Neutrophil extracellular traps (NETs) in patients with SLE and APS

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Background: neutrophil extracellular traps (NETs) are web-like structures composed of chromosomal DNA, histones, and granule proteins that are released by activated neutrophils during inflammation. It has been shown that NETs play a role in the pathogenesis of different autoimmune diseases, including systemic lupus erythematosus (SLE) and primary antiphospholipid syndrome (PAPS). There is no gold standard method of NET detection. NETs detection in blood smears is a cheap and convenient method.

The aim: to determine levels of neutrophil extracellular traps (NETs) observed in blood smears in systemic lupus erythematosus (SLE), primary antiphospholipid syndrome (PAPS), and healthy controls.

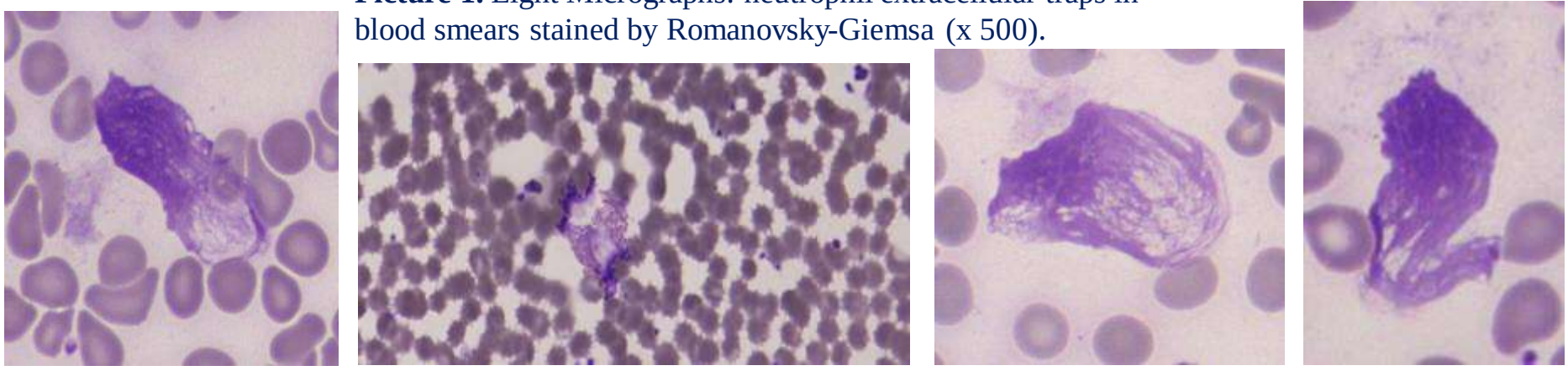
Material and methods:

- 41 patients with SLE (SLICC criteria, 2012), 29 patients with PAPS (Sydney criteria, 2006), and age and sex matched 32 healthy controls were included in the study. All patients gave written informed consent to participation.
- All patients had an established diagnosis and were on treatment.
- NETs were investigated in standardized thin blood smears produced from citrated whole blood and stained by Giemsa method.
- NETs percentage to the number of neutrophils was calculated (%NETs) using the formula: $\%NETs = N_{NETs} / (N_{native\ neutrophils} + N_{NETs}) \times 100$
- %NETs median levels between groups are compared using the Kruskal–Wallis test. Spearman's correlation was utilized to find a possible correlation between %NETs and clinical, laboratory parameters.

Table 1. Characteristics of studied patients.

Parameters	PAPS (n=29)	SLE (n=41)
Female:Male	20 (68,97) : 9 (31,03)	38 (92,68) : 3 (7,32%)
Age, years, Me [25; 75%]	41 [33,5;50]	36 [28;41]
Disease duration, years Me [25; 75%]	8 [3,1;13,5]	8 [2,4;11,1]

Picture 1. Light Micrographs: neutrophil extracellular traps in blood smears stained by Romanovsky-Giemsa (x 500).



Results.

%NETs did not differ between patients with SLE, PAPS, and healthy controls (p=0,31). There was no correlation between %NETs and SLEDAI 2K (r=0.031; p=0,85), anti-dsDNA (r=103;p=0,53), C3 (r=-0,056, p=0,73), C4 (r=-0,046, p=0,778), leukocytes (r=0,092, p=0,56), absolute neutrophil count (r=-0,005, p=0,97), neutrophil-to-lymphocyte ratio (NLR) (r=0,12, p=0,47), or erythrocyte sedimentation rate (ESR) (r=0,109, p=0,49) in patients with SLE.

There was no significant correlation between %NETs and aGAPSS (r=0,183, p=0,37), aCL IgG (r=0,212, p=0,29), aCL IgM (r=-0,007, p=0,97), aB2GP1 IgG (r=0,06, p=0,78), aB2GP1 IgM (r=0,003, p=0,98), leukocytes (r=0,12, p=0,56), absolute neutrophil count (r=0,11, p=0,61), NLR (r=0,10, p=0,64), or ESR (r=0,02, p=0,92) in patients with primary APS.

Conclusion: NET levels assessed by standardized thin blood smears were comparable between patients with SLE, PAPS, and healthy donors.

Conflict of interest: the authors have no relevant financial or non-financial interests to disclose