REVIEW ARTICLES

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The importance of matrix metalloproteinases in the prognosis of acute ischemic stroke patients

*Elena Costru-Tasnic, Mihail Gavriliuc, Elena Manole

Department of Neurology No1, *Nicolae Testemitanu* State University of Medicine and Pharmacy Chisinau, the Republic of Moldova

Authors' ORCID iDs, academic degrees and contributions are available at the end of the article

*Corresponding author – Elena Costru-Tasnic, e-mail: elena.costru@usmf.md
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Abstract

Background: Ischemic stroke is one of the leading causes of mortality and disability worldwide. Numerous studies were performed to assess the risk of clinical deterioration of acute ischemic stroke patients, including the risk of haemorrhagic transformation. The complexity of cerebral ischemia pathology raised the possibility of a multitude of candidate-molecules to be studied as stroke biomarkers. The blood brain barrier integrity biomarkers have shown promising results both in fundamental and clinical studies. Matrix metalloproteinases have been extensively analysed and gave encouraging results for predicting unfavourable neurological outcome, including the risk for haemorrhagic transformation. Matrix metalloproteinase-9 plays a crucial role in the disruption of the blood-brain barrier following focal cerebral ischemic stroke. Elevated matrix metalloproteinase-2 levels are responsible for the degradation of tight junction proteins, basal lamina and neuronal injury after ischemia, and may contribute to infarction and hemorrhagic volume. The review provides an overview of matrix metalloproteinases' role in the prognosis of acute ischemic stroke patients, regarding the stroke outcome and the risk of haemorrhagic transformation.
Conclusions: Matrix metalloproteinases, especially gelatinases, are extensively studied for their predictive value in ischemic stroke evolution. Matrix metalloproteinase-2 and matrix metalloproteinase-9 correlate with stroke severity and haemorrhagic transformation in acute ischemic stroke, but large validation studies are needed for practical translation. Future studies should focus on developing a biomarker panel for predicting outcomes in stroke patients.
Key words: cerebrovascular accident, matrix metalloproteinases, stroke outcome.

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Introduction

Ischemic stroke (IS) is one of the leading causes of mortality and disability worldwide, with an important increase in low-middle income countries for the last 20 years [1]. Moreover, stroke has the tendency to affect younger people in the less developed countries with consecutive impact on economic and social levels. There are numerous targets to improve in the management of ischemic stroke, starting with the population awareness about stroke and emergency service priority for such patients, continuing with the necessity of comprehensive stroke centres for acute/24h diagnosis and acute treatment, and finishing with appropriate long-term rehabilitation centres for in- and outpatients [2].

Given the limited therapeutic options for the acute ischemic stroke patients – chemical and mechanical thrombolysis, the multitude of exclusion criteria, and the limited time-window, only one in five patients (according to international data) or even less – one in ten patients (according to national data, in hospitals with stoke units), will receive specific treatment for cerebral ischemia [3]. These little encouraging data can become even worse when regarding the possible complications of thrombolysis, including haemorrhagic transformation (HT) [3-7]. HT of ischemic stroke represents the bleeding in the infarcted area of the brain after IS. It can occur in up to 70% of cases of cerebral ischemia – by radiologic and morphologic studies, with a various percentage of symptomatic HT (HT associated with worsening of the neurological status) – from 1 to 20% [8].

Numerous studies were performed to assess the risk of clinical deterioration of acute IS patients, including the risk of HT [9]. Various parameters were analysed, including clinical manifestations, radiologic features, laboratory factors, in a separate way, either in combined tools – scores [10-12].

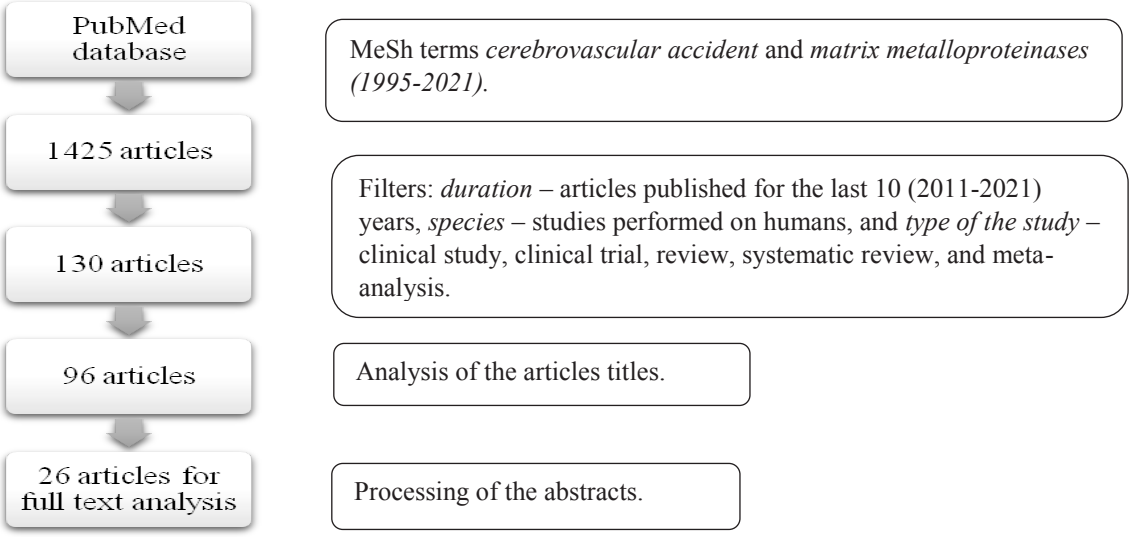

Fig. 1. Algorithm of literature research

Given the acute onset of cerebral ischemia and the limited time for diagnosis, a high percentage of the researches focused on finding the "gold-molecule" able to identify and/ or predict the evolution of patients with IS, similar to the cardiac troponins – so-called cerebral infarction biomarker [13]. *Biomarkers* are considered "signatures" of different biological processes, able to identify, stratify, predict the evolution of the assessed events.

The complexity of cerebral ischemia pathology raised the possibility of a multitude of candidate-molecules to be studied as stroke biomarkers. A PubMed search of stroke and biomarkers key words reveals an important number of studies and publications in the field (more than 15 thousand), starting with 1 article in the 80-s to up to 1662 papers in 2020. The studied molecules can be classified by the target process in the stroke pathology, but also, even more important for clinical activity, by the potential use of the biomarker.

The classification according to the affected pathway in the IS pathogenesis includes molecules related to: inflammation/oxidative stress, vasoreactivity, coagulation/ fibrinolysis disorders, and to the brain-blood barrier (BBB) disruption. The potential clinical use of the stroke biomarkers includes the following categories: prediction of stroke risk, determination of stroke mechanisms, diagnosis of cerebral vein thrombosis, prediction of infarct volume, prediction of stroke outcome, estimation of in-hospital complications risk, determination of the response to therapy [13, 14].

Regarding the response to therapy and stroke outcome, the BBB integrity biomarkers have shown promising results both in fundamental and clinical studies. Among them, matrix metalloproteinases (MMPs) have been extensively analysed and gave good results both for predicting the answer to treatment, and also for unfavourable neurological outcome, including the risk for haemorrhagic transformation [13].

The **aim** of this research was the review of matrix metalloproteinases' role in the prognosis of acute ischemic stroke patients, regarding the stroke outcome and the risk of haemorrhagic transformation.

Material and methods

The online research using the PubMed database has been conducted. There have been used the MeSh terms: cerebrovascular accident and matrix metalloproteinases, obtaining the initial number of 1425 articles for the combined searched terms (1995-2021). To restrain the number of the studies, there have been applied the following filters: duration – articles published within the last 10 years (2011-2021), species – studies performed on humans, and type of the study – clinical study, clinical trial, review, systematic review, and meta-analysis. The reasoning for the applied filters was to reveal the most recent researches, with the highest clinical application and feasibility. The selection revealed 130 articles. Individual analysis of the articles titles was thereafter done, with consecutive exclusion of the articles focusing on other cerebrovascular pathologies (e.g. cerebral sinuses thrombosis, subarachnoid haemorrhage), articles in other languages than English, studies of the different diagnosis tools (e.g. laboratory devices). For the remaining 96 articles, abstracts were processed, with final selection of 26 articles for full text analysis (fig. 1).

Nine supplementary articles on complementary data for our research – stroke epidemiological data, blood-brain barrier structure – were included.

Results and discussion

The sudden blockage of a cerebral vessel leads to immediate and delayed pathological processes linked with oxygen and glucose delivery failure – a cascade of ischemia-induced pathological events, that ultimately lead to irreversible neuronal injury – neuronal death [7, 15]. At each stage of the IS pathogenesis, numerous molecules are involved with sequential plasma increase/decrease, making them potential

biomarkers for stroke. As biomarkers, the molecules should be easily measurable, specific and sensitive [16].

Emphasising the most important steps in the cerebral ischemia, the acute energy failure caused by vessels blockage will determine at first a burst of excitotoxicity (by activation of N-methyl-aspartate receptors and calcium channels), which leads to the activation of inflammatory response (synthesis of pro-inflammatory cytokines as tumor necrosis factor α (TNF-α), interleukine 1β (IL-1β), activation of neural nitric oxide (NO) synthase, reactive oxygen species synthesis, oedema and finally neurons death (apoptosis). TNF-α and IL-1β synthesis will trigger a second line cytokine expression with release of interleukine (IL-6) and chemokine CXCL-8. These pro-inflammatory agents can increase the C-reactive protein level and start the expression of numerous matrix metalloproteinases [1, 16, 17].

Matrix metalloproteinases are a family of zinc-dependent endoproteases with multiple roles in tissue remodelling and degradation of various proteins in the extracellular matrix (ECM): collagen, proteoglycans, elastin or fibronectin. MMPs promote inflammatory response, cell proliferation, migration, and differentiation. Among MMPs, gelatinases (MMP-2 and MMP-9) are the most investigated enzymes, given the high prevalence and widespread distribution in different tissues (e.g. endothelium, intima, vascular adventitia, fibroblasts, platelets, macrophages, neutrophils, brain, heart, lungs, liver, kidney, breast, uterus, placenta, ovary, testis, prostate, tooth enamel, skin, keratinocytes) [16]. Therefore, MMP-2 and MMP-9 are studied in various medical fields. In neurology, gelatinases were investigated related to stroke, multiple sclerosis, Alzheimer disease, Parkinson disease, neuroinfections, brain tumors and others [16-18].

Characteristics of matrix metalloproteinases

In the early 1960s, MMPs were first identified by Gross and Lapiere as enzymes with collagen proteolytic activity that causes extra-cellular matrix protein degradation during resorption of the tadpole tail [5,13]. MMPs are calcium-dependent zinc-endopeptidases, which are expressed as inactive zymogens with a pro-peptide domain (pro-MMPs) that must be removed for MMP activation [18-20].

At present, time, in vertebrates, there are 28 members in the MMP family, at least 23 are expressed in human tissues [6, 21], and 14 of those – in the blood vessels. Based on the variations in MMPs biochemical structure and affinity to substrates, they were classified into several groups including [16, 17]:

1. Collagenases (MMP-1, MMP-8, MMP-13, MMP-18),
2. Gelatinases (MMP-2, MMP-9),
3. Stromelysins (MMP-3, MMP-10, MMP-11),
4. Matrilysins (MMP-7, MMP-26),
5. Membrane-type matrix metalloproteinases (MMP-14, MMP-15, MMp-16, MMP-17, MMP-24, MMP-25),
6. Other MMPs (MMP-12, MMP-19, MMP-20, MMP-21, MMP-23, MMP-27, MMP-28).

The majority of MMPs are produced and secreted from cells in the inactive form (proenzymes) with consecutive activation in the final active form within the extracellular matrix [16]. After activation, their activity is regulated mainly by natural tissue inhibitors of MMPs (TIMPs) that can bind to the active side and block the substrate availability. To date, four types of tissue inhibitors of MMPs are known, numbered from 1 to 4. Each TIMP can inhibit multiple MMPs with different efficacies [16, 17, 20]. Among four known TIMPs, TIMP-1 has the highest affinity to MMP-9, and TIMP-2 for MMP-2 [1, 22].

The role of gelatinases in the progress of IS

The plasma concentration of gelatinases varies during IS, both in the acute and later phases of cerebral ischemia [1, 16].

The concentration of MMP-9 is elevated in serum during acute phase of all types of ISs, starting from 12h up to 48h after stroke onset, and are increasingly correlated with neurological deterioration [23]. Among stroke patients, the cardioembolic stroke results in the highest MMP-2 concentrations compared to other types of stroke [16].

MMP-9 could have a prognosis value for ischemic stroke occurrence, as shown by a recent study conducted on patients with attrial fibrilation [24]. From 268 unique biomarkers, only 6 were most strongly associated with subsequent ischemic stroke/systemic embolism, among them matrix metalloproteinase-9.

The following main functions/processes are influenced by gelatinases activity during IS [16, 17, 21, 25, 26]:

1. Brain-blood-barrier (BBB) destruction – in addition to cleavage of collagen type IV, MMP-9 is able to digest occludin and claudin – essential components of tight junction proteins (TJPs) in the BBB. Finally, the high activity of MMP-9 in the blood within the acute phase of IS increases the risk of secondary bleeding within the ischemic focus. Moreover, the appearance of active enzyme form in plasma, as a result of rtPA administration, augments the risk of intracranial bleeding.

2. Inflammatory answer – TNF-α and IL-6 can activate the expression of MMP-9 which is involved into further activation of IL-1β and CXCL-8.

3. Formation of glial scar – mechanism of the second gelatinase, MMP-2.

MMP-9 plays a crucial role in the disruption of the BBB following focal cerebral ischemic stroke. Elevated MMP-2 levels were responsible for the degradation of tight junction proteins, basal lamina and neuronal injury after ischemia, and may contribute to infarction and hemorrhagic volume [1, 15, 27]. MMP-2 deficiency reduced the incidence of hemorrhage in the cortex in mouse [6]. Fundamental studies on mice with early reperfusion suggested that MMP-2 deficiency as well as MMP-2 and MMP-9 double deficiency were more protective than MMP-9 deficiency alone against HT after the early stages of ischemia and reperfusion [1].

The pro-inflammatory action of the MMPs have been shown to correlate with the atherosclerotic plaques rupture, and therefore responsible for cardio- and cerebrovascular events. Given the MMP-2 action substrate (structural components of the sub-endothelium of medium and large-size arteries, like gelatin, fibronectin, laminin-1, type IV colla-

gen and elastin), its role in vascular remodeling, neutrophil and platelet activation, numerous studies have been conducted to analyze the borderline between physiologic and pathologic hemostatic answer at the levels of atherosclerotic plaques promoted by these enzymes [28]. For example, Lenti M. et al. have demonstrated that atherosclerotic plaques of patients undergoing carotid endarterectomy determine platelet activation due to their high content in active MMP-2, the effect being confirmed by abolishment after preincubation of platelets with MMP-2 inhibitors [28].

Gelatinases and HT

HT can occur in the natural evolution of ischemic stroke, but more frequently is a complication of rt-PA thrombolysis in the treatment of acute ischemic stroke.

Radiologically, HT is defined and divided into 4 subtypes: small petechial hemorrhagic infarction (HI1), confluent petechial hemorrhagic infarction (HI2), small parenchymal hemorrhage (PH1, < 30% of infarct, mild mass effect), and large parenchymal hemorrhage (PH2, > 30% of infarct, marked mass effect) [29]. Clinically, HT is divided in: asymptomatic HT (no clinical worsening on the National Institutes of Health Stroke Scale (NIHSS) score despite HTs), minor symptomatic HT (a 1 to 3-point increase in the NIHSS score), and major symptomatic HT (a ≥ 4-point increase in the NIHSS score) [29]. Despite the "asymptomatic" term, long-term prognosis is poorer in ischemic stroke patients with all clinical types of HT.

Structurally, HT of IS occurs when blood-brain barrier is disrupted. The BBB separates the brain parenchyma and the blood circulation, providing anatomical and physiological protection for the central nervous system, supplying nutrition for brain tissue, filtering harmful substances from the brain back to the blood and protecting the brain from toxic material in the blood [25, 29].

The rupture of BBB damages the whole neurovascular unit (NVU), which consists of the extracellular matrix, endothelial cells, astrocytes, neurons, and pericytes [5, 21, 29]. The main mechanisms of HT are considered related to proteolysis, oxidative stress and leukocyte infiltration.

HT can occur spontaneously, especially in case of cerebral embolism, either induced by anticoagulants, thrombolytic therapy and endovascular procedures [23, 30]. All these situations are associated with an increased degradation of the extracellular matrix components by proteolytic enzymes, particularly MMPs, which aggravates brain edema and enhances brain damage.

Different studies applied MMP-9/TIMP-1 ratio as an indicator of MMP-9 activity *in vivo*. The proteolytic activation of MMP-9 is conducted by active forms of other metaloproteinases as MMP-2 or MMP-3. The activation of MMP-9 facilitates the BBB destruction and secondary bleeding within the ischemic focus [22, 31]. MMP-2 and MMP-9 have different temporal expression and action in the post-stroke period. It was established that MMP-2 is involved in the initial phase of BBB opening (maximum at 3h), while MMP-9 is more active in the delayed opening of the BBB after ischemic stroke [6].

Given the higher incidence of HT after thrombolytic treatment with rt-PA, numerous fundamental studies were conducted to elucidate the mechanisms underlying this phenomenon. Thus, it was established that when NVU is impaired, rt-PA may cross the brain and activate endogenous tPA signaling pathways associated with HT. t-PA mostly affects the BBB through various plasminogen independent mechanisms, such as the overexpression of MMP-2, -3 and -9 and activation/cleavage of lipoprotein receptor related protein or platelet-derived growth factor receptor alpha [15, 29].

Both fundamental and clinical studies have found an elevation of gelatinases MMP-2 and MMP-9 in the ischemic brain/plasma of IS patients within the first 24h, correlating with cerebral infarction extension, worse outcome and HT. Preclinical studies demonstrated that MMP-2 plays a key role in the initial opening of the BBB after cerebral ischemia by degradation of tight junction proteins, collagen, and occludin. According to literature data, the most extensively studied gelatinase in relation to IS and HT, MMP-9 can serve as independent predictor of HT after tPA administration [5, 22].

A systematic review (Ramos-Fernandez M. et al., 2011) analyzed 22 clinical studies, including 3289 patients, to evaluate the role of MMP-9 plasma level in acute ischemic stroke. According to this analysis, plasma MMP-9 level, measured before the administration of thrombolytic therapy in acute stroke patients, accurately predicts the development of hemorrhage [32].

Importantly, an analysis of non-selected series of patients has shown that an MMP-9 value ≥140 ng/mL within 24h of stroke onset had a high negative predictive value for future HT. The data suggest that a lower plasma level of MMP-9 is associated with a low risk of HT [23, 32, 33].

Experimental studies strengthen the correlation of both MMP-2 and MMP-9 with HT, with even a higher association of MMP-2 with HT, given its action mechanism by degradation of tight-junction proteins and the basal lamina, BBB disruption and neuronal injury [23].

The fundamental studies raised the idea of MMPs inhibition for improving clinical outcome of IS patients. Numerous molecules have been analyzed in clinical settings to inhibit gelatinases, including minocycline, (4-phenoxyphenylsulfonyl) methylthiirane (known as SB-3CT), lentiviral-mediated MMP-9 gene silencing, recombinant TIMP-1 in its native form, PLGA (poly lactic-co-glycolic acid) nanoparticles [5], majority of them showing that inhibition of MMP-9 could be a therapeutic strategy for acute ischemic stroke treatment, but with a limited time-window after which MMP inhibitors have negative impacts on stroke patients [6, 12].

Stroke severity and functional outcome versus MMPs levels

Stroke severity and final infarct volume are among the most important factors associated with functional outcomes in patients after IS. The most available and widely used tools are those clinical – NIHSS score and radiological – CT and

MRI protocols and scores. While their sensibility and specificity are high, not all hospitals, especially from rural areas, have 24h available radiological service. Therefore, the use of blood biomarkers to predict stroke patient's evolution could be a good supplement for the clinical assessment and treatment rationale in these cases.

Different biomarkers show good correlation with stroke clinical severity and infarct size, and may be useful for predicting poorer outcomes [23, 31, 32, 34].

In a review on MMPs' role in BBB breakdown during acute ischemic stroke, Lakhan S. et al., 2013, have determined that cerebral infarct size is reduced in mice deficient in MMP-9 or after treatment with MMP inhibitor [1].

MMP-2/-9 levels, when collected within 20h of stroke onset, showed a direct, significant correlation with both initial and final stroke severity, as measured by the NIHSS and infarct size. When collected earlier (within 6h of stroke onset), MMP-9 was the only predictor of infarct volume measured as a diffusion lesion [1, 23].

Consecutively, in an Italian study [31], including 327 tPA-treated patients, MMP-9 circulating level variation proved association, independent of major clinical determinants, with symptomatic HT or death. The MMP-9 serum levels correlated with the NIHSS values prior and after thrombolytic treatment.

In an extensive review by Turner R. et al., 2016, the authors highlithed that, in terms of long-term outcomes, MMP-9 was associated with a poor neurological outcome at 3 months post-stroke and hyperacute levels of MMP-9 correlated with worse Rankin outcome at 3 months post-stroke. The review mentions one single study to report that both MMP-2 and MMP-9 levels correlated with clinical severity and the extent of the infarct [21].

Iemolo F. et al., 2016, realized a prospective evaluation of a panel of blood biomarkers to assess their value in acute stroke prognosis. The panel included the following molecules: Brain Natriuretic Peptide, D-Dimers, Matrix-Metalloproteinase-9, and S100β protein generating a Multimarker index of these values. The outcome of the study was 120-day mortality. Among 244 patients included in the analysis, 161 (66.0 %) had an increase of biomarkers [34]. However, detailed statistical analysis failed to give significant correlations. The authors concluded that neither one marker nor combination of all markers was of significant benefit in acute stroke diagnostics, and DWI-MRI was the procedure with the highest diagnostic quality in case of acute cerebral ischemia.

Another study was conducted by Zhong et al., 2017, to prospectively investigate the association between serum MMP-9 levels and prognosis in patients with acute ischemic stroke using data from the China Antihypertensive Trial in Acute Ischemic Stroke (CATIS). This large study included 3.186 participants (2.008 men and 1.178 women). Study outcome data on death, major disability (modified Rankin Scale score ≥3), and vascular disease were collected at 3 months after stroke onset. The authors observed dose-response associations between higher MMP-9 levels in acute ischemic stroke and increased risk of major disability and death at 3 months after stroke onset, after adjustment for other established covariates [35].

Conclusions

It has been detected that the involvement of gelatinases into multiple physiological and pathological processes makes them an attractive target for investigations. Numerous researches revealed that both enzymes, MMP-2 and MMP-9 have their specific role during brain ischemia. Promising results are shown by both fundamental and clinical studies analyzing the predictive value for hemorrhagic transformation of gelatinases. Thus, MMP-2 and MMP-9 correlate with stroke severity and haemorrhagic transformation in acute ischemic stroke, but large validation studies for practical translation are needed.

Given the etiological heterogeneity of ischemic strokes, the variety of clinical manifestations, it is challenging to identify one single predictive biomarker for stroke outcome. Future studies should focus on developing a biomarker panel for predicting outcomes in stroke patients presenting with cerebrovascular accident.

References

1. Lakhan SE, Kirchgessner A, Tepper D, Leonard A. Matrix metalloproteinases and blood-brain barrier disruption in acute ischemic stroke. Front Neurol. 2013 Apr;4:1-15. doi: 10.3389/fneur.2013.00032.
2. Johnson W, Onuma O, Owolabi M, Sachdev S. Stroke: a global response is needed. Bull World Health Organ. 2016;94(9):634A-635A. doi: 10.2471/BLT.16.181636.
3. Manole E, Lisnic V, Groppa S, Costru-Tasnic E, Filioglo A, Odainic O, et al. Registrul RES-Q în Republica Moldova – primele rezultate naționale în cadrul unui proiect internațional [RES-Q registry in the Republic of Moldova – first national results within an international project]. [Bull Acad Sci Moldova. Sci Med]. 2017;(5):72-77. Romanian.
4. Berezowski V, Fukuda AM, Cecchelli R, Badaut J. Endothelial cells and astrocytes: a concerto en duo in ischemic pathophysiology. Int J Cell Biol. 2012;2012:176287. doi: 10.1155/2012/176287.
5. Wang W, Li M, Chen Q, Wang J. Hemorrhagic transformation after tissue plasminogen activator reperfusion therapy for ischemic stroke: mechanisms, models, and biomarkers. Mol Neurobiol. 2015 Dec 4;52(3):1572-9. doi: 10.1007/s12035-014-8952-x.
6. Sarvari S, Moakedi F, Hone E, Simpkins JW, Ren X. Mechanisms in blood-brain barrier opening and metabolism-challenged cerebrovascular ischemia with emphasis on ischemic stroke. Metab Brain Dis. 2020;35(6):851-68. doi: 10.1007/s11011-020-00573-8.
7. Li Y, Zhong W, Jiang Z, Tang X. New progress in the approaches for blood-brain barrier protection in acute ischemic stroke. Brain Res Bull. 2019;144:46-57. doi: 10.1016/j.brainresbull.2018.11.006.
8. Zhang J, Yang Y, Sun H, Xing Y. Hemorrhagic transformation after cerebral infarction: current concepts and challenges. Ann Transl Med. 2014;2(8):81. doi: 10.3978/j.issn.2305-5839.2014.08.08.
9. Sung SF, Chen SCC, Lin HJ, Chen YW, Tseng MC, Chen CH. Comparison of risk-scoring systems in predicting symptomatic intracerebral hemorrhage after intravenous thrombolysis. Stroke. 2013;44(6):1561-6. doi: 10.1161/STROKEAHA.111.000651.
10. Jha R, Battey TWK, Pham L, Lorenzano S, Furie KL, Sheth KN, et al. Fluid-attenuated inversion recovery hyperintensity correlates with matrix metalloproteinase-9 level and hemorrhagic transformation in acute ischemic stroke. Stroke. 2014 Apr;45(4):1040-5. doi: 10.1161/STROKEAHA.113.004627.
11. Kim Y, Kim YK, Kim NK, Kim SH, Kim OJ, Oh SH. Circulating matrix metalloproteinase-9 level is associated with cerebral white matter hyper-

intensities in non-stroke individuals. Eur Neurol. 2014;72(3-4):234-40. doi: 10.1159/000362876.
12. Chang JJ, Emanuel BA, Mack WJ, Tsivgoulis G, Alexandrov AV. Matrix metalloproteinase-9: dual role and temporal profile in intracerebral hemorrhage. J Stroke Cerebrovasc Dis. 2014;23(10):2498-505. doi: 10.1016/j.jstrokecerebrovasdis.2014.07.005.
13. Polivka J, Polivka J, Krakorova K, Peterka M, Topolcan O. Current status of biomarker research in neurology. EPMA J. 2016;7(1):14. doi: 10.1186/s13167-016-0063-5.
14. Lu G, He Q, Shen Y, Cao F. Potential biomarkers for predicting hemorrhagic transformation of ischemic stroke. Int J Neurosci. 2018;128(1):79-89. doi: 10.1080/00207454.2017.1349766.
15. Miao Y, Liao JK. Potential serum biomarkers in the pathophysiological processes of stroke. Expert Rev Neurother. 2014 Feb 13;14(2):173-85. doi: 10.1586/14737175.2014.875471.
16. Kurzepa J, Kurzepa J, Golab P, Czerska S, Bielewicz J. The significance of matrix metalloproteinase (MMP-2) and MMP-9 in the ischemic stroke. Int J Neurosci. 2014;124(10):707-16. doi: 10.3109/00207454.2013.872102.
17. Cui N, Hu M, Khalil RA. Biochemical and biological attributes of matrix metalloproteinases. Prog Mol Biol Transl Sci. 2017;147(617):1-73. doi: 10.1016/bs.pmbts.2017.02.005.
18. Romi F, Helgeland G, Gilhus NE. Serum levels of matrix metalloproteinases: implications in clinical neurology. Eur Neurol. 2012;67(2):121-8. doi: 10.1159/000334862.
19. Rempe RG, Hartz AMS, Bauer B. Matrix metalloproteinases in the brain and blood-brain barrier: versatile breakers and makers. J Cereb Blood Flow Metab. 2016;36(9):1481-507. doi: 10.1177/0271678X16655551.
20. Chang JJ, Stanfill A, Pourmotabbed T. The role of matrix metalloproteinase polymorphisms in ischemic stroke. Int J Mol Sci. 2016;17(8):1323. doi: 10.3390/ijms17081323.
21. Turner RJ, Sharp FR. Implications of MMP-9 for blood-brain barrier disruption and hemorrhagic transformation following ischemic stroke. Front Cell Neurosci. 2016;10:1-13. doi: 10.3389/fncel.2016.00056.
22. Gołąb P, Boguszewska-Czubara A, Michal K, Kurzepa J. The rtPA increases MMP-9 activity in serum during ischaemic stroke. Neurol Neurochir Pol. 2014;48(5):309-14. doi: 10.1016/j.pjnns.2014.07.012.
23. Maestrini I, Ducroquet A, Moulin S, Leys D, Cordonnier C, Bordet R. Blood biomarkers in the early stage of cerebral ischemia. Rev Neurol (Paris). 2016;172(3):198-219. doi: 10.1016/j.neurol.2016.02.003.
24. Hijazi Z, Wallentin L, Lindbäck J, Alexander JH, Connolly SJ, Eikelboom JW, et al. Screening of multiple biomarkers associated with ischemic stroke in atrial fibrillation. J Am Heart Assoc. 2020;9(24):e018984. doi: 10.1161/JAHA.120.018984.
25. Thomsen MS, Routhe LJ, Moos T. The vascular basement membrane in the healthy and pathological brain. J Cereb Blood Flow Metab. 2017;37(10):3300-17. doi: 10.1177/0271678X17722436.
26. Jin R, Yang G, Li G. Molecular insights and therapeutic targets for blood–brain barrier disruption in ischemic stroke: critical role of matrix metalloproteinases and tissue-type plasminogen. Neurobiol Dis. 2010;38(3):376-85. doi: 10.1016/j.nbd.2010.03.008.
27. Yang Y, Rosenberg GA. Blood-brain barrier breakdown in acute and chronic cerebrovascular disease. Stroke. 2011;42(11):3323-8. doi: 10.1161/STROKEAHA.110.608257.
28. Lenti M, Falcinelli E, Pompili M, Rango P De, Conti V, Guglielmini G, et al. Matrix metalloproteinase-2 of human carotid atherosclerotic plaques promotes platelet activation: correlation with ischaemic events. Thromb Haemost. 2014;111(6):1089-101. doi: 10.1160/TH13-07-0588.
29. Liu C, Xie J, Sun S, Li H, Li T, Jiang C, et al. Hemorrhagic transformation after tissue plasminogen activator treatment in acute ischemic stroke. Cell Mol Neurobiol. 2020 Oct 30. doi: 10.1007/s10571-020-00985-1.
30. Wang L, Wei C, Deng L, Wang Z, Song M, Xiong Y, et al. The accuracy of serum matrix metalloproteinase-9 for predicting hemorrhagic transformation after acute ischemic stroke: a systematic review and meta-analysis. J Stroke Cerebrovasc Dis. 2018;27(6):1653-65. doi: 10.1016/j.jstrokecerebrovasdis.2018.01.023.
31. Inzitari D, Giusti B, Nencini P, Gori AM, Nesi M, Palumbo V, et al. MMP9 variation after thrombolysis is associated with hemorrhagic transformation of lesion and death. Stroke. 2013;44(10):2901-3. doi: 10.1161/STROKEAHA.113.002274.
32. Ramos-Fernandez M, Bellolio MF, Stead LG. Matrix metalloproteinase-9 as a marker for acute ischemic stroke: a systematic review. J Stroke Cerebrovasc Dis. 2011;20(1):47-54. doi: 10.1016/j.jstrokecerebrovasdis.2009.10.008.
33. Castellanos M, Sobrino T, Millán M, García M, Arenillas J, Nombela F, et al. Serum cellular fibronectin and matrix metalloproteinase-9 as screening biomarkers for the prediction of parenchymal hematoma after thrombolytic therapy in acute ischemic stroke: a multicenter confirmatory study. Stroke. 2007;38(6):1855-9. doi: 10.1161/STROKEAHA.106.481556.
34. Iemolo F, Sanzaro E, Duro G, Giordano A, Paciaroni M. The prognostic value of biomarkers in stroke. Immun Ageing. 2016;13(1):1-6. doi: 10.1186/s12979-016-0074-z.
35. Zhong C, Yang J, Xu T, Xu T, Peng Y, Wang A, et al. Serum matrix metalloproteinase-9 levels and prognosis of acute ischemic stroke. Neurology. 2017;89(8):805-12. doi: 10.1212/WNL.0000000000004257.

Authors' ORCID iDs and academic degrees
Elena Costru-Tasnic, MD, PhD Applicant, Assistant Professor – https://orcid.org/0000-0001-8524-014X
Mihail Gavriliuc, MD, PhD, Professor – https://orcid.org/0000-0002-5789-2842
Elena Manole, MD, PhD, Associate Professor – https://orcid.org/0000-0003-0164-859X

Authors' contributions
ECT conceptualized the idea, conducted literature review, and wrote the first manuscript. MG and EM revised critically the manuscript and completed the final text. All the authors approved the final version of the manuscript.

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Ethics approval and consent to participate
No approval was required for this study.

Conflict of Interests
The authors have no conflict of interests to declare.