equivalent dose, age, disease duration, and C3 complement using stepwise method. Covariates included in this analysis were those variables with statistical significance in the univariate analysis or were considered with biological plausibility to P-gp serum levels

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Compliance with ethical standards

Conflict interest Authors declare that they have no conflict of interest.

Ethical approval This study was conducted according to the recommendations described by the 64th Declaration of Helsinki and was in accordance with the ethical standards of the Ethics and Research Board of UMAE Centro Medico Nacional de Occidente del Instituto Mexicano del Seguro Social (13-01 with approval code: R-2014-1301-77).

Informed consent Informed consent was obtained from all individual participants included in the study.

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