New Approach to Identify Ischemic Stroke Patients at Risk to Develop Hemorrhagic Transformation

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ABSTRACT

Some patients with ischemic stroke are subject to hemorrhagic transformation, a complication leading to increased patient morbidity and mortality. The discovery of biomarkers that can be used to identify ischemic strokes prone to this complication are very important for the clinical practice because therapy could be altered to mitigate the risk. We discuss here the results of a trial that evaluated for the first time tight junction proteins as biomarkers of blood-brain barrier disruption and hemorrhagic transformation in ischemic stroke.

Keywords: stroke, hemorrhagic transformation

Stroke is a major cause of mortality worldwide. There are two major types of stroke, ischemic stroke (IS) and hemorrhagic stroke (HS), which are caused by the occlusion and the rupture of a blood vessel, respectively (1).

A major complication that can occur in patients with IS is the hemorrhagic transformation (HT). Thrombolytic therapy with rt-PA (recombinant tissue-plasminogen activator) can improve stroke outcome, but on the other hand, in some patients it can lead to an increased risk of HT (2). Thus it is very important to identify as early as possible stroke patients at risk for HT, in order to modify their treatment to reduce the risk of HT.

In a recent issue of Neurology, Kazmierski et al. published the results of a clinical trial in which they tried to detect IS patients at risk for HT by assessing several protein markers in blood (3). They enrolled 458 IS patients (67.6 ± 12.5 y.o.). A total of 85 patients from this

group experienced a clinical deterioration (≥ 4 points NIHSS score), 33 of whom were found to HT on neuroimaging. The assessed proteins fall in one of the following categories: (i) tight junction components (occludin [OCLN], claudin 5 [CLDN5], zonula occludens 1 [ZO1]), (ii) markers of blood-brain breakdown (neuron-specific enolase [NSE], S100B), and (iii) proteins involved in the degradation of the blood-brain barrier (matrix metalloproteinase 9 [MMP-9], vascular endothelial growth factor [VEGF]). These proteins were assessed on blood samples taken at admission.

The authors found that the levels of S100B, CLDN5, and OCLN as well as the CLDN5/ZO1 ratio were significantly increased in IS patients with HT when compared to IS patients without HT. On the contrary, VEGF level were significantly decreased in IS subjects with HT when compared to IS patients without HT. Moreover, using ROC curve analysis, the authors evaluated the sensitivity, specificity, as well as the positive predictive values (PPVs) and negative predictive values (NPVs) of these proteins to discriminate between IS patients with HT from those without HT (Table 1). This study indicated that the concentration of CLDN5 within 3h from the stroke onset as well as CLDN5/ZO1 ratio within 4.5h and 6h after stroke onset could be used to discriminate between IS with HT and without HT. In conclusion, the authors suggest that some of the components of the tight junctions can be used to identify IS patients at risk to develop HT.

It is well documented by neuroimaging studies that HT is associated with BBB breakdown (4,5). The mechanism of blood-brain barrier disruption is very complex and includes the activation of several proteases: (i) MMP-2/9, (ii) cathepsin L, (iii) heparanase, and (iv) urokinase (6-8). These act upon different components of the matrix around cerebral blood vessels (type IV collagen, laminin, perlecan, fibronectin).

MMP-2/9 are responsible for the degradation of some components of the tight junctions (OCLN, CLDN5, and ZO1) (9,10). Activation of MMPs is caused, at least in part, by free radicals (11). Free radicals' scavengers such as edaravone can inhibit MMP-9 expression in vivo and thus potentially prevent HT (12). Several studies indicated a significant increase of MMP-9 level in IS patients when compared to controls and the association between this protein and the risk of HT (13). Moreover, inactivation of MMP-9 protects against HT (14).

Despite the fact that some studies indicated MMP-9 as a potential predictor for HT in IS patients, this was not shown in the study by Kazmierski et al. (13). A possible explanation for this discrepancy is the low number of patients with HT in this study. This drawback could be overcome by using a larger study population.

The novelty of the present study is represented by the possibility of using the serum concentrations of OCLN, CLDN5 and CLDN5/ZO1 ratio to discriminate between IS patients with HT and those without HT. This is supported by the high values of the calculated NPVs.

A potential marker for HT must fulfill at least four characteristics: sensitivity, specificity, high positive and negative predictive values. Also, the assay for such a marker must be very rapid (less than 4.5h). For example, even there are studies indicating that MMP-9 is a predictor of HT, it can not be used in clinical routine because the commercially available kits are time consuming requiring more than 3h to perform.

A major limitation of the present study is the low number of patients with HT (33) in which were evaluated the tight junction proteins. Also, the authors excluded from the beginning patients that took oral anticoagulants or heparin. As a consequence there is no information about the utility of these proteins in this subgroup of patients. On the other hand, the authors state that the evaluation of these markers is currently under investigation in patients which were previously exposed to thrombolytic therapy.

In conclusion, assessing in blood samples some of the tight junctions' proteins could be a

Biomarker	Sensitivity (%)	Specificity (%)	PPVs (%)	NPVs (%)
S100B	92.9	48.1	12.2	98.9
VEGF	53.3	82.4	19.0	95.8
OCLN	58.6	67.5	12.3	95.5
CLDN5	64.3	65.8	16.8	94.5
CLDN5/ZO1	66.7	60.6	11.6	95.9

TABLE 1. Sensitivity, specificity, positive and negative predictive values for some markers.

PPVs, positive predictive value; NPVs, negative predictive value

solution for the clinicians to evaluate stroke patients at risk for HT. Moreover, because patients treated with anticoagulants were excluded from the study of Kazmierski et al. a future direction is to evaluate if these proteins could be used as predictive markers for HT in patients with this medication.

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