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Progress and potential roles blood biomarkers of ischemic stroke in clinical setting

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ABSTRACT
Stroke is one of the leading causes of death and disability which involving a complex pathophysiology with multiple mechanisms. Rapid treatment is necessary to terminate the disease progression, hence minimizing CNS damage and subsequent disability. Stroke diagnostic process composed of history taking, neurological examination and supplemented with neuroimaging. Imaging modalities such as CT-scan or MRI are essential in establishing a definitive diagnosis of ischemic stroke. However, the high cost and limited number made them inaccessible for those who have low or middle income which will delay the diagnosis and treatment. On the other hand, blood biomarker has potential in either diagnostic or prognostic aspect of ischaemic stroke management. It has a promising potential to aid diagnosis, determine the subtype of stroke, predicting the outcome or early neurological deterioration, and recurrence. It also could potentially help to assess the risk of hemorrhagic transformation, treatment selection, as well as to detect salvageable ischemic penumbra. Although it could not replace neuroimaging, blood-based biomarker assessment had lower cost and faster result. However, despite its promising potential, none of the blood biomarkers is currently used in clinical practice. Therefore, further studies are needed to develop biomarkers or panels of biomarkers with better sensitivity and specificity. This review provides a highlight and summary of blood biomarkers based on their potential application in a clinical setting.

Keywords: ischemic stroke, blood biomarker, hemorrhagic, outcome

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INTRODUCTION

Stroke is the third leading cause of death and remained a major cause of disability.[1–3] It is a devastating disease with almost one-third of stroke patient die or experience disability within the first month.[4] Also, due to pathophysiologic nature of the disease with a shared risk factor such as atherosclerosis. Occlusion due to atherosclerosis in cerebral arteries is merely a symptomatic representation of the total burden of disease in the systemic arterial circulation. The result is that most stroke patients die of coronary artery disease and many also suffer from intermittent claudication from peripheral arterial disease.[5,6] Although mortality rate is declining in over the years, it is still a highly significant global health burden.[1]Due to its high incident and limited treatment options, stroke is one of the primary focus in biomedical research nowadays.

Stroke is a heterogeneous disease with complex pathophysiology involving multiple mechanisms. However, it can differentiate into a few clinical entities.[7] Stroke itself defined by WHO as "rapidly developing clinical signs of focal cerebral function lasting more than 24 hours with no apparent cause other than the vascular origin.[8] While cerebrovascular disease with duration less than 24 hours and with complete neurological recovery it is termed transient ischemic attack (TIA).[1] There are two main types of strokes, ischemic and hemorrhagic strokes. Ischemic strokes are related to reduced cerebral blood flow most commonly caused by arterial occlusion from thrombus or embolus. Hypo perfusion from decreased blood pressure or systemic hypoxia may also cause ischemic strokes.[1]Haemorhagic strokes are caused by rupture of an artery or vein and leaking of blood into the central nervous system tissue. Haemorrhagic strokes also can cause mass effect due to hematoma from the accumulation of leaked blood, and produce a direct pressure effect on the CNS tissue. Epidemiologically ischemic strokes contributes 85-87% of the cases, and it is one of the most common causes of disability worldwide.[9] While hemorrhagic strokes include intracerebral hemorrhage, and subarachnoid hemorrhage constitute the rest.[10]

Today's diagnostic method for assessing stroke and its purpose are excellent.[11] Current

diagnostic method to establish the diagnosis is history, physical examination, and biomarker study. A biomarker may be a substance/molecule measured/detected in blood, cerebrospinal fluid, or tissue, may be a recording such as ECG, EEG or an imaging test. An imaging test such as CT scan or MRI is essential in establishing a diagnosis of stroke.[11]Although other biomarkers such as blood biomarker are usually not considered important parts of the diagnostic process; it may have an additional function or benefit in stroke management.

Blood biomarkers to be applicable in clinical practice ideally needs to fulfill some criteria. It requires sufficient sensitivity and specificity, early and stable release, predictable clearance, the ability to measure rapidly and cost effective quantitatively.[10,11] Much of the current reference thought to be optimal biomarker are troponin's role in myocardial infarction.[11] Troponin and creatine kinase significantly improved diagnostics of myocardial infarction at admission in the emergency room and aided timely proper treatment. The much effort is needed to bring those same optimal blood-based biomarkers to clinical practice in stroke settings.

Most of the biomarker that has studied related to the pathophysiology of ischemic stroke such as excitotoxicity, oxidative stress, inflammation, blood-brain barrier dysfunction, apoptosis, coagulation and or thrombosis.[10,11] These biomarkers indicate the dynamic process occurring in brain and potentially complement current clinical modality in diagnosis, differentiate stroke from stroke mimics, determine etiology, prediction of stroke severity, prediction of outcome and guide appropriate treatment.[10–12]

Although many molecules have been studied and have shown promising potential, none of it currently used in routine clinical practice. It needs more extensive research before conveniently used in clinical setting. In the presence, the primary objectives of this review are to provide a summary of blood biomarkers based its potential roles in a clinical setting. This review will focus on blood biomarker related to ischemic strokes. Further reference about biomarker of hemorrhagic strokes are subject in another review.[13]

BIOMARKERS AID UNESTABLISH DIAGNOSIS

One major limitation in making an early decision on stroke management is a lack of rapid diagnostic testing.[4]Although diagnosis of ischemic stroke even in the hyperacute stage can establish with MRI-DWI (diffusion weighted imaging) with very high sensitivity and specificity; it is still not available in the most hospital.[14] This condition frequently results in a delay of treatment. Establishing diagnosis of stroke type early in the acute setting is likely to increase the number of patients receiving proper intervention in early of the disease process.[1] Intervention such as receiving thrombolytic therapy may improve overall outcome, and reduce overall health care cost. A breakthrough in new tools or method to established ischemic stroke diagnosis rapidly are necessary to reduce the time between onset and administration of effective therapy.[12]Exploration of a molecule related to stroke pathophysiology has uncovered the potential of blood biomarker to complement or even as an alternative to the present neuroimaging modalities. The benefit are significant especially in patients with localizing or transient neurological symptoms, whom neuroimaging cannot obtain or not available or those who are non-dignostic.[1]

One potential marker for establishing the diagnosis of stroke are S100 calcium-binding protein (S100B). S100B is a calcium-binding protein found primarily in mature astrocytes and NG2 cells.[15] S100B in normal condition located predominantly in intracellular and act in Ca^{2+} homeostasis, glutamate uptake in astrocytes, neurite outgrowth stimulation. Following injuries to the CNS tissue with the involvement of glutamate excitotoxicity, this protein can be released and act in complex roles in astrocytic differentiation, neuronal survival, or cell death. Detection of S100B in serum is specific for central nervous injury although it is not specifically indicating a stroke since it also released in traumatic brain injury (TBI) and ICH.[16,17] Several studies has reported that levels of S100B are higher in stroke patients compared to healthy control, and the level also significantly higher in ischemic stroke as compared to ICH or TIA.[1] It also has different temporal profile with TBI. S100B increase in 1 or 2 days following TBI, and between 2and 4 days after stroke with peak at 48 hours after symptoms onset.[1,18]

Diagnostics values of the single marker are limited, but it shows potential when combined as part of biomarkers panel for stroke.[19]Although some contradicting result are present. One study evaluates diagnostic quality of panel consisting of 4 biomarker includes Brain Natriuretic Peptide (BNP), D-Dimers (DD), Matrix-Metalloproteinase-9 (MMP9) and S100B protein yield low sensitivity and specificity. Moreover, it fails to demonstrate a correlation between combined parameter with lesion size measured with DWI-MRI.[20] Therefore more research is warranted to explore the

diagnostic quality of another potential blood biomarker or panel consisting of its combination.

BIOMARKER IN DETERMINING ETIOLOGY AND SUBTYPE

Stroke is a heterogeneous disease, with a wide range of possible etiology. A clot formed in ischemic stroke may arise from atherosclerotic large cerebral arteries (e.g., carotid, middle cerebral, or basilar arteries), or atherosclerosis small cerebral arteries (e.g., lenticula striate, basilar penetrating and medullary arteries) or may also originated from cardioembolic.[21] Therefore ischemic stroke commonly subdivided based on TOAST classification system into five etiological categories: large artery atherosclerosis, small vessel occlusion (lacunar), cardioembolism, a stroke of other determined etiology consisting rare type of stroke and stroke of undetermined etiology.[22]In most cases, atherothrombotic of artery or embolism from the heart is the cause of strokes.[10]In clinical practice, it is not always possible to identify specific cause of strokes, with the fact most registers failed to determine in 25-39% of strokes patients.[21]

Before considering therapeutics decision, the physician must determine whether the stroke is ischemic or hemorrhagic. Nowadays, stroke subtype is defined mainly by imaging data. However, availability of CT-scan still limited in the hospital. Future use of blood biomarker to differentiate between ischemic or hemorrhagic might speed up diagnosis, so proper acute treatment can be initiated even if the patient still in the prehospital setting.[9]The feasibility of the idea supported by clinical trial regarding the application of stroke emergency mobile ambulance equipped with advanced equipment such as portable CT-scan, laboratory, telemedicine can initiate earlier thrombolysis in ischemic stroke cases without increased risk for complication.[23] However; its implementation limited due to expensive and complicated equipment. In the future, a combination of portable blood biomarker as rapid diagnostic tools and early thrombolytic therapy in prehospital setting may increase the number of a patient receiving earlier treatment.

Glial fibrillary acidic protein has been reported to have predictive value to differentiate Intracerebral hemorrhage (ICH) from ischemic stroke in the setting of acute hemispheric stroke.[24] GFAP is a structural protein unique to astrocytes and lesser extent in ependymal cells. GFAP are released when cell disintegrated; the cytoskeleton degraded when neurological injury affecting astrocytes occur.[10]Levels of GFAP were higher in ICH than in a patient with ischemic stroke or stroke mimics. In its optimal cutoff, GFAP provided a sensitivity of 84.2%, the sensitivity of 96,3%.[24]Another study combination of GFAP and RBP4 (Retinol Binding Protein 4) result in dramatic increase in specificity to 100% in differentiating ICH and ischemic stroke patient during the first hour after onset. This combination yields 100% PPV, which means 0% false positive. Specificity of 100% is preferred than 100% sensitivity in context of prehospital or hyper acute setting as wrong thrombolytic treatment in ICH patient cause more harm than no treatment.[9]

Differentiate among stroke subtypes by blood biomarker seems a more difficult task to tackle. Many efforts have been conducted to differentiate subtypes based on TOAST classification, but most end with differentiation of cardioembolic from a non-cardioembolic stroke. Study biomarkers by Montaner et al. to find a biomarker which provides precise information about etiology tested several biomarkers include CRP, D-dimer, Soluble Receptor for Advanced End Glycation Product (sRAGE), Matrix metalloproteinase 9, S100B, brain natriuretic peptide (BNP), Neurotrophin 3, caspase 3, chimeric and secretagogin. Only BNP, D-dimer and sRAGE show different level (higher) in one subtype, which is cardioembolic. No significant difference of candidate biomarker among other subtypes. Within the optimal cut-off value determined from AUC curve, BNP provides a sensitivity of 72%, specificity of 68% while DD provide sensitivity 56%, specificity 64% to predict cardioembolic stroke. When BNP and DD combined, it's sensitivity increase to 87% and specificity increase to 85%.[25] Another study focus in D-dimer for the same purpose yield higher sensitivity (83,7%) and specificity (81,5%) value with a different cut-off point.[26] The difference may cause by higher levels of D-dimer in a cardioembolic patient in the later study.

BIOMARKER IN SEVERITY AND INFARCT VOLUME

Damage in CNS tissue from ischemic brain leading to release specific biomarkers from a neuron or glial cells. Some molecule has been identified and potentially be a good biomarker for severity of brain injury. Some mentioned molecule include brain-specific protein such as calcium-binding protein (S100β), glial fibrillary acidic protein (GFAP), myelin basic protein (MBP) and neuron-specific enolase (NSE).[1,10] Glutamate and Interleukin-6 in plasma at admission also reported as having significant correlation with infarct volume in patient with acute hemispheric infarction.[11]

S100β and NSE are a protein which thought to be a good indicator of infarct size. Both proteins are very low in normal condition, and when there is ischemic injury, they released into the cerebrospinal fluid (CSF) and blood through BBB, resulting in significantly increased levels.[10] Another CNS injury biomarkers are GFAP and MBP.MBP is hydrophilic protein contribute to correct structure of myelin sheath. When there are damage in CNS cells and myelin, GFAP and MBP released into CSF and blood, and their levels correlated to with the degree of injury.[10]

BIOMARKER TO PREDICT OUTCOME

Prediction patient risk for disability and mortality has important value used to inform the clinical decision, evaluate risk-benefit and optimize resource utilization. Many clinical risk scores has developed designed for predicting outcome, but it is infrequently used by clinician because its complexity, lack precision, and validation.[4]

Neuron-specific enolase, an isoform of glycolytic enzyme enolase found mainly in neuron have been reported as a predictor of neurological outcome. NSE is a specific marker for neuronal cells, except it also found in some neuroendocrine carcinoma. It assumed that being an enzyme in the cellular cytoplasm with the negligible amount of peripheral blood, elevated NSE are the result of cell destruction. Parallel to cell destruction, blood brain barrier compromised by endothelial cell death and astroglial disintegration. Hence cytosolic content has potential to cross blood brain barrier. Serum levels of NSE would be expected to rise as long as damage due to the infarction continued, and NSE leaked out of brain tissue.[2]

Few studies had reported NSE association with some neurological disorder related to brain injury including ischemic stroke and traumatic brain injury.[1] NSE elevation in first 24 hours only occurs in a small fraction of a stroke patient. Therefore it is not suitable for acute diagnostic. Meanwhile, elevated levels at 72 hours significantly correlate with worse neurological outcomes and highly predictive for determining stroke severity.[1,2,27] NSE level also positively correlated with severity of stroke at admission (r=0,919; P<0,001). Significant correlation also demonstrated between NSE level with neurological worsening after seven days of admission (r=7,06; P<0.001).[2]

A study by conducted by Berrocosa et al., describe the proteome changes in the human brain after stroke yield some biomarker candidate.[28] Advance in proteomic technology has allowed the researcher to identify previously undetected protein within the ischemic brain. From 51 identified protein in the ischemic brain, eight proteins then investigated as blood biomarker. Three of 8 protein tested shown association with poor outcome at three months. Three protein described were gelsolin, dihydropyrimidinase-related protein 2 (DRP2) and cystatin. Gelsolin is a protein which binds actin in a calcium-dependent manner to regulate the dynamics of actin polymerization within a cell or scavenge actin leaking into the blood stream after tissue injury.[29] Cystatin or stefin A is a cytoplasmic inhibitor to regulate cysteine proteinase involved in lysosomal death pathway. Cystatin able to stabilize and prevent degradation of matrix metalloproteinase, which is a notorious component in ischemic stroke.[28] Dihydropyrimidinase-Related Protein 2 mainly associated with regulation with microtubule dynamic, endocytosis in axogenesis.[30] In contrast with the other two, only DRP2 reduced in ischemic condition.[28]

All three-protein showed strong predictive value. Predictive value of each protein was Gelsolin (71.8 sensitivity, 76.5 specificities), Dihydropyrimidinase-related protein 2 (97.1% sensitivity, 28,6 specificity) and Cystatin (26.8 sensitivity, 100% specificity). All three remain independent predictor after multivariate analysis to adjust gender and NIHSS score. High cystatin also significantly associated with in-hospital mortality (62.5% sensitivity, 96% specificity). The combination of three biomarkers significantly enhanced the poor outcome discrimination of the model up to a 28% when compared to clinical model only. Moreover, additional use CYTA in model to predict in-hospital mortality enhanced the discrimination by 34%.[28]

Recent of the gene expression profiling report a gene encodes mast-cell expressed membrane protein 1 (MCEMP1) could provide a prognostic biomarker for stroke.[4] Mast cell-expressed membrane protein 1 is a transmembrane protein expressed by mast cell, macrophages, and other tissue. Its exact function is yet to be determined, but from the analysis of the gene composition, it shares similarity with many immune receptor genes.[31] MCEMP1 on average was 2,4-fold higher in patients with stroke than in healthy controls. MCEMP1 also 2,1-fold greater in a patient with intracerebral hemorrhage than those with ischemic stroke. Higher levels also associated with the higher score on *modified Rankin scale* at admission. Additionally, the expression levels correlated with disability and mortality within one month after stroke. Elevated levels MCEMP1 is associated with a disability with OR 6,6 (95%CI, 1,9-22,7). With the optimal threshold, MCEMP1 has

sensitivity of 86,2%, specificity of 80,3%, with corresponding PPV 78,1% and NPV 87,7% for disability.[4]

High levels of MCEMP1 also associated with mortality within one month with OR 20,7 (95%CI, 2.5-174,6) compared with low levels. After selecting its optimal threshold, MCEMP1 had a sensitivity of 35,1%, specificity of 97,8%, with corresponding PPV of 86,7% and NPV of 78,9%.[4]

BIOMARKER TO PREDICT EARLYNEUROLOICAL DETERIORATION

Early neurological deterioration defined as the clinical worsening or recurrence during the first 72 hours after ischemic stroke.[32] END is a common complication of acute stroke with 20-40% occurrence.[33]END has a serious complication, with the worse functional outcome at three months.[34]Predicting early deterioration is still controversial.[35]Some predictor has been known, including initial stroke severity, history of diabetes, atrial fibrillation, large vessel occlusion, hypo density in >33% of Middle cerebral artery territory, cerebral edema on early CT scan, and hyper dense MCA sign in brain CT scan.[32]The aim of blood biomarker development for END is to provide readily assessed marker in emergency department to identify high-risk stroke patient who may benefit from intensive care and obersevation.[35]

Early clinical and experimental data suggest glutamate, GABA, ferritin, nitric oxide (NO), Interleukin-6 as a biochemical mediator and predictor of early neurological deterioration.[12]Among these, glutamate is the strongest predictor of progressing stroke. Baseline CSF and blood levels of glutamate in have been reported significantly higher in END patient. A study in lacunar stroke has shown the positive predictive value of glutamate >200 µmol/L and GABA < 240 nmol/L are 67 and 84% respectively.[36] Involvement of glutamate in END seems to relate to its participation in peri-infarct spreading depression depolarization in an area with deprived energy as result of low cerebral blood flow. This event lead to increased infarct volume and neurological deterioration.[12]

The role of ferritin and nitric oxide in progressing stroke come from a study of oxidative stress involvement in stroke pathophysiology. Ferritin levels higher than 275 ng/ml independently predicted END in a patient with acute hemispheric infarction. Ferritin association with END suggest the involvement of iron-mediated excitotoxic and inflammatory mechanism in the evolution of stroke.[37]NO also play roles in oxidative stress especially in its involvement in the generation of free radicals.[12]NO levels in CSF was reported higher in a patient with progressing stroke. However, application of NO in blood biomarker seems not feasible due to technical difficulties of its measurement and not specific from stroke pathology.[38]

Recent research in fluorescent molecular peroxidation products (FMPP) report an association with early neurological deterioration.[39]FMPP are reactive oxygen species-mediated lipid and protein polymerization. It is considered as a nonspecific measurement of molecular oxidation because it reflects a mixture of lipid, protein carbohydrates and DNA peroxidation product.[40] FMPP has been found to increase in disease with major involvement of oxidative stress process. According to the study, baseline FMPP levels were higher in patients with worsened condition at 48 hours after admission. With the optimal threshold level, FMPP able to predict patient with neurological worsening at 48 hours with a sensitivity of 80% and specificity of 57,9%.[39]

Participation of inflammatory molecules such as IL-6 and TNF-α was reported to facilitate leukocyte migration and adherence from capillaries into CNS tissue and subsequent occlusion of micro vessels and reduction of already compromised blood flow. Increased infiltrating inflammatory cell to CNS tissue also worsen damage by the production of ROS, release elastase, MMP, and other inflammatory mediators exacerbate ischemic brain injury further.[41]This condition cause cell death and enlargement of ischemic damage. The evidence comes from the association of high concentration IL-6 in plasma (>21.5 pg/ml) and CSF (>6.3 pg/ml) with larger infarct volume, and neurological deterioration. Whereas high plasma TNF-α (>14 pg/ml) associated with END and poor outcome after three months.[42]

Research from the routine measurement of blood parameters of stroke patient revealed an association between the ratio of blood urea nitrogen/creatinine with neurological worsening after admission.[35] BUN/Cr ratio higher than 15 associated with stroke in evolution, and END.[35,43] Alternative interpretation of the BUN/Cr ratio is an indication of volume depletion or relative dehydration and increased risk of venous thromboembolism.[35,43,44] Hence, there is suspicion between of hydration status with early neurological deterioration mediated by venous thromboembolism.

BIOMARKERS TO AID SELECT SPECIFIC TREATMENT.

Acute stroke management focuses on stabilizing the patient and restoring cerebral blood flow as soon as possible to prevent further brain injury in the penumbral area.[1]It requires rapid assessment and early intervention to facilitate maximal reperfusion of brain tissue.[3] Thrombolytic therapy with recombinant tissue plasminogen activator, the only effective treatment approved by food and drug administration (FDA) has narrow therapeutic window (1,5-3 hours of symptom onset) and require assessment to rule out hemorrhagic strokes and assess risk of hemorrhagic complication.[1]

Arterial recanalization by intravenous tissue plasminogen activator (IV-tPA) is an important component in stroke treatment. Unfortunately, only less than half of the patient successfully achieved recanalization.[12] Some biomarkers have shown poor response to recanalization therapy. High level of plasminogen antigen inhibitor 1 (PAI-1 ≥ 34 ng/ml)have been shown to predict poor response to thrombolysis.[45]

Decompressive hemicraniectomy is another treatment for stroke which performed early in selected patients with large cortical ischemic infarcts.[46]This treatment reserved for life-threatening complication such as malignant middle cerebral artery infarction.[12]Biomarkers aid in selecting patients at risk of malignant cerebral infarction and who will benefit from such surgery would be very useful. Malignant cerebral infarction seems to involve lack integrity of the basal endothelial membrane secondary to released proteolytic enzyme.[12]Therefore molecule related to endothelial damage such as MMP-9 may provide useful information related to the occurrence of malignant cerebral infarction.

Study on matrix metalloproteinase-9 yields a confirmatory result. MMP-9 with a threshold level of> 140 ng/ml predict malignant cerebral infarction in middle cerebral artery occlusion with a sensitivity of 64% and specificity of 88%. Other biomarkers that have reported are S-100B and cellular fibronectin (c-Fn). S-100B with plasma level >0,35 µg/L can predict malignant infarction at 12 hours with 75% sensitivity and 80% specificity, and at 24 hours with 94% sensitivity and 83% specificity.[11] Cellular fibronectin measured at admission >16,6 µg/ml has even better prediction with 90% sensitivity, 100% specificity with corresponding PPV of 100% and NPV 90%.[47]

Another potential blood biomarker function to study is a biomarker to determine the stroke onset. It is helpful in deciding whether the patient is still in the time window for thrombolytic therapy or it has passed in case of stroke with unknown onset. The example is so-called “wake-up” strokes which occur during sleep. It is account for up to 25% of all strokes.[48,49] Two potential example for this function are MCEMP1 gene expression described previously. The MCEMP1 RNA Levels in peripheral blood decreased 1% for every hour passed from the symptom onset.[4]Another one example is the time-dependent increase of blood occluding with a sharp increase at after 3 to 4,5 hour after ischemic onset.[51] More about occluding discussed next. Currently study to determine stroke onset in wake stroke focus in neuroimaging, therefore more exploratory study needed in this blood biomarker category.

BIOMARKER OF HEMORRHAGIC TRANSFORMATION

Hemorrhagic transformation is a severe complication following ischemic stroke or r-spa treatment. The hemorrhagic transformation may occur as part of the natural evolution of the ischemic lesion, and the possibility is increased significantly by thrombolytic therapy.[12] Once ICH occurs following ischemia or r-tpa treatment, about 80% of the patient will die.[50]The increased risk of hemorrhagic transformation is the primary reason for withholding thrombolytics therapy in clinical practice.[51,52] Hence biomarkers aid in determining the risk for potential hemorrhagic complication and if possible justify the extension of the time window (>3 hours) for t-PA administration is critically important.[12,51] Many clinical characteristics (age, hypertension, anticoagulant use, hyperglycemia) and radiographical infarct volume, proximal occlusion, etc.) have been associated with an increased risk of hemorrhage after the institution of tissue plasminogen activator but biomarker related to the pathophysiology of hemorrhagic transformation might be more convincing at times of decision making.[11] Indeed, some molecule related to ischemic stroke pathophysiology including MMP-9, c-FN, PAI-1, TAFI, S100B and Occludin have been reported.[1,51]

MMP-9 involved in the destruction of microvascular integrity by degradation of the basal lamina and extracellular matrix, and it thought as the primary cause of hemorrhage after cerebral ischemia.[12,52,53] Administration t-PA itself activates MMP-9 and may promote hemorrhagic transformation.[54]Serum MMP-9 > 140 ng/ml independently predict hemorrhagic transformation is ischemic stroke patient who has and has not been treated with t-PA with a sensitivity of 87%, specificity of 90%, PPV of 61% and NPV of

97%.[55].Levels of MMP9 also shown association with blood-brain barrier disruption.[54]Another study in patients with cardioembolic stroke who received thrombolytic therapy, development of parenchymal hemorrhage can be predicted with sensitivity of 100%, specificity of 78%, PPV of 67% and NPV of 100% when the cut-off value is set to 191,3 ng/ml.[56]

Plasma level of cellular fibronectin has also been found to predict hemorrhagic transformation in ischemic stroke patients who receive r-tpa treatment. Cellular fibronectin (c-FN) is a factor synthesized by endothelial cells and is elevated following vascular injury. The concentration of c-Fn ≥ 3,6 µ/ml predicts the development of hemorrhagic transformation after t-PA administration with a sensitivity of 100%, specificity of 96%, PPV of 44% and NPV of 100%. MMP9 with concentration ≥ 140 ng/ml for the same type of hemorrhagic transformation yield slightly lower accuracy with a sensitivity of 81%, specificity of 88%, PPV of 41% and NPV of 98%.[57] The results may partly explain by the fact that c-Fn mainly comes from vascular endothelium, and it is likely that levels of this protein provide more accurate reflection of endothelial disruption contributing to hemorrhagic transformation.[12]

Another biomarker has been studied for hemorrhagic transformation are S100B, Plasminogen Activator Inhibitor-1 (PAI-1) and thrombin-activated fibrinolysis inhibitor (TAFI). S100B > 0,23 µg/L is associated with an increased risk of hemorrhagic transformation in ischemic stroke with lower sensitivity (75%) and specificity (97,6%) than c-FN and MMP-9.[58] Another study investigate S100B as predictor clinical deterioration caused by hemorrhagic transformation yield sensitivity of 92,9%, specificity 48,1%, PPV of 12,2% and NPV of 98,9%.[59] PAI-1 and TAFI are an endogenous fibrinolytic inhibitor that involved in hemorrhagic transformation pathogenesis. The plasma levels of PAI-1 were shown to be significantly lower, while TAFI were significantly higher in patients with symptomatic hemorrhagic transformation after r-tpa treatment. When PAI-1 (>180%) and TAFI (<21,4 ng/ml) combined, it can predict the development of symptomatic hemorrhagic transformation after t-PA treatment with sensitivity of 75%, specificity of 97,6%, PPV of 75% and NPV of 97,6%.[60]Hence the result of the combination support the idea of biomarker may use in combination to increase it prediction accuracy.

Occluding is the latest biomarker reported from an animal study in relation with hemorrhagic transformation.[51] Occluding is a structural component of tight junction protein in cerebro-microvessels to seal the blood brain barrier. As a structural protein, the basal level of occluding is low and released to blood when tight junction in the micro vessels undergo degradation possibly by the MMP-2.[51,61] Ischemic condition in infarct core and penumbra area induced a time-dependent increase of blood occluding with a sharp increase at after 3 to 4,5 hours after ischemic onset. The massive increase in peripheral blood occludin levels concurrent with loss occluding protein from cerebral micro vessels and correlated with massive blood brain barrier leakage (R=0,77, p≤0,05).[51] The result of the study suggests that pretreatment blood occluding levels may serve as a reliable biomarker to identify acute stroke patients at high risk of ICH. Future animal and clinical studies are warranted to confirm the result.

BIOMARKERS FOR RECURRENT STROKE

Prediction of early recurrent stroke after a transient ischemic attack (TIA) or stroke would be clinically useful especially to help to arranged secondary prevention.[62] Clinical risk scores for this purpose has been developed such as the ABCD score (age, blood pressure, clinical features, duration of symptoms) and possess predictive value for stroke events after TIA.[63] The predictive value improved when combined with carotid and brain imaging.[64] However, still, there are no widely used and validated scores to predict early recurrence after stroke.[62] Therefore better predictor with lower cost method required.

The Large prospective population-based study has been conducted with the goal to determine whether a panel of biomarkers related to inflammation, thrombosis, atherogenesis, and cardiac or neuronal function has prognostic value for the risk of recurrent stroke within 90 days of TIA or stroke in patients on current best medical therapy.[62] The panel investigated consist of several groups of biomarkers. The inflammatory biomarkers investigated were IL-6, CRP, tumor necrosis factor receptor-1, and neutrophil gelatinase–associated lipocalin. Thrombotic biomarkers were thrombomodulin, fibrinogen, P-selectin, D-dimer, von Willebrand factor antigen (VWF), and protein Z (PZ). Also, anti-phosphoryl choline, an anti-atherogenic antibody, was included. Markers of cardiac or neuronal function and injury and neuron regeneration used were heart type fatty acid–binding protein, neuron-specific enolase, and brain-derived neurotrophic factor (BDNF).[62]

In this large study involving 1292TIA and stroke patient with best medical therapy, no single

biomarker stood out as being a strong independent predictor of the early risk of recurrent stroke. The inflammatory biomarkers IL-6 and CRP only show the weak predictive value of recurrent stroke. Some associations inflammatory biomarker with poor outcome has reported different study. The result may reflect reverse causation (i.e., more severe strokes have greater acute phase inflammatory responses), rather than any association with risk of recurrent events.[65,66]The marginally statistically significant results must interpret with caution, and the result did not indicate biomarkers that were likely to be clinically useful yet.[62]

BIOMARKERS OF ISCHEMIC PENUMBRA

The distinction of core infarct and penumbra are an important concept in stroke pathology. The ischemic event leads to two areas with distinct tissue condition, the core infarct, and the penumbra, area, surrounds the core infarct. In the core infarct, the tissue completely loses its perfusion, leading to complete loss of energy supply for neuronal cells, hence neuronal death. In contrast, penumbra reflects the area of brain tissues that remains partially perfused from collateral blood vessels hence it is degenerate more slowly mainly through an apoptotic pathway.[67]Determining ischemic penumbra is an important task because the main goal of stroke therapy is saving the penumbra as much and as soon as possible.[50] Today visualization of core infarct and penumbra has been made possible by MRI or PET scan. The existence of blood biomarker that identifies patients with salvageable brain tissue in the setting of acute ischemic stroke could be a very important clinical utility since MRI or PET scan not yet available in the most health care facility, it is costly and requires a specific condition. No plasma biomarker has shown promising potential.

Some advance has been able to differentiate tissue in the area with reduced blood flow to an area on severely ischemic condition.[11] One of the earliest investigation in experimental model found that glucose tends to be higher in the penumbral area, while glutamate is lower.[68,69] Higher glucose probably depend to the degree of regional cerebral blood flow (CBF) while lower glutamate in penumbral is probably as a result of the upregulation of the protein that involves in glutamate transport from inter-synaptic space to astrocytes.[12,67] Cell in the penumbral area also has been shown to express some stress response protein such as heat shock protein (HSP-27, HSP-70, HSP-72), α-B-crystallin, heme oxygenase-1, neuregulin-1, cyclooxigenase-2, and HIF-1α.[70,71] Those proteins may potentially serve as tissue markers of the penumbral area, although whether or not can be detected sufficiently in peripheral blood, requires further study.

CONCLUSION

Although none blood-based biomarkers have been used routinely in clinical practice until today, many efforts have been put together to realize the blood biomarker related to ischemic stroke analog to troponin's role in myocardial infarction. Many substances have been studied and show promise to aid better diagnosis, determining etiology, predict infarct size, severity, short and long-term outcome, indication for specific therapy and complication. A panel of biomarker consist of multiple markers may require since strokes involved complex pathophysiological process and multiple mechanisms. With further study, panels of biomarkers may achieve better sensitivity and specificity to meet standard clinical modality in stroke management.

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