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ORIGINAL ARTICLE

Increased lipid and protein oxidation and lowered anti-oxidant defenses in systemic lupus erythematosus are associated with severity of illness, autoimmunity, increased adhesion molecules, and Th1 and Th17 immune shift

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Abstract This study investigated nitro-oxidative stress in patients with systemic lupus erythematosus (SLE) in association with disease activity, immune-inflammatory biomarkers, and adhesion molecules. Two-hundred-four patients with SLE and 256 healthy volunteers were enrolled in this case-control study, which measured nitro-oxidative stress biomarkers, including lipid peroxides (LOOH), advanced oxidation protein products (AOPPs), nitric oxide metabolites (NO_x), sulfhydryl (−SH) groups, products of deoxyribonucleic acid (DNA)/ribonucleic acid (RNA) oxidative degradation, and total radical-

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trapping anti-oxidant parameter (TRAP). Also measured were anti-nuclear antibodies (ANAs), antibodies against double-stranded DNA (dsDNA), plasma levels of diverse cytokines, C-reactive protein, and adhesion molecules. LOOH ($p < 0.001$) and AOPP ($p < 0.001$) were significantly higher, while TRAP was significantly lower ($p < 0.001$) in SLE patients than in controls. AOPP and LOOH were significantly and positively associated with SLE disease activity index (SLEDAI) scores, anti-nuclear antibodies, and antibodies against double-stranded DNA (anti-dsDNA) levels, while TRAP was significantly and inversely correlated with SLEDAI, ANA, and dsDNA antibody levels. There were significant positive associations between AOPP and LOOH and immune-inflammatory markers, indicating T helper (Th)-17 and Th1 bias and Th1 + Th17/Th2 ratio ($p = 0.002$ and $p = 0.001$, respectively). AOPP and LOOH (positively) and TRAP (inversely) were associated with adhesion molecule expression. A model predicting SLE was computed showing that, using LOOH, AOPP, NO_x, adhesion molecules, and body mass index, 94.2% of the patients were correctly classified with a specificity of 91.5%. Increased nitro-oxidative stress takes part in the (auto)immune pathophysiology of SLE and modulates severity of illness and adhesion molecule expression.

Keywords Systemic lupus erythematosus · Reactive oxygen and nitrogen species · Cytokines · Anti-oxidant · Adhesion molecules

Introduction

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease characterized by autoantibody production especially directed against nuclear components, leading to chronic widespread inflammation, tissue destruction, accelerated atherosclerosis, and premature mortality [1]. Although the etiology of SLE is multifactorial, it has been suggested that the increased production of reactive oxygen and nitrogen species (ROS and RNS, respectively) could produce oxidized substrates that aggravate antigenicity, contributing to immune dysregulation, organ damage, and the development of fatal comorbidities [1, 2].

Reactive oxygen species are products of cell metabolism and are generated mainly by the mitochondrial respiratory chain. When there is an imbalance between the production of ROS and RNS and their scavenging by anti-oxidant mechanisms, an excessive accumulation occurs, leading to nitro-oxidative stress (O&NS) [3]. Nitro-oxidative stress plays a major role in the development and progression of chronic and autoimmune diseases [4]. Studies have suggested that nitro-oxidative stress is associated with chronic injuries in SLE patients and that the excessive and chronic production of ROS and RNS can lead to modifications of cellular biomolecules such as proteins, lipids, deoxyribonucleic acid (DNA), and ribonucleic acid (RNA). These molecule alterations could generate neo-epitopes with the potential to produce a broad spectrum of autoantibodies, leading to inflammation, organ damage, and exacerbation of autoimmunity in SLE [5, 6]. Excessive nitro-oxidative stress and decreased anti-oxidant levels have been demonstrated in SLE patients [7, 8].

Oxidative stress may have a key role in the induction of cell adhesion molecules (CAMs) [9]. CAMs facilitate leucocyte-endothelial cell interactions and the transmigration of inflammatory cells to sites of inflammation and may act as markers of endothelial activation and dysfunction [10]. Studies have shown higher levels of adhesion molecules in patients with SLE when compared to healthy controls [11]. These elevated levels of CAMs are associated with disease activity and clinical manifestations of the illness, such as cutaneous manifestations, neurological disorders, and lupus nephritis [12–14].

T helper (Th) cells are commonly grouped into subsets Th1, Th2, Th17, and regulatory T cells (Tregs), based on the cytokines they primarily produce and their functional effects. Th cell differentiation is dependent upon the local cytokine milieu and stimulation of antigen-presenting cells [15]. Upon activation, Th1 cells secrete pro-inflammatory cytokines, which stimulate macrophages to produce ROS and nitric oxide (NO), thereby mediating innate immunity [16]. Th2 cells play an active role in the development of autoantibody-mediated autoimmunity in SLE, since several Th2 cytokines, such as interleukins (IL) IL-6 and IL-10, promote antibody production by B cells [17]. Th17, a highly proinflammatory subset, plays a major role in the initiation and development of SLE and is additionally significantly associated with SLE and severity of illness [18]. The contributions of each T helper cell subset to the pathogenesis of SLE are still a matter of debate; however, recent findings have indicated that oxidative stress may disrupt the balance of cytokine production by these subsets, enhancing severity of SLE [16, 19].

Given the important role of ROS and RON in the pathophysiology of SLE and disease activity, the aim of the present study was to delineate changes in nitro-oxidative biomarkers and anti-oxidant defenses and examine their associations with severity of SLE, autoimmunity, immune responses, and adhesion molecules. These associations could increase our understanding of physiopathology and could help identify new therapeutic targets in SLE.

Materials and methods

Subjects

A total of 460 individuals were selected from among rheumatology ambulatory patients and volunteers of the local

University Hospital to participate in the study. The study included 204 patients with SLE and 256 healthy volunteers. SLE was diagnosed using the American College of Rheumatology (ACR) 2013 revised criteria [20]. Disease activity was determined by using SLE disease activity index (SLEDAI) score [21]. Inclusion criteria were patients (both genders) aged from 18 to 65 years. Exclusion criteria were thyroid, adrenal, renal, hepatic, gastrointestinal, infectious or oncological diseases, hormone replacement therapy, and antioxidant supplements. Information on medical history was obtained at clinical evaluation. Disease duration, non-steroidal anti-inflammatory drugs, corticosteroids, anti-malarial, oral contraceptives, and anti-hypertensive medications were recorded for each patient. The individuals of both groups did not drink alcohol regularly. Sample collection and analysis as well as data evaluation were performed in a blinded fashion.

Ethics, consent, health, and safety

All procedures performed in this study were approved by the institutional Ethics Committee and were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from all the participants, who had acknowledgement that they would not be identified. All mandatory laboratory health and safety procedures have been complied.

Anthropometric and blood pressure measurements

Anthropometric measurements and laboratorial parameters were assessed. Body weight was measured to the nearest 0.1 kg in the morning by using an electronic scale, with individuals wearing light clothing and no shoes; height was measured to the nearest 0.1 cm by using a stadiometer. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Waist circumference was measured on standing subjects midway between the lowest rib and the iliac crest. Three blood pressure measurements using a calibrated sphygmomanometer were taken with a 1-min interval after the participant had been seated were recorded on the left arm. The mean of these measurements was used in the analysis.

Metabolic syndrome (MetS) was defined following the Adult Treatment Panel III criteria, where MetS is present if three or more of the following five criteria are met: (1) waist circumference over 102 cm in men and 88 cm in women, (2) fasting triglyceride levels greater than or equal to 1.7 mmol/L (150 mg/dL), (3) high-density lipoprotein (HDL) lower than 1.0 mmol/L (40 mg/dL) in men or 1.3 mmol/L (50 mg/dL) in women, (4) blood pressure over 130/85 mmHg (or anti-hypertensive medication use), and (5) fasting glucose levels greater than or equal to 5.6 mmol/L (100 mg/dL) or the use of anti-diabetic medication.

Biochemical, immunological, and hematological biomarkers

After fasting for 12 h, venous blood was withdrawn in ethylenediaminetetraacetic acid (EDTA) and BD Vacutainer® UltraTouch™ serum sterile tubes. Whole blood was allowed to stand for 30 min and centrifuged at 1500 rpm for 10 min. Plasma and serum samples were separated and divided into aliquots and then stored at −80 °C for subsequent analysis.

Total cholesterol, HDL, low-density lipoprotein, triacylglycerol, glucose, and uric acid were evaluated by a biochemical autoanalyzer (Dimension Dade AR Dade Behring, Deerfield, IL, USA) using Dade Behring® Kits. Plasma insulin level was determined by chemiluminescence microparticule immunoassay (Architect, Abbott Laboratory, Abbott Park, IL, USA).

Anti-nuclear antibodies (ANAs) were quantified using indirect immunofluorescence with HEp2 cells as substrate (IFI-ANA-HEp2-IgG, VIRO-IMMUN LaborDiagnostika, GmbH, Oberursel, Germany) and were considered significant when titers ≥1:160; antibodies against double-stranded DNA (anti-dsDNA) were quantified using enzyme-linked immunosorbent assay (ELISA; anti-dsDNA, Orgentec Diagnostika, GmbH, Germany) and were considered significant when titers ≥20 IU/mL.

Plasma cytokine levels of IFN-γ, IL-4, IL-6, IL-12, and IL-17 were measured using a sandwich ELISA (eBioscience, San Diego, CA, USA), and serum C-reactive protein (CRP) levels were measured using a turbidimetric assay (C8000, ABBOTT, Architect Abbott Laboratories, Abbott Park, IL, USA).

Oxidative and nitrosative stress measurements

All nitro-oxidative stress measurements were performed in triplicate. Lipid hydroperoxides were evaluated by tert-butyl hydroperoxide-initiated chemiluminescence (CL-LOOH), as previously described [22], and the results were expressed in counts per minute (cpm).

Advanced oxidation protein products (AOPPs) were determined in the plasma using the semi-automated method [23]. AOPP concentrations were expressed as micromoles per liter (μmol/L) of chloramine T equivalents.

Total radical-trapping anti-oxidant parameter (TRAP) was determined as reported previously [24]. This method detects hydrosoluble and/or liposoluble plasma anti-oxidants by measuring the chemiluminescence inhibition time induced by 2,2-azobis-(2-aminopropane). The system was calibrated with the vitamin E analog Trolox, and the values of TRAP were expressed in equivalent of μM Trolox/mg UA [24].

It is known that TRAP levels are in part associated with BMI and uric acid [25], and therefore, we have adjusted our TRAP data statistically for possible effects of BMI (and MetS) and uric acid where needed.

NO concentration in sample was estimated by measuring the metabolites nitrites (NO_2^-) and nitrates (NO_3^-) using cadmium beads for the reduction of nitrate to nitrite. The concentrations of these metabolites were later determined according to the method proposed by [26]. The nitric oxide metabolite (NO_x) values were expressed in μM.

Sulfhydryl (SH) groups of proteins were evaluated in plasma samples by a spectrophotometric assay based on 2,2-dithiobisnitrobenzoic acid (DTNB), as reported previously, and the results are expressed in μM [27].

DNA/RNA oxidative degradations were assessed by 8-hydroxy-2-deoxyguanosine (8-OHdG)—a product of oxidatively modified DNA base guanine—using a sandwich ELISA (eBioscience, San Diego, CA, USA).

Cell adhesion molecules and plasminogen activator inhibitor type-1

Levels of platelet endothelial cell adhesion molecule 1 (PECAM-1), vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), E-selectin, P-selectin, and plasminogen activator inhibitor type-1 (PAI-1) were determined by Human Magnetic Adhesion 6-Plex Panel (Novex Life Technologies, Frederick, USA) for Luminex® platform.

Statistics

The sample size was estimated statistically using GPower indicating that using a power of 0.80, at $\alpha < 0.05$ level, and with an effect size of 0.15 (based on mean and standard deviation for some of the parameters previously evaluated in other studies), the study sample for analysis of covariance (two groups) should be around 351. Therefore, 204 patients and 256 controls were included. Analyses of variance (ANOVAs) or the non-parametric Mann-Whitney *U* test were employed to check differences among groups in continuous variables. Analyses of contingency tables (X^2 test) were used to check associations between categorical variables. General linear model (GLM) analyses were used to assess the multivariate effects of explanatory variables (including diagnosis) on dependent variables (including O&NS biomarkers), while controlling for sex, age, BMI, and MetS. Tests for between-subject effects were employed to assess the univariate effects of significant predictor variables on the dependent variables. Estimated marginal means (±SE) were computed to interpret the inter-group differences between categorical independent variables, and parameter estimates were used to interpret the direction and impact of continuous independent variables. Automatic stepwise binary logistic regression analyses were used to delineate the most significant explanatory variables predicting SLE (controls as reference group) using the following explanatory variables: O&NS biomarkers, age, MetS, BMI with or without immune biomarkers, or CAMs. Some O&NS biomarkers were Ln transformed (lipid peroxides (LOOH), AOPP, NO_x) to normalize their distribution (assessed using the Kolmogorow-Smirnov test). We also used the *z*-transformed scores for the biomarkers to display the differences among groups and to compute *z*-unit weighted composite scores, including an index of oxidative stress as *z* value of AOPP (zAOPP) + zLOOH (zAOPP + LOOH), zNO_x (lowered NO_x indicating an increased usage of NO for nitrosylation and peroxynitrite formation) and zTRAP (lowered TRAP being an anti-oxidant) as zTRAP + NO_x, and nitro-oxidative stress index as zAOPP + zLOOH-(zTRAP + zNO_x) as zAOPP + LOOH/TRAP + NO_x.

We also used immune data as presented previously [19], namely, index of Th17 activity (Th17), that is, *z* value of IL-17 (zIL-17) + zIL-6; an index of Th17 immune activation with respect to Th2 activity (Th17/Th2), computed as zIL-17 + zIL-6 − zIL-4; an index of immune activation or Th1 + Th17 activity (Th1 + Th17), computed as zIL12 + zIFNγ + zIL-6 + zIL-17; and an index of overall immune activation versus suppression (Th1 + Th17/Th2), computed as zIL12 + zIFNγ + zIL-6 + zIL-17 − zIL-4.

All statistical analyses were performed using IBM SPSS Windows version 22. All regression results were checked for multicollinearity using VIP and tolerance. Tests were two tailed and an alpha level of 0.05 indicated statistically significant results.

Results

Descriptive statistics

Table 1 shows the socio-demographic, clinical, and biomarker data of participants with SLE and controls. The authors did not use *p* corrections to interpret the multiple results of univariate tests presented in Table 1 as these results (and the Pearson and point-biserial correlation matrices between the variables) were used to delineate the most significant predictor variables to be used as independent explanatory variables in the ultimate multivariate GLM and binary logistic regression analyses. SLE participants were somewhat older than healthy controls, while there were more women than men in the SLE group as compared to controls. BMI was significantly higher in SLE patients than in controls. There were differences in self-declared ethnicity among the groups, while there were more subjects with hypertension and MetS in the SLE group as compared with controls. Consequently, we have controlled our data for age, sex, BMI, MetS (and possible interactions, including SLE × sex), hypertension, and ethnicity by entering these variables as additional explanatory variables in our multivariate analyses (Tables 2, 3, 4, 5, and 6). Mann-Whitney *U* tests showed that SLEDAI, anti-nuclear antibodies, and antibodies against double-stranded DNA (dsDNA) were significantly increased in participants with SLE as compared to controls. LOOH and

Table 1 Socio-demographic and biomarker data in patients with systemic lupus erythematosus (SLE) and healthy controls

	Variables	Controls (n = 256)	SLE (n = 204)	F/X^2	df	p
Clinical data	Age (years)	37.4 (11.7)	41.4 (13.2)	11.30	1	*0.001**
	Sex (F/M)	183/73	191/13	36.62	1	*<0.001**
	BMI (kg/m^2)	25.4 (4.4)	27.6 (5.8)	20.38	1	*<0.001**
	Ethnicity (Caucasian/not Caucasian)	206/50	134/70	12.87	1	*<0.001**
	Metabolic syndrome (no/yes)	201/45	101/102	51.57	1	*<0.001**
	Hypertension (no/yes)	179/24	116/87	49.21	1	*<0.001**
	Uric acid (mg/dL)	4.26 (1.41)	4.22 (1.27)	0.09	–	0.771
	SLEDAI	0.0 (0.0)	3.7 (3.9)		–	–
	ANA (titer)	0.0 (0.0)	1,122 (2,032)		–	–
	Anti-dsDNA (U/mL)	0.0 (0.0)	22.9 (105.7)		1/437	–
Markers of oxidative stress	Hydrogen peroxide (RBC)	16,160 (13,848)	22,138 (17,767)	23.68	1/343	*<0.001**
	AOPP (μmol/L of chloramine T equivalents)	147.5 (60.2)	176.4 (80.2)	16.47	1/442	*<0.001**
	NO_x (μM)	33.6 (26.7)	30.5 (22.3)	0.51	1/447	0.474
	TRAP (μM Trolox/mg UA)	694.2 (151.6)	583.4 (161.0)	55.90	1/413	*<0.001**
	−SH groups (μM)	343.2 (96.6)	326.6 (86.7)	3.29	1/261	0.070
	DNA/RNA oxidative degradation	6,120 (2,299)	6,694 (4,838)	0.50	1/447	0.480
Immune parameters	CRP (mg/L)	2.89 (4.65)	6.02 (10.82)	21.72	1/443	*<0.001**
	Th17 (z score)	−0.48 (1.36)	0.69 (1.37)	51.53	1/293	*<0.001**
	Th17 / Th2 (z score)	−0.63 (1.39)	1.43 (1.79)	76.20	1/225	*<0.001**
	Th1 + Th17 (z score)	−0.72 (2.13)	1.54 (2.79)	37.35	1/223	*<0.001**
	Th1 + Th17 / Th2 (z score)	−0.87 (1.98)	2.13 (2.91)	66.43	1/216	*<0.001**
Adhesion molecules	PECAM-1 (pg/mL)	24.4 (5.8)	39.9 (11.1)	113.27	1/172	*<0.001**
	VCAM-1 (pg/mL)	635.2 (206.5)	958.9 (441.9)	36.08	1/172	*<0.001**
	E-selectin (pg/mL)	9.6 (4.2)	19.7 (11.3)	58.58	1/172	*<0.001**
	P-selectin (pg/mL)	87.7 (56.9)	165.1 (74.9)	69.50	1/172	*<0.001**
	PAI-1 (pg/mL)	44.9 (43.7)	119.7 (109.3)	45.88	1/172	*<0.001**
	ICAM (pg/mL)	641 (109)	895 (163)	3.02	*1/172*	*0.084**

Results are shown as mean (SD). F results of analyses of variance; X^2 results of analyses of contingency table

F female; *M* male; *BMI* body mass index; *SLEDAI* SLE disease activity index; *ANAs* anti-nuclear antibodies; *anti-dsDNA* anti-double-stranded DNA; *AOPPs* advanced oxidation protein products; *NO_x* nitric oxide metabolites; *TRAP* total radical-trapping anti-oxidant parameter; *−SH groups* sulfhydryl groups of proteins; *CRP* C-reactive protein; *PECAM-1* platelet endothelial cell adhesion molecule 1; *VCAM-1* vascular cell adhesion molecule 1; *PAI-1* plasminogen activator inhibitor type-1; *Th17* index for Th17 shift computed as z value of IL-17 (zIL-17) + zIL-6; *Th17/Th2* index of Th17 activation with respect to Th2, computed as Th17 − zIL-4; *Th1 + Th17* index of Th1 + Th17 immune activation, computed as zIFNγ + zIL-12 + zIL-17 + zIL-6; *Th1 + Th17/Th2* index of general immune activation computed as Th1 + Th17 − zIL4

*p < 0.05

AOPPs were significantly higher in SLE patients than in controls. There were no significant differences in deoxyguanosine, NO_x, uric acid, and levels of SH groups of proteins between both study groups, while TRAP was significantly lower in the patient group. CRP, all immune indexes, PECAM-1, VCAM-1, E-selectin, P-selectin, and PAI-1 were significantly higher in patients than in controls. There were no significant differences in ICAM between both study groups.

Aberrations in O&NS biomarkers between SLE and controls

Table 2 displays the results of GLM analysis with seven O&NS data (AOPP, NO_x, LOOH, TRAP, and the three O&NS indexes) as dependent variables and diagnosis as explanatory variable, while controlling for age, sex, BMI, and MetS. Self-declared ethnicity (F = 1.67, df = 4/381, p = 0.157) and hypertension (F = 0.55, df = 4/340, p = 0.699) did not have effects in this GLM analysis and, therefore, were not included in the GLM analysis. SLE diagnosis had a significant effect on the seven O&NS data, while there were significant multivariate effects of sex, sex × diagnosis interaction, BMI, and MetS, but not age. Tests for between-subject effects show significant sex × SLE diagnosis effects on AOPP, NO_x production, zAOPP + LOOH, and zTRAP + NO_x (see Fig. 1 for effects of the interaction sex × diagnosis). Thus, AOPP, NO_x, zAOPP + LOOH, and zTRAP + NO_x were significantly higher in male SLE patients than in female SLE patients,

Table 2 Results of multivariate general linear model (GLM) analysis with oxidative and nitrosative stress biomarkers as dependent variables

Type test	Dependent variable	Explanatory variable	*F*	*df*	*p*
Multivariate	TRAP, AOPP, NO_x, LOOH, zAOPP + LOOH, zTRAP + NO_x, zAOPP + LOOH/TRAP + NO_x	SLE	11.70	4/382	*<0.001**
		Sex	6.16	4/382	*<0.001**
		MetS	6.19	4/382	*<0.001**
		SLE × sex	4.84	4/382	*0.001**
		BMI	3.19	4/382	*0.014**
		Age	0.22	4/382	*0.927**
Between-subject effects	TRAP	SLE	10.78	1/385	*0.001**
		Sex	11.12	1/385	*0.001**
		BMI	4.38	1/385	*0.038**
	AOPP	SLE	10.90	1/385	*<0.001**
		Sex	6.74	1/385	*0.010**
		SLE × sex	6.70	1/385	*0.010**
		MetS	21.93	1/385	*<0.001**
	NO_x	SLE	4.40	1/385	*0.037**
		SLE × sex	9.63	1/385	*0.002**
	LOOH	SLE	16.37	1/385	*<0.001**
		Sex	4.07	1/385	*0.044**
		BMI	6.86	1/385	*0.009**
	zAOPP + LOOH	SLE	27.41	1/385	*<0.001**
		Sex	10.88	1/385	*0.001**
		BMI	6.12	1/385	*0.014**
		SLE × sex	5.13	1/385	*0.024**
		MetS	10.10	1/385	*0.002**
	zTRAP + NO_x	SLE × sex	10.24	1/385	*0.001**
	zAOPP + LOOH /zTRAP + NO_x	SLE	15.99	1/385	*<0.001**
		BMI	8.93	1/385	*0.003**
Estimated marginal means (SE) (all values expressed as *z* scores)					
Variables		Controls	SLE	Female	Male
TRAP (*z* score)		0.44 (0.07)	−0.09 (0.14)	−0.09 (0.06)	0.44 (0.15)
AOPP (*z* score)		−0.30 (0.07)	0.58 (0.15)	−0.09 (0.06)	0.52 (0.16)
NO_x (*z* score)		−0.12 (0.07)	0.23 (0.15)	0.10 (0.06)	0.01 (0.16)
LOOH (*z* score)		−0.17 (0.07)	0.49 (0.15)	−0.01 (0.06)	0.32 (0.15)
zAOPP + LOOH (*z* score)		−0.14 (0.11)	1.07 (0.21)	−0.08 (0.08)	0.84 (0.22)
zTRAP + NO_x (*z* score)		0.32 (0.12)	0.14 (0.23)	−0.01 (0.09)	0.45 (0.24)
zAOPP + LOOH/zTRAP + NO_x (*z* score)		−0.46 (0.16)	0.92 (0.31)	−0.07 (0.12)	0.39 (0.33)

TRAP total radical-trapping anti-oxidant parameter, *AOPPs* advanced oxidation protein products, *NO_x* nitric oxide metabolites, *LOOH* lipid hydroperoxides, *zAOPP + LOOH z* value of AOPP (zAOPP) + zLOOH (oxidative stress index), *zTRAP + zNO_x* nitro-oxidative stress and anti-oxidant index, *zAOPP + zLOOH-zTRAP-zNO_x* overall nitro-oxidative stress index versus anti-oxidant defenses, *MetS* metabolic syndrome, *BMI* body mass index

*p < 0.05

whereas in controls, no such differences were found. Tests for between-subject effects and estimated marginal means (Table 2) show that SLE is accompanied by lower TRAP, increased LOOH, and increased zAOPP + LOOH/zTRAP + NO_x ratio and that males have higher TRAP and LOOH concentrations than women. There were no significant effects of SLE on the other O&NS biomarkers, including −SH groups ($F = 0.50$, $df = 1/350$, $p = 0.481$), deoxyguanosine ($F = 2.72$, $df = 1/218$, $p = 0.100$), and uric acid ($F = 0.51$, $df = 1/379$, $p = 0.474$).

Table 3 (regression #1) shows the outcome of an automatic stepwise regression analysis with SLE as dependent variable (and controls as reference group). Entered were all O&NS variables (including the three indexes), age, sex, BMI, and MetS. We found that zLOOH + AOPP and zTRAP + NO_x together with sex, BMI, and MetS, were significantly associated with SLE ($X^2 = 120.73$, $df = 5$, $p < 0.001$, Nagelkerke = 0.408; 76.1% of the subjects were correctly classified with a sensitivity of 63.6% and specificity of 85.1%).

Table 3 Results of automatic stepwise binary regression analyses with systemic lupus erythematosus as dependent variable

Explanatory variables		OR	95% CI	Wald	*df*	*p*
Regression #1	zAOPP + LOOH	1.63	1.33–1.99	22.26	1	<0.001
	zTRAP + NO_x	0.71	0.60–0.83	18.25	1	<0.001
	MetS	4.79	2.59–8.86	24.99	1	<0.001
	BMI	1.06	1.00–1.12	4.11	1	0.043
	Sex (male)	0.09	0.04–0.20	34.67	1	<0.001
Regression #2	zAOPP + LOOH	1.62	1.06–2.50	4.89	1	0.027
	Th1 + Th17/Th2	1.84	1.50–2.26	34.19	1	<0.001
	MetS	5.66	1.70–18.87	7.95	1	0.005
	BMI	1.19	1.06–1.34	8.90	1	0.003
	Sex (male)	0.07	0.01–0.48	7.50	1	0.006
Regression #3	zAOPP + LOOH	2.59	1.49–4.51	11.37	1	0.001
	NO_x	0.009	0.03–0.28	16.97	1	0.001
	PECAM	21.56	5.26–85.97	18.38	1	<0.001
	BMI	1.20	1.04–1.38	6.20	1	0.013

AOPPs advanced oxidation protein products, *LOOH* lipid hydroperoxides, *NO_x* nitric oxide metabolites, *zAOPP + LOOH z* value of AOPP (zAOPP) + zLOOH (oxidative stress index), *zTRAP + NO_x* zTRAP + zNO_x (nitro-oxidative and anti-oxidant index), *Th1 + Th17/Th2* zIL-6 + zIL-17 + zIL-12 + zIFNγ − zIL-4, *MetS* metabolic syndrome, *BMI* body mass index

Effects of confounding variables including the drug state

Table 2 shows that both BMI and MetS have significant effects on the O&NS biomarkers. Tests for between-subject tests showed that BMI was associated with TRAP, LOOH, zAOPP + LOOH, and zAOPP + LOOH/zTRAP + NO_x ratio (all positively), while MetS was accompanied by increased AOPP and zAOPP + LOOH levels. We have also examined the effects of drug state of the patients on the O&NS biomarkers by forced entry of the separate drug state variables in the multivariate GLM analysis shown in Table 2. There were no significant effects of prednisolone (F = 1.27, df = 4/358, p = 0.283), anti-malarial drugs (F = 2.07, df = 4/381, p = 0.084), immunosuppressive drugs (F = 0.88, df = 4/381, p = 0.475), mycophenolate (F = 1.90, df = 4/381, p = 0.110), statins (F = 0.76, df = 4/381, p = 0.551), hypoglycemic drugs (F = 1.64, df = 4/381, p = 0.163), and vitamin D (F = 1.54, df = 4/351, p = 0.191) on the O&NS biomarkers.

Table 4 Results of multivariate GLM analysis with systemic lupus erythematosus disease activity index (SLEDAI), anti-nuclear antibodies (ANAs), and anti-double-stranded DNA (anti-dsDNA) as dependent variables

Tests	Dependent variable	Explanatory variable	*F*	*df*	*p*
Multivariate	SLEDAI, ANA, anti-dsDNA	TRAP	10.05	3/394	<0.001
		AOPP	3.74	3/394	0.011
		LOOH	3.14	3/394	0.025
		Sex	4.79	3/394	0.003
		BMI	7.06	3/394	<0.001
		Age	0.29	3/394	0.832
Between-subject effects	SLEDAI	TRAP (−)	26.83	1/396	<0.001
		AOPP (+)	6.02	1/396	0.015
		LOOH (+)	7.61	1/396	0.006
		Sex (F > M)	11.79	1/396	0.001
		BMI (+)	19.88	1/396	<0.001
	ANA	TRAP (−)	7.99	1/396	0.005
		AOPP (+)	4.77	1/396	0.030
		Sex (F > M)	4.70	1/396	0.031
	Anti-dsDNA	AOPP (+)	6.12	1/396	0.014
		LOOH (+)	4.87	1/396	0.028

SLEDAI SLE disease activity index, *ANAs* anti-nuclear antibodies, *anti-dsDNA* anti-double-stranded DNA, *TRAP* total radical-trapping anti-oxidant parameter, *AOPPs* advanced oxidation protein products, *LOOH* lipid hydroperoxides, *BMI* body mass index, *F* female, *M* male

Table 5 Results of multivariate GLM analyses with the immune biomarkers as dependent variables

Tests	Dependent variable	Explanatory variable	*F*	*df*	*p*
Multivariate	Th17, Th17/Th2	AOPP	4.32	4/185	0.002
	Th1 + Th17, Th1 + Th17/Th2	LOOH	5.24	4/185	0.001
	CRP	Sex	4.73	4/185	0.001
		BMI	8.02	4/185	<0.001
		Age	1.61	4/185	0.173
	Th17	AOPP (+)	6.57	1/188	0.011
		LOOH (+)	5.15	1/188	0.024
	Th17/Th2	AOPP (+)	6.02	1/188	0.011
		LOOH (+)	16.31	1/188	<0.001
	Th1 + Th17	LOOH (+)	5.82	1/188	0.017
	Th1 + Th17/Th2	LOOH (+)	13.65	1/188	<0.001
	CRP	AOPP (+)	8.86	1/188	<0.001
		Sex (F > M)	17.98	1/188	<0.001
		BMI (+)	31.50	1/188	<0.001

AOPPs advanced oxidation protein products, *LOOH* lipid hydroperoxides, *BMI* body mass index, *Th17 z* value of IL-6 (zIL-6) + zIL-17 (index of Th17 shift), *Th17/Th2* Th17 − zIL-4 (Th17 / Th2 shift), *Th1 + Th17* Th17 + zIL-12 + zIFNγ (Th1 and Th17 activation), *Th1 + Th17/Th2* Th1 + Th17 − zIL-4 (Th1 and Th17 / Th2 ratio), *CRP* C-reactive protein, *F* female, *M* male

Positive associations between nitro-oxidative biomarkers, SLEDAI, and autoimmunity

Spearman's rank order correlation analyses showed that the SLEDAI was significantly associated with TRAP ($r = -0.295$, $p < 0.001$, $n = 449$), AOPP ($r = 0.160$, $p = 0.001$, $n = 447$), NO_x ($r = -0.107$, $p = 0.024$, $n = 444$), LOOH ($r = 0.195$, $p < 0.001$, $n = 439$), zAOPP + LOOH ($r = 0.228$, $p < 0.001$, $n = 433$), and zAOPP + LOOH/zTRAP + NO_x ratio ($r = 0.318$, $p < 0.001$, $n = 415$). Spearman's rank order correlation analyses showed that

Table 6 Results of multivariate GLM analysis with adhesion molecules as dependent variables

Tests	Dependent variables	Explanatory variables	*F*	*df*	*p*
Multivariate	PECAM, VCAM, E-selectin, P-selectin, PAI-1	AOPP	2.28	5/136	0.050
		LOOH	2.36	5/136	0.043
		TRAP	3.21	5/136	0.009
		NO_x	3.70	5/136	0.004
		Sex	2.06	5/136	0.074
		BMI	4.99	5/136	<0.001
		Age	2.59	5/136	0.028
Between subject	PECAM	TRAP (−)	6.85	1/140	0.010
		NO_x (−)	13.88	1/140	<0.001
		BMI (+)	5.52	1/140	0.020
	VCAM	Age (+)	6.37	1/140	0.013
	E-selectin	AOPP (+)	10.48	1/140	0.002
		BMI (+)	16.64	1/140	<0.001
	P-selectin	LOOH (+)	7.15	1/140	0.008
		TRAP (−)	8.41	1/140	0.004
		BMI (+)	6.74	1/140	0.010
	PAI-1	LOOH (+)	9.14	1/140	0.003
		TRAP (−)	9.22	1/140	0.003
		BMI (+)	13.37	1/140	<0.001

PECAM-1 platelet endothelial cell adhesion molecule 1, *VCAM-1* vascular cell adhesion molecule 1, *PAI-1* plasminogen activator inhibitor type-1, *AOPPs* advanced oxidation protein products, *LOOH* lipid hydroperoxides, *TRAP* total radical-trapping anti-oxidant parameter, *NO_x* nitric oxide metabolites, *BMI* body mass index

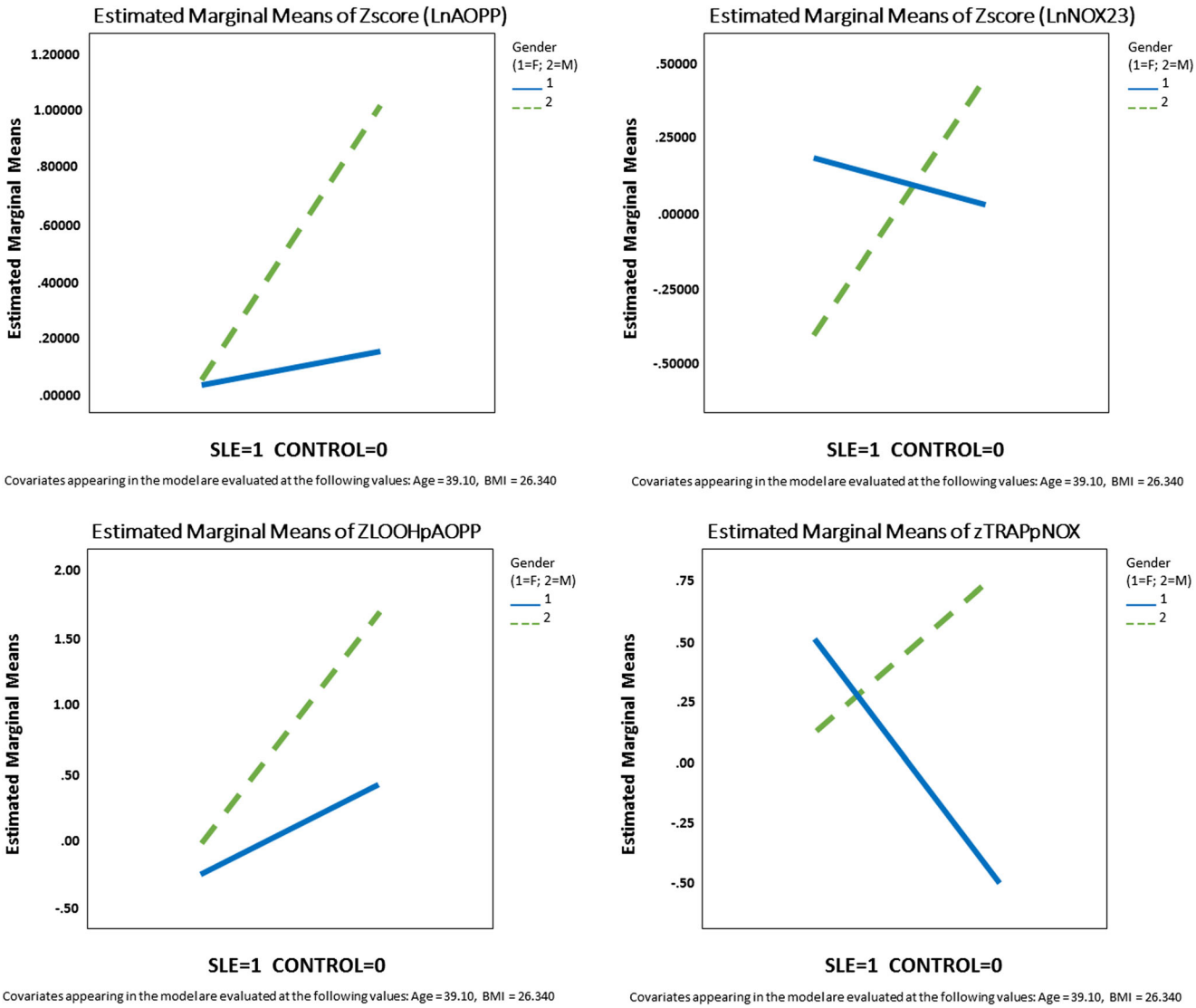

Fig. 1 Tests of between-subject effects of the interaction sex × diagnosis on oxidative and nitrosative stress markers

ANAs were significantly correlated with TRAP ($r = -0.202$, $p < 0.001$, $n = 442$), AOPP ($r = 0.117$, $p = 0.014$, $n = 440$), LOOH ($r = 0.213$, $p < 0.001$, $n = 432$), zAOPP + LOOH ($r = 0.217$, $p < 0.001$, $n = 426$), and zAOPP + LOOH/zTRAP + NO_x ratio ($r = 0.214$, $p < 0.001$, $n = 408$). Spearman's rank order correlation analyses showed that anti-dsDNA antibodies were significantly correlated with TRAP ($r = -0.136$, $p = 0.004$, $n = 449$), AOPP ($r = 0.100$, $p = 0.034$, $n = 447$), LOOH ($r = 0.125$, $p = 0.009$, $n = 439$), zAOPP + LOOH ($r = 0.135$, $p = 0.005$, $n = 433$), and zAOPP + LOOH/zTRAP + NO_x ratio ($r = 0.177$, $p < 0.001$, $n = 415$).

Table 4 shows the results of a multivariate GLM analysis with SLEDAI and ANA and anti-dsDNA antibodies as dependent variables and O&NS biomarkers as explanatory variables. We found significant effects of TRAP, AOPP, LOOH, sex, and BMI, but not age, on SLEDAI and ANA and anti-dsDNA antibodies. SLEDAI was associated with TRAP, AOPP, and LOOH after controlling for sex and BMI. ANA titers were associated with TRAP, AOPP, and female sex, while anti-dsDNA antibodies were associated with AOPP and LOOH (both positively). There were no significant effects of NO_x ($F = 1.75$, $df = 3/380$, $p = 0.0156$), −SH groups ($F = 1.73$, $df = 3/354$, $p = 0.161$), and uric acid ($F = 1.28$, $df = 3/417$, $p = 0.281$) on SLEDAI and the autoimmune biomarkers. There was a significant multivariate effect of deoxyguanosine on the three dependent variables ($F = 3.42$, $df = 3/218$, $p = 0.018$), while the tests for between-subject effects showed a significant effect (positive) on anti-nuclear antibodies only ($F = 6.37$, $df = 1/220$, $p = 0.012$).

We have also examined the effects of zAOPP + LOOH and zAOPP + LOOH/zTRAP + NO_x on the three dependent variables, after controlling for age, sex, and BMI. zAOPP + LOOH had a significant effect on SLEDAI and ANA and anti-dsDNA antibodies ($F = 8.12$, $df = 3/400$, $p < 0.001$),

while tests of between-subject effects showed associations among this oxidative stress biomarker and SLEDAI (F = 17.39, df = 1/402, p < 0.001), ANA antibodies (F = 7.56, df = 1/402, p = 0.006), and anti-dsDNA antibodies (F = 12.62, df = 1/402, p < 0.001). zAOPP + LOOH/zTRAP + NO_x ratio had a significant effect on SLEDAI and ANA and anti-dsDNA antibodies (F = 18.03, df = 3/383, p < 0.001), while tests for between-subject effects showed significant associations with SLEDAI (F = 46.64, df = 1/385, p < 0.001), ANA antibodies (F = 15.91, df = 1/385, p < 0.001), and anti-dsDNA antibodies (F = 13.95, df = 1/385, p < 0.001).

Positive associations between nitro-oxidative and immune biomarkers

Table 5 shows the results of a multivariate GLM analysis with immune-inflammatory indexes (namely, Th17, Th17/Th2, Th1 + Th17, TH1 + Th17/Th2, and CRP) as dependent variables and nitro-oxidative biomarkers as explanatory variables, while adjusting for sex, age, and BMI. We found significant effects of AOPP, LOOH, sex, and BMI but not age on the immune-inflammatory markers. We found significant between-subject effects of AOPP and LOOH (both positively) on Th17 and Th17/Th2 indexes, significant effects of LOOH on Th1 + Th17 and Th1 + Th17/Th2 indexes (all positive), and significant associations between AOPP and CRP (positive) after adjusting for sex and BMI. TRAP showed a significant effect on the immune-inflammatory variables (F = 2.93, df = 4/182, p = 0.022), while there were no significant between-subject effects. We found no significant effects of −SH groups (F = 2.36, df = 4/172, p = 0.055), NO_x (F = 2.02, df = 4/177, p = 0.094), desoxyguanosine (F = 0.86, df = 4/78, p = 0.494), and uric acid (F = 0.95, df = 4/182, p = 0.434). There was a significant effect of the oxidative index, zAOPP + LOOH, on the immune-inflammatory biomarkers, while tests of between-subject effects showed significant effects of this index on Th17 (F = 11.21, df = 1/189, p < 0.001), Th17/Th2 (F = 19.68, df = 1/189, p < 0.001), Th1 + Th17 (F = 6.02, df = 1/189, p = 0.015), and Th1 + Th17/Th2 (F = 11.02, df = 1/189, p = 0.001) indices. There was, however, no significant effect of zAOPP + LOOH/zTRAP + NO_x on the immune-inflammatory variables (F = 1.71, df = 4/177, p = 0.151).

Table 2, second regression analysis, examines the combined effects of nitro-oxidative and immune biomarkers on SLE, and therefore, we added the immune variables (Th17, Th1 + Th17, Th17/Th2, Th1 + Th17/Th2) as additional explanatory variables and found that zAOPP + LOOH, Th1 + Th17/Th2, BMI, MetS, and sex were strongly associated with SLE (X^2 = 97.63, df = 5, p < 0.001, Nagelkerke = 0.605; 88.1% of the subjects were correctly classified with a sensitivity of 65.9% and specificity of 93.8%).

Nitro-oxidative biomarkers positively correlate with upregulation of adhesion markers

There were no significant associations between any of the nitro-oxidative stress biomarkers and ICAM. Table 6 shows the results of a multivariate GLM analysis with five adhesion molecules as dependent variables and the nitro-oxidative biomarkers as explanatory variables, while adjusting for sex, age, and BMI. We found significant effects of AOPP, LOOH, TRAP, and NO_x on the five adhesion molecules. Tests for between-subject effects showed that PECAM-1 was associated with TRAP and NO_x (both negatively), E-selectins with AOPP (positively), and P-selectins and PAI-1 with LOOH (positively) and TRAP (negatively). There were no significant effects of −SH groups (F = 0.71, df = 5/130, p = 0.621), deoxyguanosine (F = 1.25, df = 5/135, p = 0.289), and uric acid (F = 1.24, df = 5/132, p = 0.296) on the adhesion molecules. There was a significant effect of zAOPP + LOOH on the five adhesion molecules (F = 4.09, df = 5/144, p = 0.002). Tests for between-subject effects showed significant effects on PECAM-1 (F = 7.37, df = 1/148, p = 0.007), VCAM-1 (F = 4.69, df = 1/148, p = 0.032), E-selectins (F = 12.84, df = 1/148, p < 0.001), P-selectins (F = 14.07, df = 1/148, p < 0.001), and PAI-1 (F = 15.37, df = 1/148, p < 0.001). There was also a highly significant effect of zAOPP + LOOH/zTRAP + NO_x on the five adhesion molecules (F = 10.18, df = 5/139, p < 0.001). Tests for between-subject effects showed significant effects on PECAM-1 (F = 36.08, df = 1/143, p < 0.001), VCAM-1 (F = 10.02, df = 1/143, p = 0.002), E-selectins (F = 19.68, df = 1/143, p < 0.001), P-selectins (F = 31.27, df = 1/143, p < 0.001), and PAI-1 (F = 21.07, df = 1/143, p < 0.001).

Table 2, regression analysis #3, examines the combined effects of nitro-oxidative biomarkers and adhesion molecules on SLE. Towards this end, we entered the five adhesion molecules together with the nitro-oxidative biomarkers as explanatory variables and found that zAOPP + LOOH (positive), NO_x (inverse), PECAM-1, and BMI were very strongly associated with SLE (X^2 = 128.52, df = 4, p < 0.001, Nagelkerke = 0.806; 93.4% of the subjects were correctly classified with a sensitivity of 94.2% and specificity of 91.5%).

Discussion

The major findings of this study are that the imbalances between ROS/RNS and the ensuing lipid and protein oxidation are strongly associated with SLE, and impact severity of illness, and the immune profiles, adhesion molecule levels, and autoimmune responses in SLE. Moreover, this study established a model predicting increased risk towards SLE versus controls indicating that lipid and protein oxidation, lowered NO_x, and increased PECAM-1 are important features of SLE.

Differences among SLE and controls

Patients with SLE showed significantly higher lipid and protein oxidation, hs-CRP levels, indices of immune activation and CAMs, and lower anti-oxidant defenses as compared with controls. Previous reports found increased protein oxidation [8] and AOPP levels [28] in SLE as compared with controls. Furthermore, chronically elevated oxidation was reported in SLE patients with inactive and active diseases, indicating that protein oxidation could play an important role in the pathogenesis of SLE [8]. AOPP production results from oxidation of amino acid tyrosine residues on plasma proteins, including albumin, fibrinogen, and lipoproteins [23]. Modifications in the amino acid sequence or structure can generate neo-epitopes from self-proteins, leading to autoimmune responses [29]. These amino acid modifications may also alter enzyme functions and oxidative signaling, thereby further aggravating SLE pathophysiology [30].

SLE patients show a significant increase in lipid peroxidation as determined by lipid hydroperoxides, which originate mostly from phospholipids, cholesterol esters, and free fatty acid oxidation triggered by the action of free radicals. Lipids are the main oxidative target in cell membranes, causing damage in cell structure and functions [31]. Prior studies reported increased lipid peroxidation in SLE [16]. Patients with SLE show a significant decrease in plasma anti-oxidant capacity as measured by TRAP methodology. Our findings are in line with previous studies reporting impaired anti-oxidant status in SLE [32]. It is important to note that uric acid has anti-oxidant properties, which are in part responsible for the free radical scavenging by TRAP. However, uric acid levels were not significantly altered in SLE.

SLE patients also showed significant increases in hs-CRP and indices of immune activation. An exacerbation of immune-inflammatory responses is known to initiate and sustain SLE disease activity [33]. In agreement with previous studies, adhesion molecules were significantly higher in patients than in controls [12, 13].

The nitro-oxidative biomarkers were significantly associated with increased BMI and the presence of MetS. MetS is generally defined as a complex condition represented by a combination of risk factors such as central obesity, dyslipidemia, hypertension, and disturbed glucose metabolism [34]. Therefore, in accordance with other investigations, this study found that both BMI and MetS contribute to increased inflammation and oxidative stress [35, 36].

A newly computed oxidative stress index using the sum of z scores of LOOH and AOPP (reflecting lipid and protein oxidation), NO_x, and PECAM-1 showed a good diagnostic performance for SLE versus controls. ANA is highly sensitive (~95%), but lacks specificity, while other parameters such as anti-dsDNA, anti-SSA/Ro, and anti-SSB/La are specific but less sensitive for SLE [37, 38]. However, we underscore that our new algorithm cannot replace useful conventional markers of SLE. Indeed, elevations in oxidative stress and adhesion molecules also occur in other inflammatory and BMI-dependent diseases and are therefore not specific. Furthermore, any prediction obtained in a training set should be checked in a validation set.

To our knowledge, this is a first study to demonstrate significant diagnosis × sex interaction effects on nitro-oxidative biomarkers in SLE. Thus, these biomarkers were significantly higher in male than in female patients, while no such differences were detected in controls. This indicates a sexual dimorphism in the contributions of nitro-oxidative stress to the pathophysiology of SLE. Thus, in male SLE patients, lipid and protein oxidation may play a more important role than in females, while in the latter, increased utilization of NO and lowered TRAP values may be more important. The increased oxidative processes in males may perhaps explain that male patients have a more severe course of illness [39]. Nevertheless, other factors may explain sexual dimorphism in autoimmune diseases [40]. In addition, male patients are usually less aware of SLE as a disease and, therefore, may have their illness diagnosed later as compared to women leading to a greater impact of oxidative stress [39, 41].

Positive associations between O&NS biomarkers, SLEDAI, and autoimmunity

Our results show that imbalances between pro-oxidant (LOOH and AOPP) and anti-oxidant (TRAP) biomarkers are associated with increased disease activity. Increased lipid peroxidation is associated with higher SLEDAI scores in several studies [7, 16, 42]. Lipid peroxidation produces highly reactive aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE), which may bind covalently to proteins, leading to changes in molecular structure and biological functions [7]. These reactive aldehyde-modified proteins are immunogenic and may induce T cell-dependent antibody response that may have a causative role in SLE [42]. Oxidation of amino acid residues of proteins, such as cysteine and methionine, also alter the molecular structure and biological functions of these proteins [6]. Multiple markers of protein oxidation are associated with increased disease activity in SLE [6].

The human anti-oxidant defense system consists of enzymatic anti-oxidants such as SOD, CAT, glutathione-related enzymes, and non-enzymatic reactions [5]. Biomarkers of impaired anti-oxidant capacity have been associated with disease activity in SLE [7, 43]. In this study, we found a significant inverse association between disease activity and TRAP, but not NO_x. These findings do not corroborate previous findings reporting a small but significant effect of NO_x on SLEDAI [44].

In the present study, BMI had a significant effect on SLEDAI score. This has been previously described even in patients with inactive or mild disease [35]. Visceral fat

contributes to systemic inflammation and promotes oxidative stress [36]. Our study also showed that the female sex impacted the SLEDAI score, while previous studies investigating gender disparities in SLE yielded mixed results [41, 45].

Anti-dsDNA positivity was associated with increased lipid and protein oxidation. Morgan et al. (2005) reported that increased anti-dsDNA antibody positivity was associated with several markers of protein oxidation [6]. Another study demonstrated an association between anti-dsDNA positivity and lipid peroxidation and suggested that oxidative stress may damage cell membranes and nucleus, increasing antigenicity thereby generating autoantibodies, including anti-dsDNA and ANA [46]. Our findings that female sex was significantly associated with antinuclear antibodies corroborate those of previous studies [47].

Positive associations between nitro-oxidative and immune biomarkers

In our study, increased peroxides and protein oxidation were associated with a Th1 and Th17 immune shift. These findings extend previous reports indicating skewing towards Th17 with higher Th1/Th2 and Th17/Th2 ratios in SLE [15]. Shah et al. suggested that increased oxidative stress in SLE is associated with a shift towards Th1 cytokine production [16]. We also found an association between oxidized proteins and CRP in SLE, findings which corroborate a study reporting an in vitro association between oxidized phospholipids and CRP [48]. Increased body weight was strongly associated with CRP, supporting the notion that this proinflammatory protein is produced by the visceral adipose tissue [35, 36, 49]. We found that female sex was associated with higher CRP levels, which is in agreement with previous reports showing significant higher CRP levels in women than in men [50].

Positive associations between nitro-oxidative stress biomarkers and adhesion molecules

This is also a first study reporting that in SLE nitro-oxidative and anti-oxidant biomarkers are significantly associated with increased adehesion molecules levels, including PECAM, E-selectin, P-selectin, and PAI. A study using cultured human umbilical vein endothelial cells demonstrated that two antioxidants (pyrrolidine dithiocarbamate and N-acetylcysteine) were able to partially repress gene transcription and expression of some adhesion molecules [9]. Another human endothelial cell study showed that ROS provoked a significant increase in the expressions of ICAM-1 and VCAM-1 in a time- and dose-dependent manner [51]. Altogether, these findings indicate that oxidative stress may play an role in CAM expression.

The main limitation of our study is that this is a case-control study, which does not allow inferences on causal relationships. A second limitation is that our study sample shows a relatively lower disease activity as indicated by a SLEDAI score of 3.7, and therefore, our findings may not be applicable to patients with more severe phenotypes. Thirdly, we did not distinguish between different SLE presentations, including cutaneous SLE, lupus nephritis, and central nervous and psychiatric presentations. Moreover, most SLE patients received medications with anti-inflammatory and/or immunomodulatory properties that could affect the results. Nevertheless, we found that treatments with these immunosuppressive drugs did not significantly interfere with the results of our study. Another limitation of this study was that subjects were not placed on a 24-h low nitrate diet, which may have influenced variability in serum levels of nitrate and nitrite [52]. Strengths of our study are that we used multivariate statistical analyses, thereby adjusting for many confounding variables including BMI, MetS, and sex. A second strength is that we recruited a large study sample and sampled blood in the same month of the year, thereby minimizing possible effects of seasonality [53].

Conclusion

Increased protein and lipid oxidation and lowered anti-oxidant defenses in systemic lupus erythematosus are associated with severity of illness, autoimmunity, increased adhesion molecules, and Th1 and Th17 immune shift. A model predicting SLE diagnosis was proposed, in which lipid and protein oxidation, nitric oxide metabolites, adhesion molecule PECAM-1, and BMI correctly classified SLE patients with high sensitivity and specificity. These results show that nitro-oxidative stress is a possible new drug target and that new drugs could be developed targeting nitro-oxidative stress and immune-inflammatory processes.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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

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