Author Notes: Neutrophil Extracellular Traps Profiles in Patients with Incident Systemic Lupus Erythematosus and Lupus Nephritis

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Title and Introduction. Neutrophil Extracellular Traps or NETs is one of the first defense utilized by Neutrophils against bacteria, virus, protozoa, etc. In this sense, NETs should be defined as a first phase of innate immunity. It starts with de-condensation and release of nuclear chromatin outside the cell that lead to the formation of a physical net where pathogens are entrapped and killed by elastase, defensin, reactive oxygen species (ROS), myeloperoxidase (MPO).

Slide 1. Dynamic of NETs release from Neutrophils stimulated in vitro with bacteria: neutrophils after stimulation release the green material that is constituted by DNA. It is impressive how much NETs are produced that gives an idea of the importance of the mechanism. It seems that NETs are indeed an extended net that is able to block and destroy bacteria, virus, etc.

Slide 2. Topics of the video are circulating NETs, whether they can be traced in serum of patients and whether they can accumulate in some conditions. A second topic is the mechanism of accumulation that derives from an equilibrium between production and removal *in vivo*. A third point is the composition of NETs and finally potential link with autoimmunity in lupus and lupus nephritis. The presence of DNA and of other post-translationally modified proteins is a characteristic of NETs that renders NET components potential autoantigens for autoimmune conditions,

Slide 3. Levels of NET remnants in serum of patients with Lupus and Lupus nephritis; the method for determination is an ELISA for the myeloperoxidase (MPO)-DNA complex in which MPO is first blocked with specific antibodies on solid phase and the free edge of the complex consisting in dsDNA is determined with unconjugated anti-dsDNA antibodies. Serum NETs remnant levels were significantly higher in patients with incident lupus nephritis and with incident lupus compared to healthy controls and compared to patients with other primary glomerulonephritis. NETs levels were not correlated with the timing of the disease

Slide 4. ROC curves show AUCs of 0.82 and 0.92 of the DNA-MPO assay (measuring NETs remnants) in SLE and LN, respectively.

Slide 5. '*ex vivo'* NETs production by neutrophils (release of elastase and DNA) obtained from subsets of patients recruited in the main study (neutrophils from 15 with lupus, 18 with lupus nephritis and 27 normals). Resting neutrophils from patients with SLE and LN release lower levels of elastase than control cells; after stimulation with phorbol-12-myristate-13-acetate (PMA) the difference in favor of normal cells is maintained. These results suggest that the production of NETs is not increased in lupus nor in lupus nephritis compared to normal resting cells

Slide 6. At the same time, and in the same patients, serum DNase activity is reduced in lupus nephritis patients compared to other lupus patients and to controls reaching, in some cases, levels one half the normal activity.

Slide 7. The lowest levels of DNase activity can be found in patients with high circulating NETs; 20% of LN patients with serum NETs > 0.5 (RU/ml) had DNase under the limit of normality. Sera with DNase activity in the lower range (that are indicated in squares) were pre-treated with Protein A and G to remove potential inhibitors. This pre-treatment increased DNase activity up to normal levels in 5 samples, implying that the removal of serum elements with affinity for the dye had restored DNase activity in patients with very low functional levels.

Slide 8. Serum levels of DNases1, that is the enzyme deputed to DNA removal, are normal excluding a quantitative defect of DNAse.

Slide 9. Proteins composing NETs produced by neutrophils ex vivo. Several groups of patients were considered (lupus nephritis, lupus and controls) and analysis was done by Mass spectrometry. Results show quite a number of proteins varying from 5 to 600 in NETs with important differences among groups.

Slide 10. Volcano plot shows in fact proteins high in lupus patients and others high in control cells and variations between lupus and lupus nephritis.

Slide 11. In lupus and lupus nephritis there are many proteins associated with inflammatory processes and with autoimmunity

Slide 12. An important aspect related to proteins in NETs is that in many cases, they undergo deep post-translational modifications for oxidation and other processes. For example, Enolase contains methionine 93 that is transformed into Methionine sulphoxide (in yellow).

Slide 13. Steric relationship between Enolase and DNA in NETs filaments with STED microscopy: in red Enolase and in green the DNA filament.

Slide 14. Conclusions.