Supplementary Methods

DNASE1L3 ELISA. For DNASE1L3 we utilized a specific for DNase 1L3 kit purchased from LSBio Inc. (Seattle, USA) according to the manufacturer´s instructions. Briefly, serum previously diluted 1:50 or standard solutions (100 µl) were added to each well and incubated 2 hours at 37°C. Wells were, then, incubated with Reagent A for 1 hour; after 3 washings, Reagent B was added for 1 hour at 37 C° and removed. After 5 washing steps, 100µl of substrate solution was added and then blocked with 50 µl of stop solution; reaction was read at 450 nm as above. Results are given as ng/ml.

Anti DNA antibodies determination was done with 2 methods, one is a commercial assay, DNA-DIAMEDIX (Delta Biologicals, Rome, Italy) and the second is a home-made Western-blot assay. In the home-made assay, dsDNA were loaded onto nitrocellulose membrane using Bio-Dot apparatus (Bio-Rad). After membrane blocking in 3% BSA in PBS, 100 µl of diluted serum (1:50) were added per well and incubated overnight at 4°C. Then, the membranes were washed 3 time, in and 0.05% v/v tween-20 (PBS-T) and incubated 4 hours with HRP anti-Human IgGs. Chemiluminescence was used for detection. Images were acquired with the ChemiDoc Touch Imaging System (Bio-Rad). The agreement between DIAMEDIX and home-made assay was measured using the Cohen kappa (k) and Sperman's correlation coefficients. Home-made assay showed good agreement (k=0.61; 0.50-0.72 CI at 95%) with DIAMEDIX assay. Sensitivity, and specificity were 90% and 70%, respectively. The Sperman's coefficient was 0.67 with 0.59-0.75 CI at 95%.

Isolation of neutrophils. Neutrophils were isolated from heparinized peripheral blood under sterile conditions, using a dextran sedimentation followed by Ficoll gradient centrifugation (1). One volume of heparinized blood was mixed with 0.8 volume of Dextran Plander 70000 (Fresenius Kabi Italia s.r.l, IsoladellaScala, VR, Italy) and RBC were allowed to sediment for 45 minutes at room

temperature. The granulocyte-rich supernatant was layered onto Ficoll-Histopaque 1077 and centrifuged at 800g for 30 minutes. Residual RBC were removed from by hypotonic lysis. Neutrophils were then re-suspended in RPMI with 1 mM Calcium Chloride and 1% Human Serum Albumin (Albital 200 g/l, Kedron, Castelvecchio Pascoli, Borgo, LU, Italy).

Ex vivo NET production with resting and PMA neutrophils. Neutrophil suspensions were allowed to adhere for 1 hour at 37°C onto 24-well plastic dishes at the density of $1x10^6$ cells/ml in RPMI medium, supplemented with Calcium Chloride and Human Serum Albumin. Resting and PMA stimulated cells were utilized. NETs formation was induced by treating cells for 3 hours with 20nM PhorbolMyristate Acetate (PMA). Cells were then washed with PBS and incubated with 15U/ml S7 Nuclease for 15 minutes at 37°C. Reaction was stopped with 2mM EDTA. Cellular debris were then pelleted by centrifugation at 300 g and supernatants saved for Elastase assay.

Determination of NETs production. To quantify NETs production, it was used the Cayman's NETosis assay kit (cat. No 601010, Cayman Chamical, MI, USA) according to the manufacturer´s instructions. Briefly, 100 µl of standard or culture supernatants per well, pre-heated to 37°C, were incubated with 100 µl of the 1:30 diluted NET assay neutrophil elastase substrate for 2 hours at 37°C before reading at 405 nm.

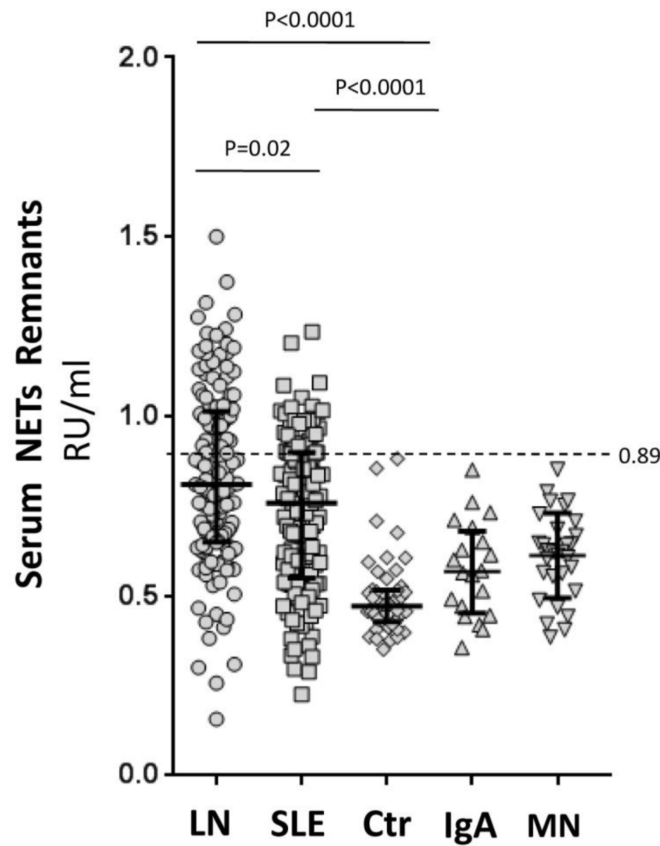
Characterization of NETs from serum-stimulated neutrophils. Neutrophils obtained from SLE patients were re-suspended in PBS containing 2% Human Serum Albumin and 0.5% donor human serum and allowed to adhere on poly-L-lysine–coated glass slides for 40 min, incubated with 0,05 ml SLE and control sera and then fixed with 3,7% paraformaldehyde in PBS at room temperature for 15 minutes. Cells were then washed in PBS and non-specific epitopes were blocked by incubation with 5% goat serum in PBS, for 30 minutes at 4°C. Slides were then incubated overnight at 4°C

with a polyclonal rabbit anti-human Histone 1 and 3 antibodies (AbDSerotecMorphoSys Ltd, Endeavour House, Kidlington Oxford, UK). As secondary antibodies we used Alexa 568-conjugated goat anti-rabbit IgG (Molecular Probes, Eugene, OR, USA). Incubation was performed for 1 hour at room temperature. Nuclei were stained with Draq5 (Cell Signaling Technology, Beverly MA). Negative controls were processed in parallel using PBS or an equivalent concentration of non-immune rabbit or mouse serum as primary antibody. The images were acquired using LSM 510 Meta confocal system scan integrated with Axiovert 200M inverted microscope (Carl Zeiss, Jena, Germany). Samples were observed through a 40×, 1.3 NA oil objective and image acquisition was carried out in multitrack mode, namely through consecutive and independent optical pathways. For 3D reconstruction, stacks of digital images were processed employing the “surface” algorithm of the “Inside 4D” module of Axiovision (release 4.5) software (Carl Zeiss, Jena Germany)

REFERENCES

1. Weiss J, Kao L, Victor M, Elsbach P. Oxygen-independent intracellular and oxygen-dependent extracellular killing of escherichia coli s15 by human polymorphonuclear leukocytes. J Clin Invest 1985;76:206-12.

Supplementary Figure 1



Supplementary Figure 1. Circulating Neutrophil Extracellular Traps (NETs) Remnants.

Serum NETs were determined using an ELISA measuring the DNA-MPO complex. Results are Relative Unit/ml given as median and interquartile range. The dotted line indicates the upper limit of normality (0.89). Control sera included in this figure both normal people and patients with Membranous nephropathy and IgA glomerulonephritis.

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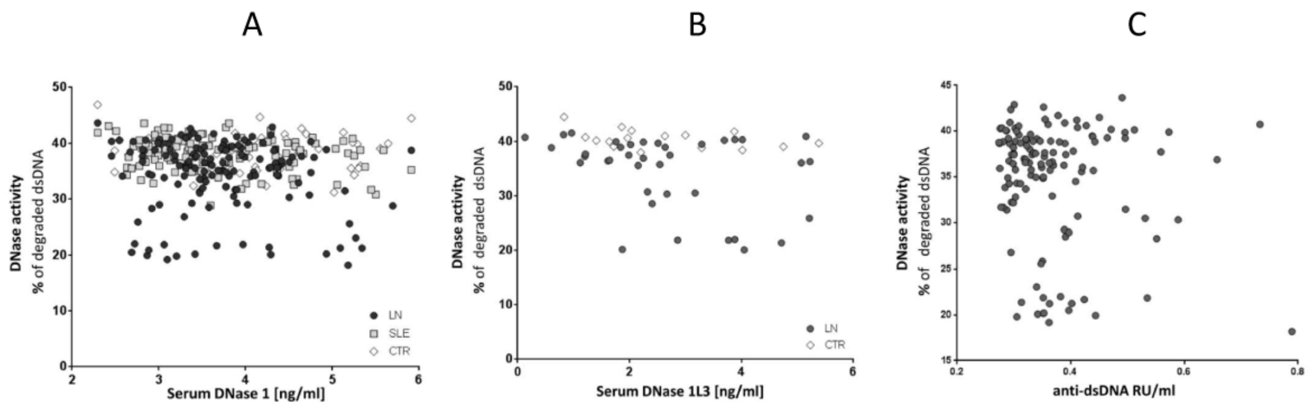
Supplementary Table

Supplementary Table 1. **SLEDAI SCORE.** The SLEDAI- K2 score did not correlate with circulating NET levels and with a modest score with DNase activity in the whole population of LN and SLE patients. High statistical correlations were instead found with anti-dsDNA ab levels, RCP.

SLEDAI SCORE	Spearman Correlation Coefficient	P-value
DNAse1 concentration RU/ml	-0.072	0.3
DNAse1L3 concentration ng/ml	0.103	0.5
Serum NETs Remnants	0.109	0.1
anti-C1q	0.016	0.8
DNase activity	-0.145	0.03*
anti-dsDNA	**0.374**	**<0.00001***
ESR T0	-0.031	0.7
CRP T0 mg/dl	**0.334**	**<0.00001***
C3 T0 mg/dl	**-0.215**	**0.003***
C4 T0 mg/dl	-0.147	0.04*

Supplementary Figure 2



Supplementary Figure 2. Correlations between DNAse levels and activity

(**A**) lack of correlation between DNASE1 levels and DNase activity. There was a scattered distribution of DNASE1 levels without correlation with activity in all the cohorts of patients.

(**B**) lack of correlation between DNASE1L3 levels and DNase activity. As above, there was a scattered distribution of DNASE1L3 levels. The results allow to conclude that there is no correlation between DNASE1L3 levels and DNase activity.

(C) lack of correlation between DNase activity and anti-DNA antibody levels in patients of the LN cohort.