

NEUTROPHILS EXTRACELLULAR TRAPS RELEASE DURING GRANULOMATOSIS WITH POLYANGIITIS

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One of the mechanisms of neutrophil action is the formation of neutrophil extracellular traps (NETs). The process of NET generation, called netosis, is a specific type of cell death, different from necrosis and apoptosis. It is suspected that the overproduction of extracellular traps is responsible for immune system hyperactivity.

The main aim of our study was to check out Netosis process in neutrophils obtained from patients with Wegener's Granulomatosis.

Neutrophils were obtained from peripheral blood collected into test-tube containing 3.2% sodium citrate. Pure cell population were seeded on 96-well plate and activated with potent Netosis activator, PMA (phorbol-12-myristate-13-acetate). The quantitative assay was performed with a use of fluorometer and DNA-binding dyes. In addition microscopically visualisation was performed.

For our studies we used 22 samples derived from WG patients (15 freshly diagnosed and 7 undergoing drug therapy) and 19 from healthy donors. Shapiro-Wilk test ($p > 0.05$) showed normal distribution in all used samples. Average release of extracellular DNA in time (values in Relative Fluorescence Units) for patients with untreated autoimmune disorder is 13983 RFU, while healthy group show release at 11891 RFU level ($p < 0.05$). Extracellular DNA quantification showed that WG-neutrophils release approximately 15% more DNA than "healthy" ones. All differences between WG patients and control group are statistically relevant based on T-student test ($p < 0.05$).

Our research have shown that neutrophils derived from Wegener's patients were more potent to release extracellular traps than neutrophils obtained from healthy volunteers. In addition we noticed that drug therapy severely diminish NET production in vitro.