ABSTRACT

Çetin Ç. The role of NETosis in Behçet's disease with vascular involvement and other rheumatologic diseases. İstanbul University, Institute of Health Science, Aziz Sancar Institute of Experimental Medicine, Department of Immunology. PhD Thesis. İstanbul. 2026.

This study aimed to evaluate neutrophil activation, reactive oxygen species (ROS) production, and NETosis responses in normal-density and low-density neutrophil subgroups of newly diagnosed, treatment naive Behçet's disease patients presenting with vascular thrombosis. To distinguish the neutrophil response in vascular Behçet's disease from other vasculitic and thrombotic conditions, patients with newly diagnosed active granulomatosis with polyangiitis, vascular thrombotic primary antiphospholipid syndrome, and idiopathic vascular thrombosis were included as comparison groups.

Normal-density and low-density neutrophil subgroups were isolated from peripheral blood using a density-based separation method. Magnetic negative selection was applied to isolate low-density neutrophils with high purity. Neutrophils were then stimulated with phorbol myristate acetate (PMA), a classic chemical stimulant, and 23-valent pneumococcal polysaccharide vaccine antigen (PSV-23), mimicking microbial stimulation. ROS levels, citrullinated histone H3 (citH3), myeloperoxidase (MPO), and MPO+/citH3+ NETosis markers were evaluated by flow cytometry. NET-related markers (citH3, MPO, NE, PAD4) in serum and neutrophil culture supernatants were assessed by ELISA, while cf-dsDNA was measured fluorometrically.

Findings revealed increased ROS production and NETosis response in vascular thrombotic Behçet's disease, particularly in the low-density neutrophil subgroup. This increase, particularly marked following PSV-23 stimulation, suggested that Behçet's neutrophils exhibit a highly primed, low-activation-threshold, and hyperactivated phenotype toward microbial stimuli

Our findings support that the enhanced NETosis response, particularly within low-density neutrophils of newly diagnosed vascular Behçet's patients, may be associated with a distinct thromboinflammatory process that differentiates it from other vasculitic and thrombotic diseases.

Key Words: Behçet's disease, vascular thrombosis, neutrophil extracellular traps, low-density neutrophils, granulomatosis with polyangiitis

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