The new applications of ceramide and sphingosine-1-phosphate system in diagnosis of ischemic stroke

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Background and Purpose: A diagnosis of ischemic stroke is entirely based on patients neurological outcomes and the brain imaging. Using computer tomography (CT), an identification of early ischemic incident (less than 3 hours from onset) may be challenging and frequently needs the use of brain contrast agents. In our study, we propose ceramide (Cer) and sphingosine-1-phosphate (Sph-1-P) as potential ischemic stroke biomarkers, which biological features could allow to follow the several stroke-related processes in the future. Cerebral ischemia alters both ceramide and Sph-1-P concentrations in affected tissues. Ceramide is accumulated in the brain shortly after ischemia and are generally considered as mediator of neurodegeneration.12,13,14 Ceramide created in the brain can potentially get through the impaired BBB to the circulation, changing ceramide profiles in blood serum/plasma. In contrast to ceramide, content of Sph-1-P tends to decrease in ischemic stroke.15 Sph-1-P reaches the particularly high concentrations in blood, wherein it is mainly produced by red blood cells and endothelial cells.16 However, during clot formation, Sph-1-P stored in high amounts in platelet is released extracellularly and increases substantially the Sph-1-P content in blood serum.

The main goal of this study was to establish whether ceramide and Sph-1-P levels are altered in serum of patients after ischemic brain stroke and TIA and whether they can be considered as potential ischemic stroke biomarkers in the future. Moreover, calculating the ratios of chosen SFs, we attempted to find a method of SF data presentation, which would improve an informative value of obtained results for their potential use in stroke diagnostics.

Methods: Levels of individual ceramide species and Sph-1-P in blood serum of patients with acute ischemic stroke (n=42), TIA (n=27) and age-matched neurological patients without cerebral ischemia (n=34), were assessed by means of LC-MS/MS technique. We found significant increases in levels of several SFs, with particularly strong elevations of Cer-C20:0 in patients with acute stroke in comparison with non-stroke subjects. In opposite, Cer-C24:1 was the only ceramide species, which concentrations decreased as a result of acute stroke. Moreover, its levels inversely correlated with the number of days after stroke onset, but did not correlate with any other analyzed parameters. It suggests that Cer-C24:1 is an independent parameter related to the course of stroke. To increase the accuracy of SF system in stroke diagnostics, we calculated values of ratios of Sph-1-P/individual ceramide species and individual ceramide species/Cer-C24:1. We found several ratios significantly changed in stroke patients. Two ratios, Sph-1-P /Cer-C24:1 ($p<0.001$) and Cer-C24:0/Cer-C24:1($p<0.001$), presented especially strong increments in patients with acute stroke. Moreover, Sph-1-P /Cer-C24:1 values were augmented in TIA patients

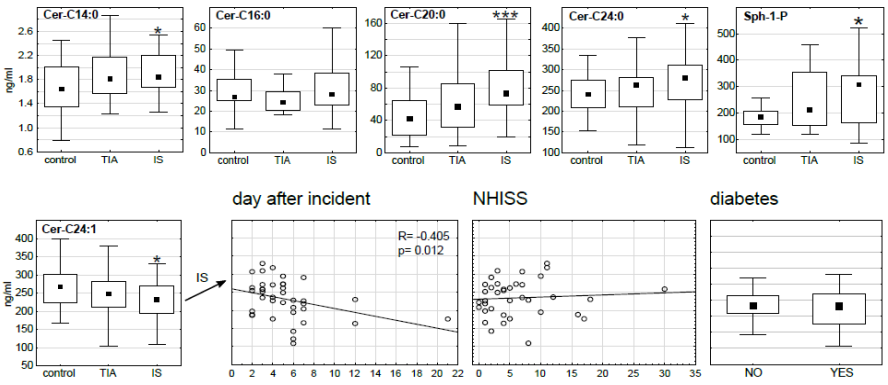


Fig.1 Levels of individual ceramide species and Sph-1-P in blood serum (A, B) and their selected correlations with number of days after ischemic stroke onset, NIHSS and diabetes (C). The concentrations of studied SF species were presented as median (Q1-Q3) values. Selected Spearman's rang correlations were shown as scatter graphs (number of days after ischemic stroke onset and NIHSS). Correlations between levels of selected SFs and diabetes were presented on plots of SF concentration median (Q1-Q3) values in ischemic patients with and without diabetes. *p <0.05, ***p < 0.001 versus control. TIA-transient ischemic attack, IS - ischemic stroke.

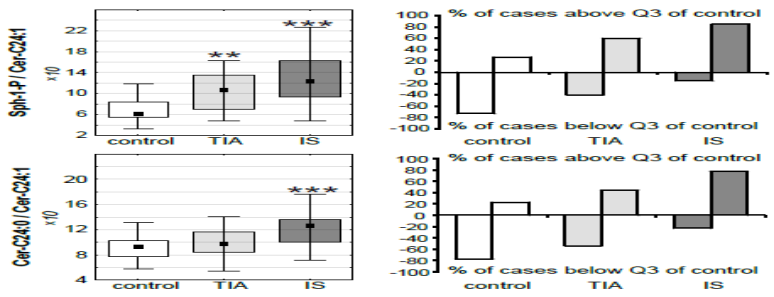
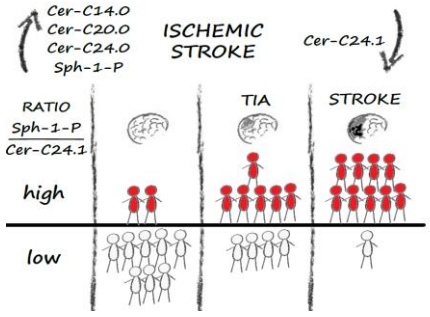


Fig.2 Selected ratios of blood serum SFs. Values of SF ratios were presented as median (Q1- Q3) values. Percentage of patients presented selected SF ratio values in relation to Q3 of control as a reference was shown as % of cases above and below Q3 of control. **p < 0.01, ***p < 0.001 versus control. TIA-transient ischemic attack, IS - ischemic stroke.



Conclusion: We found that serum SFs can be good candidates for the ischemic stroke biomarkers. We identify two SF ratios, Sph-1-P /Cer-C24:1 and Cer-C24:0/Cer-C24:1, with strong diagnostic potential in ischemic stroke. We found Sph-1-P /Cer-C24:1 ratio as possibly useful in TIA diagnostics, also in long-term after ischemic incidence, which is essential in further anti-stroke preventive treatment.