




# Neutrophil Extracellular Traps (NETs): A New Therapeutic Target for Neuroinflammation and Microthrombosis After Subarachnoid Hemorrhage?

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## Abstract

Neutrophil extracellular traps (NETs) play a major role in intrinsic immunity by limiting and killing pathogens. Recently, a series of studies have confirmed that NETs are closely associated with vascular injury and microthrombosis. Furthermore, NETs play an important role in neuroinflammation after ischemic and hemorrhagic stroke. Neuroinflammation and microthrombosis after subarachnoid hemorrhage are key pathophysiological processes associated with poor prognosis, but their crucial formation mechanisms and interventions remain to be elucidated. Could NETs, as an emerging and important pathogenesis, be a new therapeutic target after subarachnoid hemorrhage?

**Keywords** Subarachnoid hemorrhage · Neutrophil extracellular traps · Neuroinflammation · Microthrombosis

Neutrophil extracellular traps (NETs) are reticulated DNA structures attached to histones and multiple granulocyte granule proteins released by activated neutrophils that can limit and eliminate pathogens through a range of immune responses [1]. Since their discovery as an intrinsic immune response in 2004, NETs have demonstrated an emerging therapeutic role in infectious and non-immune diseases, many of which are associated with a risk of thrombosis. NETs were found in 2010 to directly activate the coagulation cascade and limit the fibrinolytic effect of tissue-plasminogen activator (tPA), and the cell-free DNA of NETs can act as a reaction platform for fibrin polymerization and blood cell adhesion [2]. In recent years, numerous studies have identified neutrophils and NETs present in the perivascular space of infarcted lesions in specimens from ischemic stroke patients and corresponding animal models. Following ischemic stroke, NETs are involved in the impairment of neurological function by promoting thrombosis and

disrupting the blood–brain barrier structure. Furthermore, Middleton et al. reported that NETs contribute to COVID-19-associated lung injury and microthrombosis, and lung autopsy confirmed microthrombi containing NETs with neutrophil-platelet infiltration [3]. We reported that in a rat model of intracerebral hemorrhage and intraventricular hemorrhage, where large numbers of NETs are formed in and around the hematoma, the use of tPA in combination with recombinant deoxyribonuclease1 (DNase1) to break down NETs accelerates hematoma clearance and reduces hydrocephalus, whereas cell-free DNA, the main skeletal structure of NETs, caused delayed hematoma clearance and aggravated brain damage [4, 5]. In neuroinflammation after subarachnoid hemorrhage (SAH), neutrophils play an important and complex role [6]. Therefore, it is worth exploring whether NETs, a key pathological link in emerging inflammation and microthrombosis, also play an important role after subarachnoid hemorrhage.

The focus of SAH treatment is to find appropriate neuroprotective approaches in the early stages to prevent secondary brain injury in the later stages of the disease [7]. As treatment of vasospasm has not yielded a good prognosis, molecular models of neuroinflammation and early microthrombosis prevention are emerging as research foci and treatments [8]. Microcirculation is affected early in SAH, including ultra-early recruitment of peripheral inflammatory cells, neuroinflammatory

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activation, endothelial damage, platelet aggregation, and microthrombosis. Microthrombosis of the parenchymal vessels in the hyperacute phase of SAH has been observed by T2\* magnetic resonance imaging and is highly suspected to be the cause of delayed cerebral ischemia [9, 10]. Zeng et al. have reported significant NET formation in peripheral blood and intracranially in SAH mice, which exacerbates neuroinflammation and neuronal injury after SAH, and that inhibition of NET formation attenuates the neuroinflammatory response after SAH [11]. Recently, we also observed that early microthrombosis and NETs are well co-localized in mice after SAH, and that clearance of NETs by neutrophil depletion as well as DNase I can reduce cortical microthrombosis and brain injury, reducing brain edema, and improving neurological function (unpublished data). Taken together, NETs may play an important role in neuroinflammation and microthrombosis after SAH, providing a new perspective for understanding microcirculatory disorders after SAH. While non-specific anti-inflammatory drugs such as cortisol have shown a good prognosis in animal studies, they have performed disappointingly in clinical trials for the prevention of DCI and poor prognosis. This suggests that suppression of neuroinflammation requires the development of targeted anti-inflammatory therapies and that the selection of specific therapeutic time windows and specific molecular patterns of neuroinflammation are necessary. The key challenge for clinical trials in the prevention of microthrombosis is how to manage the conflict between the use of anti-platelet or anti-coagulant agents and the risk of rebleeding. The important role of NETs provides us with ideas for treating neuroinflammation and microthrombosis through fine modulation of cellular immunity, which holds exciting research promise and may become a therapeutic target.

Methods of preventing or inhibiting the formation of NETs depend on the structure of the NETs and the mechanism of their formation. The dissolution of the NETs skeleton using DNase I is the most classical intervention, but the limitation is that it does not effectively remove the released histones and multiple proteases. The neonatal NET-inhibitory factor (nNIF) is an endogenous regulator of NETs found in the umbilical cord blood, which not only effectively inhibits the formation of NETs but also does not affect the recruitment of neutrophils after stroke and has shown good therapeutic effects in animal models. Since the release process of NETs involves several key reactions, there are a wide range of compounds that could theoretically intervene in the formation of NETs. These therapeutic modalities require more research and clinical trials to enable the protection of the anti-inflammatory function of neutrophils while regulating and NETs.

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## Declarations

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**Human and Animal Ethics** Not applicable.

**Consent for Publication** Not applicable.

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