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Special Topic

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Neutrophil Extracellular Traps in Coronavirus Disease-19-Associated Ischemic Stroke: A Novel Avenue in Neuroscience

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Ischemic stroke is one of the catastrophic neurological events that are being increasingly recognized among Coronavirus Disease (COVID)-19 patients. The recent studies have revealed about a possible connection among COVID-19, ischemic stroke, and excessive Neutrophil Extracellular Traps (NETs) formation. This paper establishes an overview of coronaviruses and NETs, NETs in pathogenesis of COVID-19 induced-ischemic stroke, and future directions using related recent literatures. NETs are normally functioned for a defense against pathogens, but in immoderate amount, they can trigger series of destructive events. Vasculopathy and neuroinflammation are the pathological mechanisms of NETs suggested to link COVID-19 and ischemic stroke. Based on newly discovered possible mechanisms, the potential clinical implications that could be applied consists of inhibition of NET formation, disrupting cholesterol synthesis, and interfering inflammatory pathway. A considerable number of scientific works are needed in order to complete the current understanding of the emerging relationship among COVID-19, NETs, and ischemic stroke. Although the exact mechanism is still unknown, these novel findings are a worthwhile contribution in defining future studies, suitable future frameworks, and therapeutic strategies.

Key words: Stroke, Coronavirus, Neutrophil, Extracellular traps

INTRODUCTION

For these past few months, our world has been facing the catastrophic spread of the newest member of the coronavirus family called Severe Acute Respiratory Syndrome-Coronavirus 2 (SARS-CoV-2), which has been identified as the etiology of Coronavirus Disease-19 (COVID-19). Countries are currently facing its devastating domino effects, which have affected all sectors of lives. Healthcare professionals are taking extensive efforts to make a quick and precise diagnosis in order to make the optimal decisions related to the therapy. Studies have revealed that SARS-CoV-2 may induce ischemic strokes in human brains. Recently, five cases of large vessel stroke-related COVID-19 in young patients were reported in New York. Indeed, these cases have raised concerns among neurologists [1].

As global efforts to fight the current coronavirus pandemic along with its deteriorating complication, the ischemic stroke, continue, new studies have placed the potential role of overactive neutrophils under the spotlight [2, 3]. Recently, despite there being some currently established mechanisms of ischemic stroke, researchers have become curious to explore a newly discovered role of Neutrophil Extracellular Traps (NETs), which are networks of DNA, histones, and proteolytic enzymes produced by activated neutrophils in stroke pathogenesis [3]. Indeed, an association between the SARS-CoV-2 infection and the formation of NETs were investigated in some recent studies. Moreover, a study that documented

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high levels of NETs in patients with COVID-19 revealed that excessive NETs contribute to inducing disseminated inflammation, cytokine release, and microvascular thrombosis [4]. On the other hand, the biomarkers of NETs formation; citrullinated histone H3 (H3Cit) and cell-free DNA (cfDNA), were elevated during ischemic stroke. These findings propose a novel link among COVID-19, the formation of NETs, and ischemic stroke.

Although the exact mechanism is still unknown, understanding the role of NETs in the pathogenesis of stroke-related COVID-19 is a worthwhile contribution to defining suitable future therapeutic strategies. This paper presents an overview of coronaviruses and NETs, suggests the pathogeneses of NET-induced COVID-related ischemic stroke, and the future directions.

COVID-19-INDUCED ISCHEMIC STROKE

Acute Ischemic Stroke (AIS) is a catastrophic neurological emergency which induced by various risk factors, either modifiable or non-modifiable. Infection is one of established modifiable risk factors that do elevate the odds of getting stroke by 1.4-fold [5]. A recent case series study in New York comparing two groups; stroke patients who have confirmed with COVID-19 infection and another group of stroke patients without infection, revealed that COVID-19 was an independent risk factor for stroke in hospitalized patients [6]. Recent findings have reported several events of stroke in severe COVID-19 infection, whereas ischemic stroke is more frequent than intracerebral hemorrhage (Table 1).

A systematic review evaluating studies correlating AIS and COVID-19 revealed a high mortality rate [12]. Large vessel occlusion has determined as the underlying pathology in majority of COVID-19-related AIS cases. On the other hand, the traditional trends of AIS subtypes demonstrated higher incidence of small vessel stroke [13]. The common imaging patterns of ischemic stroke found in COVID-19 patients are stenosis and multiple territory pattern [12]. Another difference found between conventional AIS and COVID-related AIS is on the outcome. COVID-19 patients with AIS shows more significant incidences of hemorrhagic transformation, higher National Institutes of Health Stroke Scale (NIHSS) score, and increased mortality rate [9]. Factors related with higher possibility of in-hospital death in COVID-19-related AIS are ICU admission, high levels of C-reactive protein, lactate dehydrogenase, and D-dimer [14]. A systematic review revealed that there is a prolonged time of AIS onset in COVID-19 patients, ranged from 8 until 10 days [12]. Furthermore, a study observed a prolonged time to symptom recognition and presentation of COVID-19-related AIS, limiting the optimization of acute reperfusion therapy [15]. Owing to the tendency of having a large vessel occlusion and higher mortality on COVID-19 patients, the prothrombotic and cytokine storm are the possible underlying mechanisms of direct relationship between COVID-19 and ischemic stroke, although further researches need to be elucidated. Early detection of specific markers involved in the pathogenesis of COVID-related AIS can be a groundbreaking invention to choose the appropriate therapeutic interventions.

NEUTROPHIL EXTRACELLULAR TRAPS

Neutrophil Extracellular Traps (NETs) was initially characterized in 2004 [16]. They are extracellular structures, web-like in shape, containing nuclear DNA, histones, and proteolytic enzymes expelled from cell death of activated neutrophils [3] which has essential function to bind, degrade, and eliminate pathogens [2, 16]. It was extensively discussed in vast amount of studies related to bacterial infection before being explored in the pathogenesis of viral infection [17]. The mechanism of cell death performed by NET (Neutrophil Extracellular Trap-osis; NETosis) is a distinguishable mechanism from either apoptosis or necrosis [2]. Main function of NET is related to host defense against pathogens including viruses and bacterium, which are killed by NET-associated proteins. On the other hand, excessive NET formation triggers the destructive inflammatory cascades which lead into organ damage and promotes other catastrophic events such as metastatic process of

Table 1. Clinical studies of strokes in COVID-19

Year	Country	Patient population	Total number of COVID-19 patients	Incidence of each stroke subtype (%)		Citation
				Ischemic	Hemorrhagic	
2020	United States	Laboratory confirmed COVID-19	86	83.7	16.3	Katz et al. [6]
2020	China	Laboratory confirmed COVID-19	214	2.3	0.4	Mao et al. [7]
2020	Italy	Laboratory confirmed COVID-19	388	2.5	0	Lodigiani et al. [8]
2020	United States	Laboratory confirmed COVID-19	3,556	0.9	0	Yaghi et al. [9]
2020	France	Severe COVID-19	150	2.0	0	Helms et al. [10]
2020	Netherlands	Severe COVID-19	184	2.7	0	Klok et al. [11]

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cancer and endothelial dysfunction [18].

The formation of NET is a series of regulated processes involving some key enzymes; Neutrophil Elastase (NE), Myeloperoxidase (MPO), Peptidyl Arginine Deiminase type 4 (PAD4), and gasdermin D. NE is a neutrophil serine protease resides in azurophilic granules which breaks down intracellular proteins and induce disintegration of nucleus [3]. Together with MPO, NE started its role upon activation and Reactive Oxygen Species (ROS) production. Translocation of NE into nucleus followed by histones cleavage and promotes decondensation of chromatin. In the late stage of the process, MPO binds to chromatin and promotes further decondensation. Final result of these cooperative enhancement of chromatin decondensation leads into cell rupture and NET release [19]. Along with other enzymes, Gasdermin D (GSDMD), a pore-forming protein, takes an important role in disrupting cell membrane. It also triggers the ejections of DNA and associates with molecules required in NETosis [20].

Another key molecule is Peptidyl Arginine Deiminase 4 (PAD4), a 74kDa protein [3]. It promotes the decondensation and release of the chromosomal DNA. PAD4-deficient mice were unable to exhibit all those processes linked with formation of NETs [21, 22]. PAD4 citrullinates arginine residues of histone H3 –resulted in formation of H3Cit– prior to NETosis or formation of NETs [23]. Together with cell-free DNA (cfDNA), H3Cit considered as a surrogate biomarker to show the presence of NETs formation even after cerebral ischemia [24]. H3Cit serves as a specific marker of NETs formation, while cfDNA has less specificity as a NET-related biomarker [25].

COVID-19 AND NETS

There are increasing evidences to suggest that in the case of COVID-19, NETs play a significant role as the potential drivers of exacerbation and worsening of outcome [26]. Neutrophilia is a hallmark of acute infection in COVID-19 and could also be a source of excess NETs [3]. A recent autopsy findings from a COVID-19 patient revealed that there was a high density of neutrophil infiltration found in pulmonary blood vessels [27].

There are some reasons believed to be the cause of those findings. The elevation of Granulocyte-Colony Stimulating Factor (G-CSF) levels found among COVID-19 patients, even higher in severe cases [28]. The up-regulation of G-CSF, thus, obtains neutrophil out of bone marrow into circulation. It also reduces levels of C-X-C motif ligand (CXCL)-12, an important compound from the bone marrow which functions to clear out aged PMN [29]. CXCL2 and CXCL8, the NF-κB driven neutrophil attractant chemokines, increased in sera of COVID-19 patients [30].

Patients with severe COVID-19 infections have higher neutrophil counts and less amount of lymphocytes [31]. Along with that, high level of neutrophil-to-lymphocyte ratio (NLR) has known as an independent risk factors of severe COVID-19 [32, 33]. However, several meta-analysis have shown that elevated NLR indicated bad prognosis of AIS and spontaneous intracerebral hemorrhage [34, 35]. Neutrophils have known to interact with platelets to enhance immune system for fighting against pathogens. The interaction of them will stimulate neutrophil to release more neutrophil mediators and produce NETs [36]. The problem will be started if there is a massive formation of NETs which induces proinflammatory cascades along with tissue damages. Surprisingly, this idea was

Table 2. Studies of NETs in COVID-19

Author, year	Patients	Biomarkers	Method used in measurement of NETs	Results
Nicolai et al., 2020 [37]	38 COVID-19 patients and 24 non-COVID-19 controls	H3Cit, MPO	Immunofluorescence	NETs detected in inflammatory microvascular thrombi located in the lung, kidney, and heart
Zuo et al., 2020 [38]	22 COVID-19 patients who developed thrombosis and 22 COVID-19 patients without clinical thrombosis	cfDNA, MPO, H3Cit	Quant-iT Pico Green dsDNA Assay Kit (for dsDNA) and ELISA (for MPO and H3Cit)	All NETs markers associated with higher risk of morbid thrombotic events
Middleton et al., 2020 [39]	33 COVID-19 patients and 17 healthy controls	MPO	ELISA	Plasma MPO-DNA complexes significantly elevated in COVID-19 patients and correlated with illness severity
Veras et al., 2020 [40]	32 COVID-19 patients and 21 healthy controls	MPO, H3Cit	Immunostaining	Neutrophils from COVID-19 patients released statistically higher levels of NETs compared with healthy controls

AIS, Acute Ischemic Stroke; dsDNA, double-stranded DNA; ELISA, Enzyme-Linked Immunosorbent Assay; NE, Neutrophil Elastase; MPO, myeloperoxidase; NET, Neutrophil Extracellular Traps; H3Cit, citrullinated histone H3; cfDNA, cell-free DNA.

supported by some recent evidences about the presence of NETs biomarkers in severe cases of COVID-19 (Table 2).

ISCHEMIC STROKE AND NETS

As high levels of neutrophils identified in cerebral thromboemboli, it brings out a suspicion about the contribution of NETs in thrombogenesis and its role in decreasing efficacy of intravenous thrombolysis among Acute Ischemic Stroke (AIS) patients [41]. The neutrophils being the first blood-borne immune cells to arrive at ischemic brain tissues, binding various adhesion molecules in order to attaching themselves to endothelium within 15 minutes of ischemic stroke [42, 43], produces proinflammatory cytokines, chemokines, and cytotoxic molecules that accelerate the progression of brain damage [44]. It has known that the recruitment of neutrophils into the brain was orchestrated by IL-1. After being translocated, neutrophils will activate themselves, changes their phenotypes and releases decondensed DNA threads known as NETs [45]. These events have seen in a study conducted by Perez-de-Puig et al. which observed the presence of H3Cit, the specific marker of NETs formation, in the ischemic brain after 24 hours [24]. Kang et al. found that Ly6G-positive neutrophils, F4/80-positive macrophages/microglia, Iba1-positive microglia, NeuN-positive neurons, and glial fibrillary acidic protein (GFAP)-positive astrocytes express H3Cit after stroke. Studies that investigated NETs in ischemic stroke, either in human or animal models, identified significant difference of the amount of NETs related-markers positive cells found in ischemic brain and healthy controls (Table 3 and 4).

COVID-19, NETS, AND ISCHEMIC STROKE: A POSTULATED LINK

Revealing the link among COVID-19, NETs, and ischemic stroke-related COVID is similarly to constructing a picture from puzzle pieces. Kang et al. shown that H3Cit positive cells existed inside blood vessels and cerebral parenchyma [50]. Thus, the main points of the link between COVID-19 and ischemic stroke are closely related to two events: atherosclerosis along with thrombus formation in blood vessels and neurotoxicity in cerebral parenchyma [52].

Table 3. Human studies of NETs in stroke

Author, year	Patients	Biomarkers	Method used in measurement of NETs	Results
Essig et al., 2020 [46]	37 acute ischemic stroke (AIS) patients undergone mechanical thrombectomy	H3Cit, NE, MPO	Immunofluorescence	NETs found in more than 90% of thromboemboli. NETs were almost exclusively found within fibrin-rich areas
Zhou et al., 2020 [47]	55 AIS patients undergone endovascular thrombectomy and 35 healthy controls	H3Cit, NE, MPO, cfDNA	Immunofluorescence	All NET-related markers found in plasma were higher in AIS compared with control group
Lim et al., 2020 [48]	58 AIS patients and 25 healthy adults	Double-stranded DNA (dsDNA), DNA-histone complex	Immunofluorescence (for dsDNA) and ELISA (for DNA-histone complex)	Circulating levels of NETs was significantly higher in AIS patients at initial presentation compared with control group
Laridan et al., 2017 [49]	68 AIS patients undergone thrombectomy	H3Cit	Immunostaining	NETs was observed in almost all thrombi

Table 4. Animal studies of NETs in stroke

Author, year	Species	Biomarkers	Method used in measurement of NETs	Results
Kang et al., 2020 [50]	Mice	H3Cit	Western Blot (ischemic cortex) and immunostaining (peri-infarct cortex)	Cortex of ischemic mice have shown higher amount of $H3Cit^+$ neutrophils. Peri-infarct cortex was extensively labelled with $H3Cit^+$ cells after 3 days
Kim et al., 2019 [51]	Rat	H3Cit	Immunofluorescence	The first $H3Cit^+$ cells entry occurred at 12 hours after middle cerebral artery occlusion through leptomeninges
Perez-de-Puig et al., 2015 [24]	Mice	H3Cit	Immunofluorescence	$H3Cit^+$ neutrophils found in perivascular spaces, cerebral parenchyma near blood vessels, lumen capillaries, and striatum

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NETs and procoagulant activity in blood vessels

Impact of high levels of intravascular NET in SARS-CoV-2 can be explained in the vital role of circulating NETs to initiate and accrete thrombosis in artery and veins [53], also trigger occlusion of small vessels in lungs, heart, and kidneys [54]. In addition, NETs tend to form large aggregates which accelerate formation of thrombi in blood vessels at high neutrophil densities. Prothrombotic events and mechanism behind them are highly related to fibrin, tissue factor, and coagulation processes. The central role in thrombosis induced by NETs is promoting fibrin deposition and the establishment of fibrin networks [55] resulted in elevation of D-dimer level, the global indicator of coagulation activation and fibrinolysis [56], along with its procoagulant activities that induce coagulation system [18].

Coagulation increases the risk of getting thrombotic complications including ischemic stroke [57]. The proposed underlying pathological mechanism of NET-induced coagulation in COVID-19 is the ability of NETs to activate the plasma kallikrein-kinin system as the contact pathway of coagulation and its interaction with platelet. The kallikrein-kinin system can be activated through electrostatic interactions between histones of NET and platelet phospholipids [58]. Indeed, histones also act as ligands for Toll-like receptors on platelets, which induce platelet activation [59]. There is a well-established loop involving platelets and neutrophils. High Mobility Group protein B1 (HMGB1), a highly conserved nonhistone nuclear protein existed in platelet is a major endogenous inducer of NET formation. Activated platelets present HMGB1 to neutrophils and stimulate them to form NETs [18]. After adhesion process occurred, the chromatin of NET acts by disrupting epithelial lining, triggers platelet aggregation, so that it enhances further NET formation along with recruitment of neutrophils.

The foundation of NET-induced thrombus formation is by promoting platelet adhesion and concentrating coagulation factors to make a though clot. Extension of thrombi can be achieved by binding of thrombus-resident neutrophils and factor XII. In addition, activation of factor XII is supported by NETosis [60]. Apart from aforementioned mechanisms, there is an evidence for the release of tissue factor through NETs [61]. These lines of prothrombotic and hypercoagulable state-based mechanisms considered as the potential underlying events behind elevated levels of D-dimer as a reliable coagulation marker of severe COVID-19 cases. Supporting this idea, there is a study reported that patients with higher D-dimer levels show a significantly higher level of leukocytes and platelets in peripheral blood cell count test [62].

NET-induced endothelial dysfunction is a vital point in atherosclerosis [18], the fundamental events for pathogenesis of ischemic stroke. Two components of NETs; Cathepsin G and cathelicidins, induce the attraction of monocyte in atherosclerotic plaques [63]. Moreover, NETs are recognized as the major sources of Cathelicidin-related Antimicrobial Peptide (CRAMP). CRAMP is an endogenous antimicrobial polypeptide that is situated in secondary granule of neutrophil and deposited on inflamed endothelial surface [64]. NET-derived CRAMP anchors to endothelial cells then promote further monocyte recruitment. Another pathological mechanism underlying NET-induced endothelial dysfunction has done by MPO. After binding, MPO elevates the level of Reactive Oxygen Species (ROS). Further, ROS induces the production of oxidized Low Density Lipoprotein (oxLDL) which encourages the buildout of foam cells. Along with ROS, proteinase from NETs affects plaque instability [65].

Another proposed link in thrombus formation is the hyperinflammatory state induced in COVID-19. The complex mechanism of inflammation results in hyperviscosity, thrombogenesis, and higher stroke risk [52]. Severe COVID-19 is associated with a cytokine storm characterized by increased plasma concentrations of IL1β, IL2, IL6, IL7, IL8, IL10, IL17, Interferon (IFN)γ, IFNγ-inducible protein 10, monocyte chemoattractant protein 1 (MCP1), G-CSF, Macrophage Inflammatory Protein 1α, and Tumor Necrosis Factor (TNF)α [28]. In addition to IL-17, IL-1β also induces the blood level of IL-6. Plasma IL-6 is widely studied as a strong predictor of ischemic stroke outcome [66]. Significant elevation in plasma, as well as brain tissue concentration of IL-6 or mRNA have been described in either clinical or experimental studies about ischemic stroke and transient ischemic attacks [67-69]. A potent prothrombogenic properties of IL-6 presents in its ability to promote thrombocytosis secondary to stimulation of thrombopoiesis [70], induction of platelet-leukocyte aggregation [71], platelet activation [71], expression of tissue factor, fibrinogen, factor VIII and von Willebrand Factor (vWF), and thrombin generation. Another influence of IL-6 on coagulation system is by reducing level of anti-thrombin and protein S as the inhibitors of hemostasis [72].

Histone H4, one of main enzymes of NETs, has known to facilitate NETs to bind to host cell membranes by its high amount of cationic amino acid residues. This mechanism allows NETs to make pores on cell membranes, causing host smooth muscle cell lysis that leads to destabilization of plaques [73]. This role will exacerbate clot formation in blood vessels that leads into depletion of oxygen and nutrition in brain parenchyma.

The NETs-induced neuroinflammation

The interesting role of intravascular NETs after enhancing coagulation and prothrombotic pathway is orchestrating neuroinflammation, one of main pathological processes in every acute

brain injury, including ischemic stroke. Disruption of blood flow after clot formation depletes the amount of cerebral oxygen and nutrients level. That deprivation of oxygen and nutrients results in neuronal damages followed by subsequent damage of BBB, glial cell activations and influx of circulating immune cells into cerebral parenchyma, worsening the progress of brain ischemia inducing neurological deficits [74-77]. Dying neurons and glial cells releases Damage Associated Molecular Pattern (DAMP) called HMGB1. Upon released, HMGB1 will interact with Toll-like Receptor4 (TLR4), results in activation of neutrophils, enhancement of NETosis in brain parenchyma, and activation of microglial cells. Activation of microglia will trigger more NETs release [45].

The mechanism of NETs-induced neuro inflammation seems to correlate with the action of neutrophil granules-derived enzymes along with its inflammatory events. In the brain parenchyma, extruded NETs components induce brain damage [44]. Allen et al. identified cathepsin-G and NE as the culprits of neuronal toxicity, and their inhibitors, when administered as a mixture, reported to reverse the neurotoxic effects of neutrophil. The study also revealed that the viability of cultured neurons depleted after 3 hours of administration of transmigrated neutrophils [78]. Another NETs enzyme, PAD4, showed a contribution in progression of brain damage upon NETosis. Inhibition of PAD4 using Cl-amidine, the pan-PAD4 inhibitor, results in decrease of microglial activation, neuronal death, and infarct volume in a hypoxic ischemic mouse model [79].

Another interesting insight is NETs-IL-1β loop that exacerbates neuroinflammation. It is especially noteworthy that SARS-CoV-2 appears to act on the activation and maturation of IL-1β. NETs trigger macrophages to secrete IL-1β [80]. The significance of IL-1β in stroke-related COVID pathogenesis is its role in inducing levels of IL-17 and IL-6. IL-1β induces the release of IL-17 by activating Th17 cell [81]. Subsequently, pathogenic effect of IL-17 could be achieved through the interaction of IL-17 with its receptors existed on astrocytes, microglia, and neurons. The IL-17–IL-17R signalling on astrocytes and microglial cells will activate the Nuclear Factor kappa-B (NFκB) transcriptional factors which is responsible for elevating the levels of proinflammatory cytokines (IL-6, TNFα, and IL-1β), chemokines (eg, CXCL1, CCL2, CXCL2, CCL7, CCL11, and CCL20), and metalloproteinases [82], amplifies the release of ROS, and enhance neutrophils accumulation in the brain parenchyma [83]. An experimental study using ischemic stroke model revealed that IL-17A-producing γδ T cells have a pivotal role to enlarge infarct size [84]. Fig. 1 summarized the proposed pathological mechanism underlying NET-induced COVID-19-related ischemic stroke.

THERAPEUTIC IMPLICATIONS AND FUTURE DIRECTIONS

The ideas of NET involvement in COVID-related ischemic stroke has opened new horizon of therapeutic modalities. Based on its pathogenic mechanism, there are some different options could be offered as the potential therapeutic approaches related to NETs formation.

The inhibition of NET formation has established as either by blocking fundamental process involved in NETosis or disrupting the structure of NETs. However, the first option would not be feasible. The reason behind it is the high possibility of developing catastrophic adverse events. Inhibition of citrullination elevates the risk of carcinogenesis because citrullinated histones are not only important for chromatin decondensation, but also acts as an apoptotic code for detecting damaged cells [85]. On the other hand, targeting the dark side of NET either by disrupting DNA backbone of NET via DNase or antihistone modalities have been shown to be promising candidates to demolish pathological process in underlying diseases with excessive NET formation including Transfusion-Related Acute Lung Injury (TRALI) [86], Systemic Lupus Erythematosus (SLE) [87], and ischemic stroke [88]. Interesting result from a study revealed that administration of recombinant human DNAse 1 reduced infarct volumes and alleviated ischemic stroke outcome in mice [88].

However, the DNAse strategy does not optimally reduce the amount of NETosis because toxic histones remain attached to blood vessel wall [89], thus, next therapeutic approaches supporting pathological action of histones need to be considered. The histone depletion strategies can be achieved by blocking of extracellular histone toxicity using activated protein C [90]. Another approaches are heparin forms modification [91], C1 esterase inhibitor [92], antihistone antibodies, or blocking peptides [73]. Intranasal administration of PAD4 inhibitor results in less amount of NETosis, thus reduces inflammation by reducing immune cell infiltration and alleviates vasculogenesis [44].

A considerable number of scientific works are needed in order to complete the current understanding of the emerging relationship between COVID-19, NETs, and ischemic stroke. Preliminary studies about level of NETs and related factors in COVID-19-stroke patients can be designed in order to strengthen the foundation of this novel knowledge, indeed, with the consideration of ethical aspects for preparing such studies. We have already suggested a correlation between NETs, viral infection, and ischemic stroke by synthesizing studies. Targeting NETs in COVID-19 ischemic stroke, mechanistically, is a potential novel approach without any risks of general neutrophil depletion. Thus, whether the COVID-19 pandemic is still going on or not, studies correlating NETs

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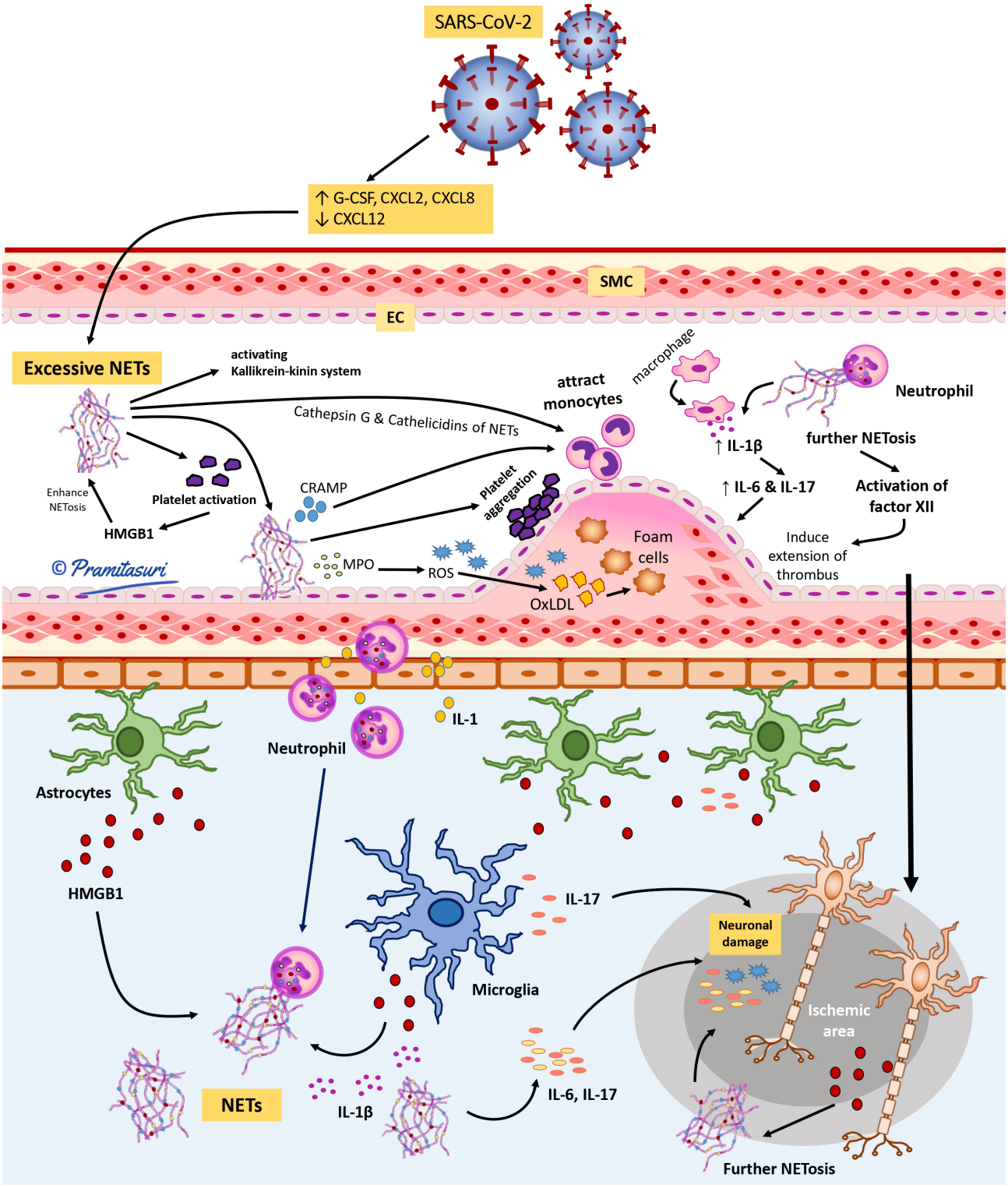

CRAMP	: Cathelicidin-related Antimicrobial Peptide	MPO	: Myeloperoxidase
CXCL	: C-X-C motif Ligand	NETs	: Neutrophil Extracellular Traps
EC	: Endothelial Cells	OxLDL	: Oxidized Low Density Lipoprotein
G-CSF	: Granulocyte-Colony Stimulating Factor	ROS	: Reactive Oxygen Species
HMGB1	: High Mobility Group protein B1	SARS-CoV-2	: Severe Acute Respiratory Syndrome-Coronavirus-2
IL	: Interleukin	SMC	: Smooth Muscle Cells

Fig. 1. NETs in the pathogenesis of COVID-19-associated ischemic stroke.

and ischemic stroke is a worth-to-explore topic in neuroscience field.

CONCLUSION

The establishment of NET as a potential driver in a series of pathological mechanisms in COVID-19-related ischemic stroke, including coagulation, inflammatory state, and endothelial dysfunction, is an important novel finding in neurology. Inhibition of NETs formation are potential clinical implications in the sphere of NETs-induced COVID-19-related ischemic stroke. Beyond of its fascinating role and aside of COVID-19, NET is a strategic research object in either exploring pathogenesis, drug prospecting studies, and even reversing rTPA resistance among ischemic stroke patients.

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