Circulating neutrophil extracellular traps in cutaneous lupus erythematosus

Both SLE (systemic lupus erythematosus) and CLE (cutaneous lupus erythematosus) immunopathogenesis involves neutrophil extracellular traps (NETs) [1, 2], composed of neutrophilic DNA and antimicrobial proteins. The release of NETs by activated neutrophils, called "NETosis", is a cell death process applied in order to ensnare and kill pathogens, by providing a high local concentration of antimicrobial agents included inside the NETs [3-5]. However, NETs are also released in sterile environments in autoimmune diseases such as lupus erythematosus (LE) [4].

Moreover, SLE is characterized by over-production of autoantibodies against nuclear antigens including double-stranded DNA (dsDNA) and histones, and NETs contain these antigens [3]. Therefore, a defective clearance of NETs may result in long-term exposure to these autoantigens [3].

Despite the abundance of studies demonstrating a correlation between increased NET production and SLE, very little is known about a possible association with CLE. Namely, there are no studies in the literature demonstrating the presence of circulating NETs in subjects with CLE, which was the aim of this study.

This cross-sectional study was conducted from October 2021 to March 2022 at the Dermatology Unit of San Martino Policlinic Hospital. Thirty-six patients with active, histologically confirmed CLE were enrolled. Patients with concurrent or previous serious infections or immunodeficiency status, taking medications that may induce neutrophilia or neutropenia, or who met the ACR–EULAR 2019 criteria for SLE [6], were considered ineligible. Thirty-four healthy controls matched for age and sex were selected. Participants underwent blood tests to dose anti-nuclear antibodies (ANA) and extractable nuclear antigens (ENA). Informed consent was obtained from all subjects. All methods were carried out according to relevant guidelines and regulations.

Citrullinated histone H3, myeloperoxidase (MPO)-DNA and neutrophil elastase (NE)-DNA complexes were used as markers to measure the NETs via ELISA. MPO-DNA and NE-DNA were quantified as previously reported [7, 8]. The absorbance at 405 nm wavelength was measured and results were reported as percentage compared to healthy adult serum (arbitrarily set at 100%) ± SD. Citrullinated histone H3 was quantified using the Citrullinated histone H3 (Clone 11D3) ELISA kit (Cayman, 501620) according to the manufacturer's instructions. The cut-off value to define the positivity was set at ≥0.156 ng/mL. Continuous variables were described as mean with standard deviation, and categorical variables as frequency with percentages. Differences in continuous variables were assessed using Student t test or corresponding non-parametric Mann Whitney U test based on data distribution. Any relationship between discrete categorical data was explored using the Chi-Square Test, or Fisher's exact test, as appropriate. Correlation degree between continuous variables was investigated using Spearman's rank correlation coefficient. A 2-tailed *P* value of 0.05 was considered significant. All statistical analyses were performed using the SPSS 23.0 (SPSS, Chicago, Illinois). Results are reported in *table 1*.

Patients enrolled belonged to three categories of CLE: 25 (69.4%) patients with discoid LE, seven (19.4%) patients with LE tumidus, and four (11.1%) patients with subacute CLE. Twenty (55.5%) patients had a duration of disease ≤10 years, nine (25.0%) patients had a duration of disease >10 years but ≤20 years, and seven (19.4%) patients had a duration >20 years. Seven (19.4%) patients presented with joints symptoms besides skin involvement. No other extracutaneous manifestation of disease was recorded. Fourteen patients were non-smokers, 10 patients smoked from one to 10 cigarettes daily, eight patients smoked from 11 to 20 cigarettes daily, and four patients smoked more than 20 cigarettes daily. Twenty (55.5%) patients were on systemic treatment for LE; medications included hydroxychloroquine, corticosteroids, methotrexate, mycophenolate mofetil and mepacrine. Sixteen (44.4%) patients were not on systemic

Table 1. Baseline characteristics and serum concentrations of the three components of NETs.

		Healthy controls (*N* = 34)	CLE patients (*N* = 36)	*p* value
Age		53.7 ± 16.72	51.6 ± 15.08	0.46
Sex	Females	16 (47.1%)	23 (63.9%)	0.16
	Males	18 (52.9%)	13 (36.1%)	
MPO-DNA (405 nm)		100.1 ± 47.11	380.3 ± 412.04	<0.001
	Negative	30 (88.2%)	10 (27.8%)	<0.001
	Positive	4 (11.8%)	26 (72.2%)	
NE-DNA (ng/mL)		100.0 ± 47.59	338.7 ± 329.06	<0.001
	Negative	28 (82.4%)	7 (19.4%)	<0.001
	Positive	6 (17.6%)	29 (80.6%)	
Histone H3		0.6 ± 0.42	1.1 ± 2.78	0.51
	Negative	6 (17.6%)	11 (30.6%)	0.21
	Positive	28 (82.4%)	25 (69.4%)	

therapy. ANA and ENA were positive in 25 (69.4%) and 14 (38.9%) patients, respectively. A strong positive correlation was found between MPO-DNA and NE-DNA (Spearman's Rho = 0.64; $p<0.001$). The results of our study show a correlation between the production of circulating NETs and CLE, as shown by other studies for SLE [5, 9-12].

The novel finding from this study is that NETs are present in serum in a higher percentage of CLE patients than healthy controls. More precisely, MPO-DNA and NE-DNA showed a statistically significant difference, while citrullinated histone H3 did not, suggesting a lower specificity of this marker. Of note, there was also a strong correlation between high values of MPO-DNA and NE-DNA. Overall, together with the study by Safi *et al.* [10], these results might raise the hypothesis that CLE, as well as SLE, may be a "NETosis". Moreover, recent findings revealing that ultraviolet light induces *in vitro* NET formation [13] strengthen the relationship between CLE lesions and NETs. The findings of increased NET concentration in the serum of patients with cutaneous lesions even without signs of systemic involvement may suggest that SLE and CLE share pathogenic mechanisms, giving strength to the hypothesis of a continuum within the spectrum of LE, which would explain why some patients with CLE have a potential to develop SLE [1]. Of course, all these hypotheses should be confirmed by studies on larger populations. Lastly, in the present study, the serum concentrations of NETs did not show a clear correlation with other demographic and clinical variables.

There are, however, some limitations to our study. Notably, the small number of patients and controls, with subsequent low statistical power and impossibility to perform subgroup analysis. Consequently, more investigation is needed to confirm our results on larger samples, also including patients with other immunological diseases – especially SLE. Having tested citrullinated histones, and not as a complex with DNA, may also represent a limitation. Moreover, measuring markers from serum, and not from isolated PMNs, is also a limitation.

However, this is the first study evaluating circulating NETs in patients with CLE. If confirmed in bigger samples, our results could have intriguing implications for the investigation of NETs as diagnostic, prognostic or follow-up markers, or as targets for new treatments. ■

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Papuloerythroderma of Ofuji associated with thymic carcinoma

Papuloerythroderma of Ofuji (PEO) tends to affect older Japanese men, with various underlying aetiologies implicated, including drugs, infections, and malignancy [1]. PEO differs from ordinary erythroderma by forming a distinctive pattern of erythroderma characterized by flat-topped papules known as the "deck-chair sign" that are usually absent from the face and skinfolds. Cancers have been reported in 20-54.5% of patients with PEO [1]. PEO is reported more often in Japan than in any other country, with gastric cancer as the most common comorbidity [2]. To the best of our knowledge, there are no reported cases of PEO