

Immune biomarkers in acute ischemic stroke

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Abstract of the Ph.D. thesis

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Stroke is one of the most prevalent causes of death and ranks as the second common cause of disability-adjusted life-years (DALYs) in Romania, thus being an enormous burden for society. Given the high mortality rate and the high degree of disability caused by this pathology, it is important to identify reliable biomarkers that would help diagnosis, evolution or prognosis of ischemic stroke patients. Until now, there is no peripheral biomarker included in the evaluation of patients with ischemic stroke.

The aim of the thesis was to identify a panel of biomarkers related with stroke severity, outcome and degree of disability after an acute episode of ischemic stroke, and to evaluate the added value of these parameters in the clinical basal model.

Study 1. The experimental research on adult male rats underwent middle cerebral artery occlusion, which analyzed the influence of Omega 3 preconditioning on post-stroke inflammation, revealed the reduction in the profile of peripheral pro-inflammatory cytokines. The plasma concentration of TNF- α in the pre-treated group was significantly lower, and declined after 24 hrs, while in the saline-treated group TNF- α had an insignificant reduction in dynamics. MCP-1, a proinflammatory chemokine, involved in monocytes migration, was significantly downregulated after 24 hrs in preconditioning group (0.798 ± 0.06 pg/ml) compared to saline-treated group (1.627 ± 0.19 pg/ml), $p=0.0001$. In the same experiment, plasma concentration of tissue inhibitor matrix metalloproteinase-1 (TIMP-1), an endogenous inhibitor of matrix metalloproteinases, was lower in the pre-treated group (14.11 ± 1.79 pg/ml compared to saline group (37.28 ± 4.85) pg/ml, $p < 0.0001$, suggesting a potential response in relation with the magnitude of MMP-9 expression in *penumbra*, evaluated by immunohistochemistry (7.10 ± 0.26 in the Omega 3 preconditioning brain tissue compared to 7.54 ± 0.23 in saline brain tissue, $p=0.002$). Moreover, expression of the factor XIII was found significantly higher in the saline-treated group 1.72 ± 0.39 compared to Omega 3 group (1.06 ± 0.39), $p=0.001$, the *penumbra* being the area with the most significant differences. Besides the influence on coagulation cascade, recent studies revealed the link between FXIII and inflammation, alternative activation of macrophages, or stroke severity.

Study 2. Lipid profile of the patients in relation to healthy controls and stroke outcome revealed a different pattern, both in fatty acids and in lipids fractions (total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol). The fatty acids profile of the plasma and red blood cell membranes were analyzed by LC/MC method; docosahexaenoic acid (DHA), was lower both in plasma ($p<0.001$) and RBC membrane ($p=0.029$) in ischemic stroke patients compared to controls. Additionally, AA/DHA+EPA ratio was higher in the ischemic group compared to controls, turning the balance in favor of pro-inflammatory actions of arachidonic acid. Omega 3 index was far below the recommended range, in all studied groups, much lower in ischemic group 1.35 (0.73-2.30) compared to control group 1.97 (1.67-2.51), $p=0.002$.

Using the path analysis, a hypothetic model involving relationships of associations and dependencies between lipid profile and modified Rankin Scale (mRS) at 3 months was tested. Outcome measured with mRS at 3 months was positively influenced by age ($\beta^*=0.22$, $p=0.001$), and NIHSS score at admission ($\beta^*=0.55$, $p<0.001$), higher values for these variables being associated with an unfavorable outcome. Also, a linear dependence between the total cholesterol levels and mRS at 3 months was found, as lower cholesterol was significantly correlated with higher mRS at 3 months ($\beta^*=-0.17$, $p=0.031$).

Study 3. Using a biochip array technology (BAT), the study aimed to evaluate the utility of a panel of five peripheral biomarkers of inflammatory status (CRP, NGAL, sTNFR-1), neuronal destruction (NSE), and secondary fibrinolysis (D-Dimers) in the acute phase of ischemia and prediction of functional

outcome at 3 months after hospital discharge of patients. D-Dimers were significant predictors after controlling for age, gender and stroke severity (OR=10.40; 95% CI: 1.38-78.16); and dyslipidemia, atrial fibrillation, and history of stroke (OR=10.85; 95% CI:1.28-91.84). After D-Dimers were included in a multivariate model, they remained independent predictors, significantly related to the risk of unfavorable outcome of ischemic stroke. Plasma levels of D-Dimers above 246.93 ng/mL were identified as a risk factor for unfavorable outcome. Similarly, the sTNFR-1 remained a significant predictor after controlling for demographical parameters, stroke severity (OR=7.43; 95% CI:1.12-49.18), dyslipidemia, atrial fibrillation, and stroke history (OR=7.16; 95% CI:1.03-49.76). CRP was significantly associated with unfavorable outcome after controlling for age, gender and stroke severity (OR=4.42; 95% CI:1.06-20.42), but in the presence of dyslipidemia, atrial fibrillation, and history of stroke, there was a tendency towards statistical significance (OR=4.43; 95% CI:0.95-21.11). NGAL was a predictor with a tendency toward statistical significance after adjusting for age, gender and stroke severity (OR=3.20; 95% CI:0.70-14.58) however, in the presence of dyslipidemia, atrial fibrillation, and history of stroke (OR=3.10; 95% CI:0.65-14.68) the significance was lost.

The model containing multiple biomarkers taken together had a higher discrimination power than models containing any individual stroke independent predictor, like CRP ($p=0.011$), D-Dimer ($p=0.019$), NGAL ($p=0.007$) and sTNFR-1 ($p=0.031$).

Study 4. The main objective of the study was to identify from a panel of 10 inflammatory markers and chemokines, biomarkers with a potential prediction role in the evolution of disability and functional dependence after an acute ischemic stroke episode. Peripheral biomarkers were measured using the xMAP technology, which allows the evaluation of multiple parameters in a small sample volume. Dynamic changes in biomarkers profile, as well the comparison with healthy controls were evaluated. Plasma concentration of RANTES and NCAM were significantly lower in ischemic stroke patients compared to healthy controls, while MPO and sICAM were significantly higher in stroke patients vs controls. Plasma concentrations of sICAM, sVCAM, and RANTES significantly decreased during the analyzed period of time. Lower plasma levels of PDGF-AA ($p=0.013$), PDGF-AB/BB ($p=0.008$), BDNF ($p=0.014$), RANTES ($p=0.012$), were associated with higher NIHSS at discharge. Univariate and multivariate logistic regression indicated basal NCAM as a significant predictor for NIHSS > 7 points, five days after disease onset. PDGF-AA (per 1 unit increase), PDGF-AB/BB (per 10 units increase), and BDNF (per 10 units increase) were univariate and multivariate predictors of functional dependence in daily life activity (BI $\leq$ 80), having a protective effect (OR < 1). There was no significant differences in the discriminatory capacity of models improved with significant predictors of functional dependence, compared to basal clinical model, although markers with significant effect in the prediction of functional dependence had a clinically relevant prognostic value, the area under the ROC curves related to each biomarker being greater than the threshold value of 0.80.

Conclusions

The Omega-3 index was found very low in the investigated population, far below the recommended range, with a significantly lower value in ischemic stroke patients. The $\omega 6/\omega 3$ ratio was higher in patients compared to the control group. For the 3 months prediction of functional outcome, NIHSS at admission, age, and total cholesterol were the most relevant predictors.

Omega-3 supplementation reduces the expression of inflammatory cells in the *penumbra*, FXIII expression in the *penumbra* and MMP-9 expressions in the *core* and the *penumbra*, as well as the level of peripheral pro-inflammatory cytokines TNF-alpha and MCP-1.

D-Dimers and sTNFR-1 were independent predictors for the unfavorable outcome at 3 months after an ischemic stroke episode. The addition of CRP, D-Dimers, NGAL, and sTNFR-1 to the basal clinical model offers greater sensitivity and specificity in the prediction of functional deficits.

PDGF-AA/AB and BDNF neurotrophins were identified as significant independent predictors, negatively associated with unfavorable mRS at 3 months, but without significant improvements in the clinical model of functional deficit estimation. RANTES, PDGF-AA/AB, PDGF-AA, and BDNF were positively associated with a low degree of functional dependence.