Review Article

Current progress in searching for clinically useful biomarkers of blood–brain barrier damage following cerebral ischemia

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Abstract:

Ischemic stroke is a leading cause of death and disability. Fear of intracranial hemorrhage (ICH) has been the primary reason for withholding tissue plasminogen activator (tPA) and thrombectomy, the only two widely accepted treatments for ischemic stroke. Thrombolysis treatment is only allowed in a very narrow time window (within 4.5–6 h). However, so far, other than the time window guideline, there is no reliable indicator available in the clinic to predict ICH before thrombolysis treatment. Recently, extensive research efforts have been devoted to the development of reliable indicators to predict ICH and safely guide the thrombolysis treatment. Accumulating evidence suggests that ischemic brain regions with a compromised blood–brain barrier (BBB) before tPA treatment develop ICH at the later time during thrombolytic reperfusion. Assessing BBB damage before thrombolysis could potentially help predict the risk of ICH after thrombolysis. This article reviews the literature reports on BBB damage biomarkers that have been developed in recent years, including biochemical markers such as BBB structural proteins, circulating brain microvascular endothelial cells, plasma albumin, and brain parenchyma proteins, as well as image markers such as magnetic resonance imaging assessment for BBB damage.

Keywords:
Biomarkers, blood–brain barrier, cerebral ischemia, stroke

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Introduction

Stroke is one of the most common diseases causing death and disability. Ischemic stroke is the most common type of stroke, which approximately accounts for three-fourths of all strokes.[1] Fear of symptomatic intracranial hemorrhage (ICH) has been the primary reason for withholding tissue plasminogen activator and thrombectomy, the only two effective treatments for acute ischemic stroke. Currently, a fixed time window of within 4.5–6 h from stroke onset is used as the primary factor for ICH risk prediction in the thrombolytic therapy treatment guideline.[2,3]

Studies have demonstrated that ischemic brain regions with blood–brain barrier (BBB) breakdown during the early phase of stroke often develop ICH at later time points following thrombolytic reperfusion.[4-6] Therefore, an individualized measurable factor based on BBB damage is likely to be more accurate than using a fixed time window in predicting ICH risk. In this review, we focus on the potential clinically available indicators of BBB damage as biomarkers of ICH following thrombolytic reperfusion.

Background

The function of blood–brain barrier

The central nervous system is formed by the neurons and glial cells in vertebrates.

However, neurons are not renewable cells; therefore, it is essential to maintain homeostasis to protect neurons. BBB is a solute exchange barrier between the brain and blood to preserve this microenvironment, which was discovered and named by bacteriologist Ehrlich and Goldman in the late 19th century.[7] BBB is not only a mechanical barrier but also has essential physiological functions, including: (1) restricting free exchange of material between blood and the brain, (2) delivering the essential nutrients to the brain and excreting the metabolites from the brain into blood, (3) removing the toxic or unnecessary substances from brain to blood for protecting brain function, and (4) regulating hormonal functions. However, under the pathophysiological conditions, such as being affected by a tumor, inflammation, ischemia, or other factors, BBB is vulnerable to damage, causing increased permeability and losing "barrier" function.[8]

The structure of blood–brain barrier

The integrity of BBB is essential for maintaining its functions. Thus, a comprehensive understanding of the BBB structure is critical for studying the mechanism of BBB damage. Here, we briefly introduce the structure of BBB, which is composed of brain microvascular endothelium cells (BMECs), pericytes, and astrocyte foot processes around the capillaries and basement membrane.

Brain microvascular endothelium cells
BMECs are the first barrier that constitutes the BBB. The tight junctions between cells tightly bind the gap between endothelial cells like zippers, and there is no pore structure on the surface of the cell membrane on which some specific ions and transporters are expressed. Endothelial cells not only maintain the integrity of BBB but also act as a barrier to toxins and pathogens. Moreover, BMECs can regulate the microcirculation in the brain by producing and releasing a variety of vascular regulators such as endothelin, NO, and vascular endothelial growth factor (VEGF). Cerebral ischemia can cause BMECs swollen, apoptosis, further leading to BBB disruption. Besides, BMECs participate in the inflammatory cascades of cerebral ischemia to increase BBB damage.[9-11] The tight junctions connect the BMECs by binding one transmembrane protein, one cytoplasmic attachment protein, and one cytoskeletal protein. This structure is the foundation of the BBB. The transmembrane proteins include occludin, claudin, and junctional adhesive molecule. The cytoplasmic adhesion proteins include ZO-1, ZO-2, and ZO-3. Moreover, the cytoskeletal protein is actin.[12,13] There are two pathways involved in the transtight junction flux. One is the pore pathway, which allows ions and small solutes pass through the tight junction. The other is the leak pathway, which is controlled by cytoskeletal dynamics or factors that affect cell homeostasis.[14,15] Tight junction is an important factor in maintaining the integrity of the BBB. The structural changes in tight junctions lead to the BBB damage and increase vascular permeability, causing toxic substances flux into the brain and contributing to brain damage.[16] Numerous studies have shown that the expression of the structural components of the BBB, such as occludin, claudin-5, and ZO-1, decreases after cerebral ischemia.[17-19]

Pericytes
Pericytes, which are known as vascular smooth muscle cells, is an important cellular constituent of postcapillary venule and capillaries.[20] It is intimately connected to the endothelial cells by N-cadherin, gap junction, and tight junctions outside the blood vessels and shares the same basement membrane with endothelial cells.[21] The synthesis and release of albumin by pericytes have been considered as an important step in BBB differentiation.[22] Pericytes are involved in many crucial functions during a stroke, including the regulation of blood flow and BBB permeability as well as repair of the neurovascular unit.[23]

Astrocytes
Astrocytes form the bridge that connects neuronal signaling and the vasculature in the central nervous system. Astrocytes are involved in the maintenance of BBB integrity mainly through the release of active substances, especially the growth factor, such as VEGF and glial cell line-derived neurotrophic factor. Moreover, the regulation of intracellular cAMP and other protein expressions by astrocytes also affects BBB integrity. In addition, there is a complicated relationship between astrocytes and brain capillaries, which plays a vital role in the maintenance of BBB function.[24-26]

Basement membrane
Basement membranes contain laminin, Type IV, collagen, and fibronectin. The extracellular matrix, as the main component of the basement membrane, is composed of macromolecules which are synthesized and secreted into extracellular space. Fibronectin binds the basement membrane to the surrounding tissues and the extracellular matrix, suggesting its role in maintaining BBB functions. The basement membrane can be degraded by matrix metalloproteinases (MMPs). Degradation of basement membrane components can increase the permeability of BBB, causing edema, hemorrhage, or even death.[8,27]

The clinical significance of studying blood–brain barrier damages

The current research has shown that pathological BBB destruction is closely related to various diseases, including acute and chronic cerebral ischemia, brain trauma, multiple sclerosis, brain tumors, epilepsy, and

dementia.[28] Here, we focus on the relationship between ischemic stroke and BBB injury. So far, thrombolysis and endovascular treatment are the only two FDA-approved effective therapies for the acute ischemic stroke. However, both of the procedures are associated with increased ICH.[29] Therefore, according to the American Heart Association/American Stroke Association guidelines, the patients can receive thrombolysis only within 4.5 h of stroke onset and/or receive endovascular treatment within 6 h of stroke onset.[30] The latest clinical research of DAWN (diffusion-weighted imaging or computerized tomography perfusion assessment with clinical mismatch in the triage of wake up and late presenting strokes undergoing neurointervention) has shown that the patients, who have a 6–24 h stroke with a mismatch between clinical deficits and infarct, could benefit from the thrombectomy treatment.[31] Moreover, a DEFUSE3 (Endovascular Therapy Following Imaging Evaluation for Ischemic Stroke phase III) study has demonstrated that thrombectomy improves the outcome for the patient who has a 6–16 h ischemic stroke with a region of ischemic tissue but not yet infarcted.[32] Both of these latest studies have greatly extended the time window for treatment of recanalization for certain subgroups of stroke patients. It suggests that the "one-size-fits-all" time window may not be the best rule to select the candidates for recanalization treatment. Thus, it is urgent and critical to find reliable indicators to include currently "noneligible patients" for recanalization, allowing more stroke patients to benefit from the treatment.

The relationship between blood–brain barrier injury and cerebral ischemia-induced brain damage

Ischemic stroke causes endothelial cells damage, leading to the release of free radicals, cytokines, and other proteases. Then, the accumulation of free radicals and inflammatory reactions activates MMP, leading to tight junction proteins degradation and causing BBB disruption.[33,34] Destruction of the BBB leads to the nonselective access of multiple inflammatory factors, proteases and various ions into the brain parenchyma, resulting in disruption of brain tissue environment and producing a series of secondary chemical reactions, such as energy failure, ion imbalance, acidosis, intracellular calcium overload, neuronal excitability, free radical-mediated lipid oxidation, inflammatory cell infiltration, and glial activation. These events eventually lead to brain edema, cerebral hemorrhage, and neuronal apoptosis and necrosis.[6,33]

Biomarkers of blood–brain barrier damage

An ideal biomarker capable of responding to BBB injury should have the following characteristics: (1) high specificity, (2) high sensitivity, (3) high reliability, (4) easy and fast assessment, and (5) minimally invasive. So far, many biomarkers have been reported to indicate BBB damage; however, none of them meets all the characteristics for an ideal biomarker. Each of these reported biomarkers has its advantages and disadvantages. These biomarkers are summarized in Table 1.

Blood–brain barrier structural proteins

BBB structural protein degradation is the first step of BBB destruction. Following BBB damage, the degraded proteins are released into blood circulation. Thus, measuring the BBB structural proteins in blood may reflect the extent of BBB damage.

Occludin

Occludin is composed of 504 amino acids with a relative molecular weight of 64,000.[35] Occludin is an integral membrane protein localized at tight junction.[36] Clinical studies have shown that occludin levels in the serum of the patients with poststroke hemorrhage are significantly higher than those without hemorrhage, suggesting occludin has the potential to be used as a biomarker for predicting the risk of hemorrhage after cerebral ischemia.[36] Liu *et al.* have demonstrated that MMP-2 induces rapid degradation of occludin in the cerebral microvasculature during the early stage of ischemic stroke and causes the destruction of BBB *in vitro* and *in vivo*.[65] Our recent results, obtained in animal models and pilot clinical studies, show that cerebral ischemia/reperfusion-induced degradation of occludin in the microvascular endothelium causes increased BBB permeability. In blood samples of stroke animals, blood occludin level increases sharply at 4.5 h after ischemic stroke onset and remains at a highly elevation compared to their basal levels. These findings indicate that serum occludin may be a clinically feasible biomarker for assessing early BBB injury after ischemic stroke.[37,38] Importantly, the blood occludin level reflects BBB damage with high specificity and sensitivity. Moreover, it is easy to access blood with low invasiveness. Although it is still in the experimental stage, it has significantly high potential to be used in clinical diagnosis in the future.

Cellular fibronectin

Cellular fibronectin (c-Fn) is a dimer composed of two 250-kDa subunits linked by two c-terminal disulfide bonds.[66] c-Fn is an essential component of the basement membrane, which is synthesized and secreted by endothelial cells. When the basement membrane is disrupted, c-Fn is released into the plasma, leading to the movement of polymorphonuclear leukocytes to the vascular injury site.[39] Since c-Fn is primarily localized in vascular endothelial cells, an elevation of this molecule in

Table 1: Biomarkers of BBB damage following cerebral ischemia

Biomarker	Description	Major findings	Advantages	Disadvantages	Reference numbers
Occludin	An integral membrane protein localized at tight junction	Blood occludin level increases sharply at 4.5 h after ischemic stroke onset and remains at a highly elevation compared to their basal levels	A prospective marker to predict BBB damage with high specificity and sensitivity, ease, low cost, quantifiable	Single time point cannot be reflected immediately	[34-38]
c-Fn	Major component of extracellular matrix	Plasma c-Fn level is elevated in patients with hemorrhagic transformation after acute ischemic stroke	High sensitivity, ease, low cost, quantifiable	Single time point, cannot be reflected immediately, poor specificity	[39-43]
MMPs	Calcium-dependent proteolytic enzyme, involved in degradation of basal lamina, and extracellular matrix	MMP-9 concentration is independently associated with BBB damage; a potential biomarker for predicting the risk of ICH	High sensitivity, ease, low cost, quantifiable	Single time point, cannot be reflected immediately, poor specificity	[44-48]
cBMECs	The major structural components of the BBB	Measuring the amount of cBMECs in the blood may reflect the BBB damage level	High specificity, ease, low cost	Single time point, cannot be reflected immediately, poor sensitivity, not a good candidate as a biomarker for BBB damage	[49,50]
S100B	Homodimeric glial protein that regulates intracellular calcium levels, also a marker of BBB	S100B can be detected in peripheral blood after BBB injury and its concentration is related to the extent of BBB opening	High sensitivity, ease, low cost, quantifiable	Single time point, cannot be reflected immediately, not a specific biomarker for BBB damage after ischemic stroke	[51-55]
UCH-L1	Expressed in neurons and neuroendocrine cells in vertebrates and widely distributed in the nervous system BBB dysfunction	UCH-L1 level increases in CSF and blood circulation; Serum UCH-L1 concentration is associated with abnormal BBB status 12 h after moderate-to-severe brain injury	Ease, low cost, quantifiable	The specificity and sensitivity might be too low, Single time point	[56-58,75]
Albumin	After BBB disruption, albumin enters the CSF across the damaged BBB from blood plasma	CSF/serum albumin ratio has been used as a reliable parameter for assessing the impairment of BBB], including poststroke BBB injury	A reliable parameter, low cost, quantifiable, high specificity	Invasive test, CSF/serum albumin ratio hasn't been wildly used	[59-61]
HARM	Utilized DCE-MRI to observe a HARM	HARM was the most influential factors predicting early BBB injury	A sensitive and low invasive method	Expensive and time-consuming, not realistic within the limited time window before treatment; not widely used	[62-64]

BBB: Blood–brain barrier, c-Fn: Cellular fibronectin, MMPs: Matrix metalloproteinases, cBMECs: Circulating brain microvascular endothelial cells, S100B: S100 calcium-binding protein B, UCH-L1: Ubiquitin carboxyl-terminal hydrolase isozyme L1, HARM: High-intensity acute reperfusion marker, DCE-MRI: Dynamic contrast-enhanced magnetic resonance imaging, CSF: Cerebrospinal fluid

plasma may indicate endothelial damage. A clinical study has shown that plasma c-Fn level is elevated in patients with hemorrhagic transformation after acute ischemic stroke.[40] Additional evidence indicates that serum c-Fn level is highly associated with malignant middle cerebral artery infarction.[41] Therefore, high serum c-Fn may reflect the BBB injury and hemorrhagic transformation with high sensitivity. However, plasma levels of c-Fn are also increased in patients with secondary vascular lesions, such as vasculitis, sepsis, major acute trauma, diabetes, and ischemic stroke.[42,43] Thus, c-Fn may not be a highly specific indicator of BBB damage.

Matrix metalloproteinases

MMPs are a family of endogenous proteolytic enzymes that degrade almost all extracellular matrixes. Currently, MMP-9 is considered to be closely related to BBB disruption. Using the specific inhibitor metallopeptidase inhibitor 1 to block MMP-9 *in vivo*, the researchers demonstrate that MMP-9 is responsible for the degradation of Type IV collagen, layer proteins, and fibrin, which are the major components of the basement membrane.[44,45] Disruption of the basement membrane is the primary cause of cerebral edema and hemorrhagic transformation.[46] In addition, several studies have shown that MMP-9 causes degradation of tight

junction proteins, such as occludin, claudin-1, and ZO-1.[67] Since the degradation of tight junction proteins leads to loss of BBB integrity,[19,47] MMP-9 is closely related to the destruction of BBB. A recent clinical study has revealed that MMP-9 concentration is independently associated with BBB damage, indicating MMP-9 may be a potential biomarker for predicting the risk of ICH after thrombolysis.[48] However, the specificity is poor since MMP-9 is also increased in a variety of diseases, such as cancer, heart diseases, diabetes, epilepsy, and neurodegenerative diseases.[68-71]

Circulating blood–brain microvascular endothelium cells
Brain microvascular endothelial cells (BMECs) are the major structural components of the BBB. BBB damage causes a dynamical exfoliation of BMEC. The exfoliated BMECs flux into the peripheral blood and become circulating blood BMECs (cBMECs).[49] Huang *et al*. have reported a correlation of BMECs and BBB disorders.[50] Therefore, measuring the amount of cBMECs in the blood may reflect the BBB damage level. However, the sensitivity of measuring cBMECs to assess BBB damage is relatively low, and the detection process is cumbersome. Thus, cBMECs is currently not a good candidate as a biomarker for BBB damage.

Other proteins in circulation
The highly selective semi-permeability of BBB separates proteins in the brain parenchyma from circulation. However, when BBB is disrupted, the brain parenchyma proteins can flux into blood circulation. Thus, the detection of specific brain parenchyma proteins in blood circulation should reflect BBB disruption.

S100 calcium-binding protein B (S100B)
S100 proteins are a family of acidic calcium-binding proteins. There are at least 21 different S100 proteins.[51] S100B is expressed primarily by mature astrocytes. In physiological conditions, S100B cannot across BBB. However, when BBB is extensively damaged, S100B can be released into the blood circulation through the compromised BBB. Clinical studies have found that S100B can be detected in the peripheral blood after BBB injury, and its concentration is related to the extent of BBB opening.[52,53] Clinical and initial studies have demonstrated that blood S100B level is correlated with BBB damage level, suggesting that S100B may serve as a biomarker of BBB injury.[54] However, elevated S100B levels have been found not only when BBB permeability increases due to ischemic stroke but also when trauma occurs to the head.[55] Thus, S100B is not a specific biomarker for BBB damage after ischemic stroke.

Ubiquitin carboxyl-terminal hydrolase isozyme L1
Ubiquitin carboxyl-terminal hydrolase isozyme L1 (UCH-L1), also known as protein gene product 9.5 (PGP9.5) and PARK5, is discovered in 1987,[56] with a molecular weight proximately 24.8 kDa.[72] UCH-L1 is abundant in the brain parenchyma; approximately 2% of soluble proteins in the brain parenchyma are UCH-L1.[57] UCH-L1 is expressed in neurons and neuroendocrine cells in vertebrates and widely distributed in the nervous system.[58,73,74] UCH-L1 level increases in the cerebrospinal fluid (CSF) and blood circulation following traumatic brain injury and ischemic stroke. However, according to BJ Blyth's research,[75] serum UCH-L1 concentration is associated with abnormal BBB status 12 h after moderate-to-severe brain injury but not in mild brain injury. Based on these studies, both the specificity and sensitivity of UCH-L1 might be too low to be a reliable indicator of early BBB damage after ischemic stroke.

Plasma albumin
Albumins are commonly found in blood plasma, and the amount of albumin in CSF is negligible under the normal physiological conditions. However, after BBB disruption, albumin enters the CSF across the damaged BBB from blood plasma. Thus, the CSF/serum albumin ratio has been used as a reliable parameter for assessing the impairment of BBB,[59] including poststroke BBB injury.[60,61] However, to acquire the CSF/serum albumin ratio, it requires obtaining both blood and CSF sample. The clinical acquisition of CSF through lumbar puncture is invasive. Thus, assessing CSF/serum albumin ratio to assess BBB damage hasn't been wildly used.

Brain imaging assessment
Besides molecular and cellular markers, imaging techniques are wildly used to assess BBB injury in the clinic. The most widely used brain imaging assessment is magnetic resonance imaging (MRI), especially T1-weighted dynamic contrast-enhanced MRI (DCE-MRI).[62] DCE-MRI is a sensitive and low invasive method to quantify the functional integrity of the BBB.[63] Hjort's study has shown that parenchymal enhancement (PE) is measurable by T1-weighted MRI as early as 2 h after thrombolytic therapy. All the patients, who have PE, showed ICH subsequently.[76] Warach *et al*. utilized DCE-MRI to observe a high-intensity acute reperfusion marker (HARM) and found that HARM was the most influential factors predicting early BBB injury.[64] However, MRI examination is expensive and time-consuming, and not all hospitals are equipped with MRI machines. In addition, contrast agent is required to obtain the BBB leakage signal by MRI. BBB leakage signal is only visible postthrombolysis treatment. Thus, using MRI to assess BBB damage before thrombolysis treatment to predict the risk of ICH after thrombolysis is not realistic within the limited time window before treatment.

Conclusions
This systematic review has highlighted the biomarkers that have been developed to assess BBB damage
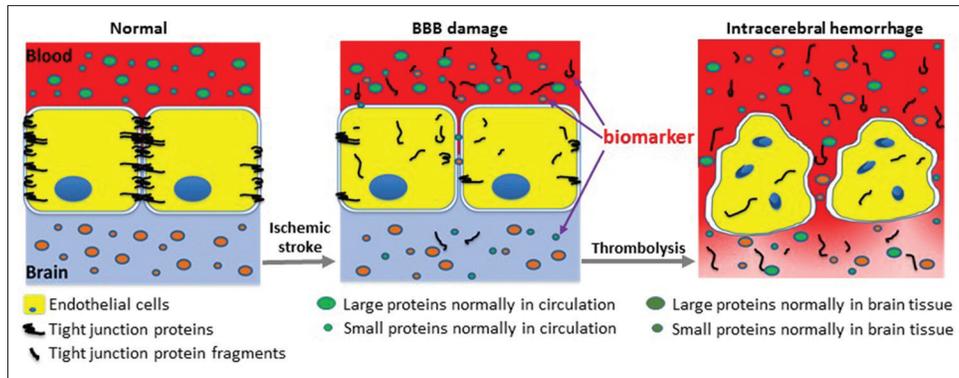
Figure 1: Schematic diagram for the structure of blood–brain barrier and the generation of biomarkers for blood–brain barrier damage and development of symptomatic intracerebral hemorrhage following thrombolytic therapy for acute ischemic stroke

following the acute ischemic stroke. The development of these biomarkers is based on the destruction of barrier functions of BBB [Figure 1]. With further understanding of the mechanism of BBB damage, it has been observed that the degradation of BBB structural proteins is directly related to compromised BBB integrity. These proteins have a high potential to become sensitive and specific biomarkers for BBB damage. However, because of the complexity and heterogeneity of the ischemic stroke, adequate clinical information and data are needed to verify the feasibility and reliability of these potential biomarkers.

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Conflicts of interest

There are no conflicts of interest.

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