PhD Thesis

Novel biomarkers in the era of modern stroke management

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I. Introduction

Stroke was defined by WHO in 1970, as rapidly developing clinical signs of focal (or global) cerebral deficits, with symptoms lasting 24 hours or longer, or leading to death, with no apparent cause other than vascular origin. Of all strokes, 85% are ischaemic (IS), 10% intracerebral hemorrhage and 5% subarachnoid hemorrhage (SAH). Stroke is the second leading cause of disability and death worldwide, affecting 15 million people per year, 5 million of them die, and another 5 million are permanently physically challenged. Beyond doubt, stroke has serious economic and social consequences. The public health burden of stroke is likely to increase in the future decades, therefore, there is an urgent need to find reliable markers that predict complications, support decision-making, and be promising in treatment.

In 2001 the National Institute of Health defined biomarkers as „objectively measured characteristics evaluated as an indication of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention". Biomarkers play an important role in screening for diseases, diagnosis, drug discovery and development, and can also be useful to predict disease course and potential outcome. Over the past decade, the pathophysiological role of several blood biomarkers in the development of IS and SAH has been elucidated, furthermore, diagnostic, and prognostic markers have been identified.

Periostin is a 93 kDa secreted N-glycoprotein that mediates cell-matrix interactions and cell functions in the extracellular matrix. It has been identified in many pathological conditions such as cardiovascular diseases, gastrointestinal diseases, tumors, and respiratory inflammatory diseases. Periostin under physiological conditions, is expressed at low a level in human tissues but can be rapidly upregulated by various pathophysiological signals. The neuroprotective and neurogenic effects of exogenous periostin have been demonstrated in both in vitro and in vivo IS models. Other studies have shown that increased serum periostin levels following IS are associated with higher NIHSS scores and more extensive stroke volume in patients with large artery atherosclerotic stroke. Several studies have proven that the ASPECT score is a simple and effective tool for assessing the collateral system by helping to estimate the core:penumbra ratio and thus the extent of the collateral network. Based on an analysis of a large stroke registry database, we know that better collaterals are associated with lower core volume and higher ASPECT score, but not with higher penumbra volume. This suggests that collaterals play a significant role in early tissue loss.

Medical complications such as rebleeding, hydrocephalus, cerebral vasospasm (CV), increased intracranial pressure, seizures and delayed cerebral ischemia (DCI) may occur following aneurysmal subarachnoid hemorrhage (aSAH). DCI is defined as a symptomatic vasospasm and/or infarction attributable to vasospasm. The prognosis for patients with aSAH is heavily influenced by the development of DCI. Diagnosing and managing this condition is particularly difficult. A laboratory biomarker that would allow timely, specific, and sensitive diagnosis of DCI, would be an ideal solution. In contrast to other diseases where serum biomarkers are routinely used, there are still no effective serum biomarkers in clinical practice to predict DCI or monitor therapeutic efficacy. However, analysis of a comprehensive biomarker profile may help to gain a broader view of possible pathophysiological processes.

II. Aims

The overall aim of this thesis is to explore the clinical utility of biomarkers potentially associated with acute IS and aSAH.

2.1. Ischemic stroke

We aimed to measure serum periostin levels in the hyperacute phase of ischemic stroke in order to reveal its predictive power in identifying patients with poor collateral network.

(i) How does serum periostin level correlate with 90-day outcome?

(ii) How ASPECT score and NIHSS correlate with systemic concentration of periostin?

(iii) How associate the periostin level with ASPECT score < 6 calculated on admission CT scan?

2.2. Aneurysmal subarachnoid hemorrhage

As a single biomarker study is not suitable for the complex characterization of the mechanisms underlying DCI, we aimed to create a biomarker profile to investigate the relationship of biomarkers to DCI and the functional outcome and their relationship to each other.

(i) Are MCP-3 and CX3CL1 levels associated with the occurrence of DCI?

(ii) Are IP-10, MCP-3, and MIP-1b levels correlating with Day 30 adverse outcome?

(iii) Is the serum level of IL-4 significantly higher in TCD-positive patients than in TCD-negative ones?

III. Methods

3.1. Ischemic stroke

This was a prospective observational study from a tertiary stroke treatment center in Pecs, during the period between July 2019 and April 2021, a total of 122 patients. Acute IS was diagnosed according to WHO criteria. The following inclusion criteria were applied: (i) first-ever ischemic stroke, (ii) admission within 6 h after the index event. Exclusion criteria were as follows: (i) <18 years; (ii) previous ischemic or hemorrhagic stroke; (iii) premorbid modified Rankin score (mRS)> 1; (iv) active malignant or autoimmune disease; (v) immunosuppressive therapy; (vi) acute or chronic infection at study enrollment; (vii) severe hepatic or renal insufficiency; (viii) and pregnancy. Stroke severity was assessed using GCS and NIHSS scores, while the early ischemic changes were evaluated by ASPECT score calculated on admission. The unfavorable outcome was defined as an mRS score > 2 at 90 days after IS. As controls, we recruited fifteen age- and sex-matched healthy individuals. The venous blood samples were collected on admission to the stroke unit immediately after NCCT scan, but not later than 6 h after symptom onset. The blood samples were immediately centrifuged at 3500 r/min for 15 min and aliquots of the samples were immediately stored at −80 ◦C before assay. Biomarker concentrations were measured by using ELISA-based kits. Patients received the standard stroke care: (i) within 4.5 hours, if there were no contraindications, they received IVrtPA; if the suspicion of large vessel occlusion arose

(NIHSS>8), CT angiography was performed; if a large vessel occlusion was confirmed (MCA—M1, ICA or basilar artery), thrombectomy was also performed after thrombolysis (ii; EVT + IVrtPA) or without systemic thrombolysis (iii; EVT alone). Patients with an ASPECT score < 6 on admission were considered to have a poor collateral network.

3.2. Aneurysmal subarachnoid hemorrhage

This was a prospective observational study from a tertiary stroke treatment center in Pecs, during the period between November 2018 and December 2021. All patients ≥ 18 years of age with a newly diagnosed aSAH admitted to our hospital were offered enrollment into this study. Exclusion criteria were: (i) traumatic SAH, (ii) pregnancy, (iii) hospital admission later than 24 h after ictus, (iv) no aneurysm treatment, (v) absence of a signed consent form, (vi) underlying SARS-CoV-2 infection, and (vii) systemic diseases (chronic neurological disease, tumors, liver and/or renal insufficiency, and chronic lung disease). All included patients underwent CTA or MRA before admission and conventional cerebral angiography after admission, and received treatment according to clinical treatment guidelines. The severity of aSAH was assessed using WFNS grade and mFisher scale. Samples were collected from the patients at two-time points: (i) 24 h after ictus (Day 1) and (ii) 5–7 days after ictus and were centrifuged at 3500 r/min for 13 min and stored at −80 ◦C until measurement. Serum concentrations of CCL11, FGF-2, FLT-3L, CX3CL1, IL-1b, IL-4, IP-10, MCP-3 and MIP-1b were determined using multiplex assay according to the manufacturer's protocol. In all cases, the patient spent at least 12–14 days in the neurointensive care unit, so that expected complications (e.g., DCI) could be detected in time. We used the widespread, consensus definition of DCI. DCI was screened by using TCD from admission every day of hospital care. TCD spasm indicated TCD positivity (TCD+) was diagnosed by daily transcranial Doppler measurements and defined as a peak-value increase by >50 cm/s/24 h compared with the previous result or a mean value >120 cm/s in one of the main supply branches. If DCI was suspected, MRI and catheter angiography were performed to confirm macrovascular vasospasm and DCI. If vasospasm was confirmed, intra-arterial nimodipine was administered. The favorable outcome was defined as an mRS score 0-2 while the unfavorable was 3-6.

IV. Results

4.1. Ischemic stroke

4.1.1. Clinical characteristics

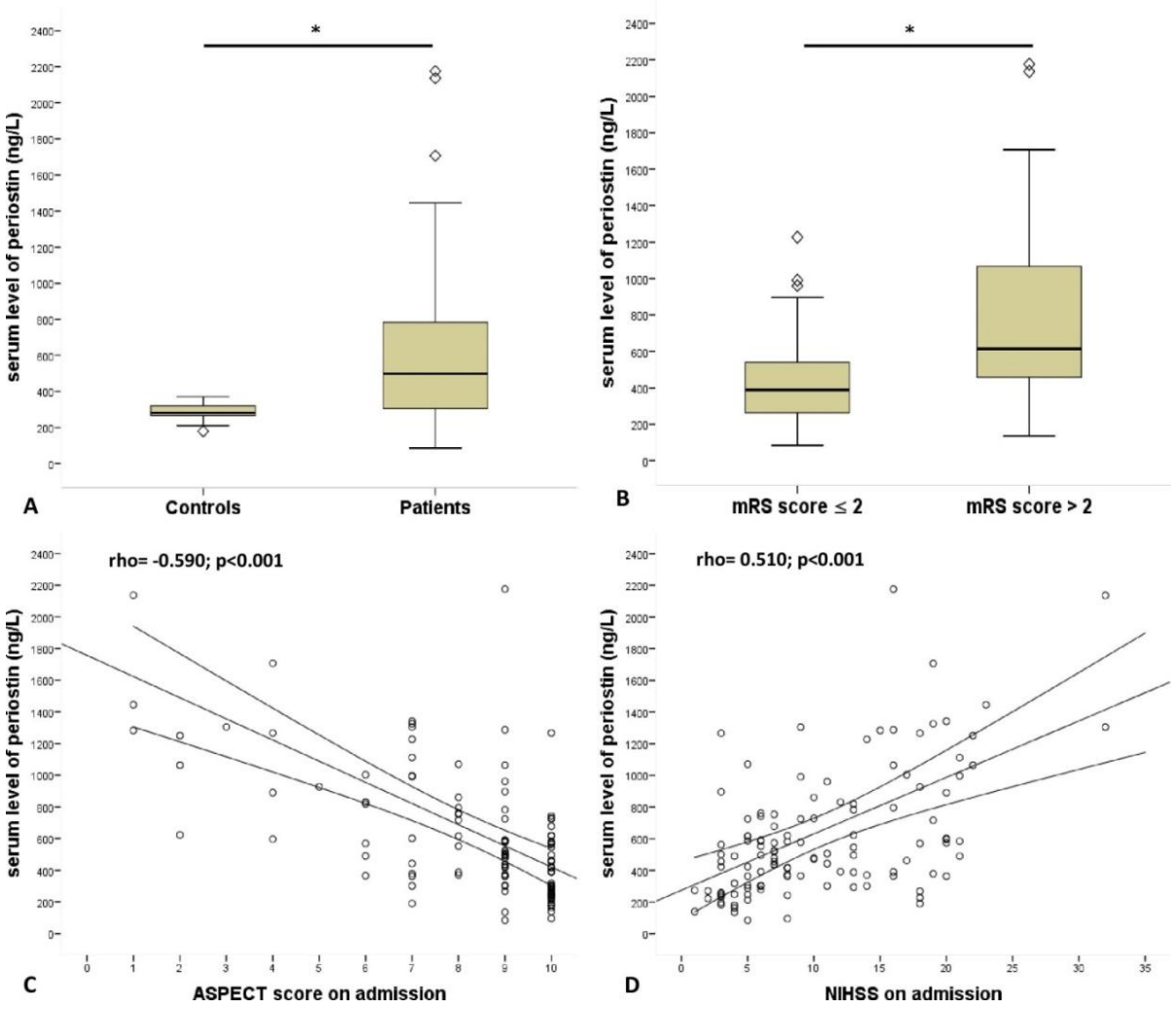
This cross-sectional study enrolled 122 patients with first-ever acute IS. **Table 1** shows clinical profiles of patient groups based on their best mRS scores at 3 months as the primary outcome measure. The median age of the patients was 71 years (interquartile range: 63–79, min-max. values: 30–91) and 39.3% were female. Fifteen healthy volunteers served as age-matched normal controls. The median age of controls was 66 (interquartile range (IQR): 55–73, range 46–82), and 46.7% were female. The age and sex differences between patients and controls were non-significant. Regarding comorbidities, 100 patients (82%) had hypertension, 35 patients (29%) had diabetes, and 34 patients (27.9%) presented with AF. The median admission NIHSS score was 8 (IQR: 5–16, min-max: 1–32), and the median systolic and diastolic blood pressure on admission was 150 mmHg (IQR: 130–170, range: 90–240) and 84 mmHg (IQR: 80–93.5, range: 48–118). The median ASPECT score was 9 (IQR: 7–10, min-max: 1–10). In total, 29 patients (24%) underwent EVT, 51 (42%) received IVrtPA treatment, and 17 (14%) underwent a combined EVT + IVrtPA treatment. A total of 25 patients (20.5%) were not eligible either for EVT or for IVrtPA; therefore, they received conservative treatment. The median serum level of periostin was 498.4 ng/L (IQR, 305–783) in patients with IS, and 280.4 ng/L (IQR, 259–332) in healthy controls ($p < 0.001$, **Figure 1A**).

Table 1. Baseline characteristics of the study population.

Characteristics	Total (n=122)	Favorable* outcome (n=59)	Unfavorable* outcome (n=63)	*p*=value
Age, y, median (IQR)	71 (63-79)	71 (62-77)	73 (64-79)	0.127
Male, n (%)	74 (61)	35 (59)	39 (62)	0.770
Hypertension, n (%)	100 (82)	48 (81.4)	52 (82.5)	0.865
Diabetes, n (%)	35 (29)	17 (29)	18 (29)	0.976
Smoking, n (%)	52 (3)	19 (32)	33 (52)	0.024*
Atrial fibrillation, n (%)	34 (28)	7 (12)	27 (43)	<0.001*
GCS, median (IQR)	15 (12-15)	15 (15)	14 (11-15)	<0.001*
NIHSS , median (IQR)	8 (5-16)	6 (4-8)	13 (8-19)	<0.001*
SBP, median (IQR), Hgmm	150 (130-170)	148 (130-170)	160 (138-180)	0.237
DBP, median (IQR), Hgmm	84 (80-94)	82 (80-90)	86 (80-100)	0.463
ASPECTS, median (IQR)	9 (7-10)	10 (9-10)	8 (6-9)	<0.001*
WBC, median (IQR), G/L	8.4 (6.9-10.7)	7.7 (9-10)	8.8 (7-11)	0.264
NLR, median (IQR)	2.9 (2-5.6)	2.5 (1.7)	3.6 (2.5-7.3)	0.002*
platelet, median (IQR), G/L	242 (188-306)	245 (196-300)	238 (185-305)	0.625
creatinine, median (IQR), μmol/L	86 (73-102)	83 (70-97)	87 (74-104)	0.411
glucose , median (IQR), mmol/L	7.2 (6.2-8.9)	6.8 (5.9-8.1)	7.8 (6.8-9)	0.004*
CRP, median (IQR), mg/L	3.7 (1.4-9.5)	2.6 (1.4-5.4)	5.1 (1.7-16)	0.042*
Thrombectomy, n (%)	29 (23.8)	14 (23.7)	15 (23.8)	0.856
Intravenous tPA, n (%)	51 (41.8)	28 (47.5)	23 (36.5)	0.190
Thrombectomy plus intravenous tPA, n (%)	17 (13.9)	6 (10.2)	11 (17.5)	0.260
Conservative, n (%)	25 (20.5)	11 (18.6)	14 (22.2)	0.658
serum level of periostin, median (IQR), ng/L	462 (297-735)	390 (260-563)	615 (443-1070)	<0.001*

Abbreviations: GCS, Glasgow coma scale; NIHSS, National Institutes of Health Stroke Scale; SBP, systolic blood pressure; DBP, diastolic blood pressure; ASPECTS, Alberta stroke programme early CT score; WBC, white blood cell; NLR, neutrophil-lymphocyte ratio; CRP, C-reactive protein; tPA, tissue plasminogen activator; * at Day 90 follow-up. *Note:* Continuous variables are expressed as medians (interquartile ranges). Categorical values are given as frequencies (percentages).

Figure 1. Comparison of serum periostin level (A) among patients with stroke and controls, (B) between patients with favorable outcome (mRS≤2) vs. unfavorable outcome (mRS>2) on Day 90 follow-up. Correlation of admission serum periostin level with (C) ASPECT score measured on admission and (D) NIHSS score recorded on admission.



Abbreviations: ASPECT, Alberta stroke programme early CT score; mRS, modified Rankin score; NIHSS, National Institutes of Health Stroke Scale. *Note:* Serum periostin level was measured at 24 hours after stroke onset. *p-value <0.001. Statistical analysis was performed with Mann-Whitney U-test (A,B) and using Spearman rank correlation (C,D).

4.1.2. Admission periostin level, comorbidities and outcome

The admission serum periostin concentration was significantly higher in those patients who later had an unfavorable 90-day outcome compared to patients with favorable outcome (**Figure 1B** and **Table 1**). Moreover, the admission concentration of serum periostin showed an inverse correlation with ASPECT score (**Figure 1C**), while it was positively correlated with the admission NIHSS score (**Figure 1D**). In multivariate analysis, we found positive associations between admission level of periostin andAF, admission white blood cell (WBC) count, NLR, creatinine, CRP, and glucose level (**Table 2**). In contrast, serum periostin level was negatively associated with GCS and ASPECT score, both measured at admission.

Table 2. Spearman correlation between admission clinical parameters and serum periostin level measured at 24 hours after admission.

Variable	Spearman correlation coefficient (r)	*p*-value
Atrial fibrillation	0.335	**<0.001**
Systolic blood pressure	0.068	0.459
Dyastolic blood pressure	0.119	0.193
Glasgow Coma Scale	-0.308	**<0.001**
ASPECT score	-0.590	**<0.001**
White blood cell count, G/L	0.239	**0.01**
Neutrophil-lymphocyte ratio	0.328	**<0.001**
Creatinine, μmol/L	0.277	**0.003**
C-reactive protein, mg/L	0.285	**0.002**
Glucose, mmol/L	0.257	**0.007**
Platelet, G/L	-0.059	0.534
Carbamide, mmol/L	0.245	**0.01**

Abbreviations: ASPECT, Alberta stroke programme early CT score. *Note:* Coefficient (r) values > 0 indicate a positive association; values < 0 indicate a negative association. Statistically significant values are given in bold.

4.1.3. Variables associated with poor collaterals

ASPECT score < 6 indicating patients with poor collaterals on admission were positively associated with admission GCS in univariate analysis. In contrast, diabetes, AF, admission NIHSS, periostin level, NLR, CRP as well as creatinine levels were inversely associated with ASPECT score < 6 calculated in univariate analysis. Age and sex were not shown correlation with ASPECT < 6. Although GCS, NIHSS, AF, CRP, diabetes, creatinine and NLR were adjusted in a binary logistic regression model, serum periostin level remained a significant predictor for ASPECT < 6 status on admission (OR, 5.911; CI, 0.990–0.999; p = 0.015, **Table 3**). Next, another binary logistic regression analysis was performed to explore independent predictors of the outcome: NIHSS on admission and atrial fibrillation were independently associated with a favorable 90-day outcome (mRS0–2). Based on ROC (receiver operating characteristic) analysis, both NIHSS score (AUC, 0.817; 95% CI, 0.743–0.892, cutoff: 8.5, sensitivity: 75%, specificity: 78%, p < 0.001) and admission serum concentration of periostin (AUC, 0.757; 95%CI, 0.672–0.841, cutoff: 466.7 ng/L, sensitivity: 75 %, specificity: 65%, p < 0.001) showed similar sensitivity and specificity in the prediction of unfavorable 3-month outcome. In contrast, the combination of these two variables had significantly greater predictive power (AUC, 0.842; 95% CI, 0.773–0.911, p < 0.001) (**Figure 2**). The serum concentration of periostin with a cut-off value of ≥594.5 independently predicted admission ASPECT < 6 reflecting the poor collateral status with a sensitivity of 84.2% and specificity of 72%.

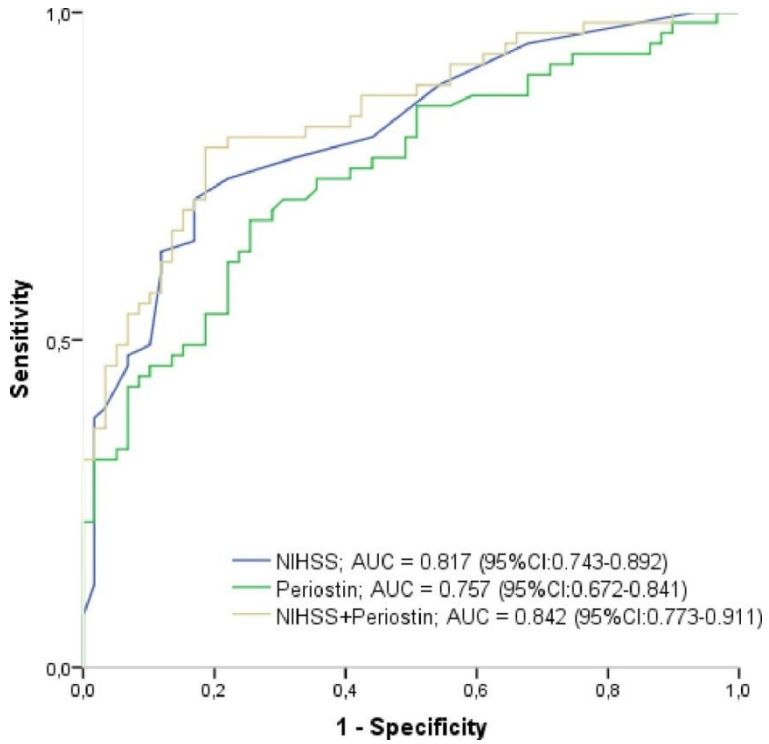
Table 3. Binary logistic regression analysis of predictors for admission ASPECT score <6 in patients with acute ischemic stroke.

	Odds ratio	**95% CI**	**P-value**
periostin	15.532	0.995-0.998	<0.001
Model 1	6.339	0.995-0.999	0.012
Model 2	5.917	0.993-0.999	0.015
Model 3	5.911	0.990-0.999	0.015

Abbreviations: CI, confidence interval.

Note: Model 1 included Glasgow Coma Scale and National Institute of Health Stroke Scale. Model 2 included variables in model 1 plus atrial fibrillation and admission level of C-reactive protein. Model 3 included variables in model 2 plus diabetes, admission serum level of creatinine and admission neutrophil-lymphocyte ratio.

Figure 2. Receiver operating characteristic curve analysis of prognostic predictive ability of serum NIHSS score on admission, serum level of periostin on admission and the combination of NIHSS score and periostin for 3-month unfavorable outcome in patients with acute ischemic stroke.



Abbreviations: AUC, area under the curve; CI, confidence interval; NIHSS, National Institute of Health Stroke Scale

4.2. Aneurysmal subarachnoid hemorrhage

4.2.1. Clinical characteristics

One hundred and twelve patients with aSAH (**Table 4**) were included in this study. Patients were enrolled between November 2018 and December 2021. All (100%) of the aneurysms were secured by coiling. Patients had a mean age of 57 (SD13) and 62% were female. Of them, 38 patients with aSAH (34%) presented to the emergency department with a WFNS Grade I. Almost half of the patients had a history of arterial hypertension (43.8%) and 11% had a history of smoking. Nearly one-third of the patients had DCI (29.1%) during their in-hospital stay. A description of these aSAH patients is shown in **Table 4**.

Table 4. Patients characteristics.

Number of patients with aneurysmal SAH, *n*=112		
Age	(years, mean±SD)	57±13
Female	(N,%)	69 (61.6)
Hypertension	(N,%)	49 (43.8)
Diabetes mellitus	(N,%)	11 (9.8)
Smoking	(N,%)	12 (10.7)
Aneurysm location		
– Internal carotid artery	(N,%)	16 (14.3)
– Middle cerebal artery	(N,%)	22 (19.6)
– Anterior communicating artery	(N,%)	31 (27.7)
– Posterior communicating artery	(N,%)	13 (11.6)
– Anterior cerebral artery	(N,%)	14 (12.5)
– Vertebrobasilar	(N,%)	16 (14.3)
WFNS		
– 1	(N,%)	38 (33.9)
– 2	(N,%)	24 (21.4)
– 3	(N,%)	8 (7.1)
– 4	(N,%)	14 (12.5)
– 5	(N,%)	28 (25)
modified Fischer grade		
– 1	(N,%)	1 (0.9)
– 2	(N,%)	18 (16,1)
– 3	(N,%)	57 (50.9)
– 4	(N,%)	36 (32.1)
Glasgow coma scale, on admission	median, IQR	13 (6-15)
Neutrophile-lymphocyte ratio, on admission	median, IQR	5.9 (4-10)
C-reactive protein, on admission, mg/L	median, IQR	13 (4-61)
Creatinine, on admission, µmol/L	median, IQR	61 (50-72)
Extraventricular drainage	(N,%)	53 (47.3)
Infection, CSF	(N,%)	7 (6.3)
Infection, systemic	(N,%)	18 (16.1)
Infection, CSF+systemic	(N,%)	5 (4.5)
Mechanical ventillation	(N,%)	50 (44.6)
Decompressive craniotomy	(N,%)	14 (12.5)
Lumbal drainage	(N,%)	14 (12.5)
Delayed cerebral ischemia	(N,%)	32 (29.1)
Angiographic vasospasm	(N,%)	28 (28.3)
Transcranial Doppler positivity	(N,%)	41 (41.8)
Ischaemic laesion on MRI	(N,%)	16 (15.8)
Favorable outcome on Day 30 (mRS=0-2)	(N,%)	58 (51.8)
In-hospital death	(N,%)	15 (13.4)

Abbreviations: SAH, subarachnoid hemorrhage; WFNS, World Federation of Neurological Societies Score; MRI, magnetic resonance imaging; CSF, cerebrospinal fluid. *Note:* The categorical variables are displayed presented as frequency (%) and the continuous variables are displayed presented as mean ± standard deviation (SD) or median with interquartile range (IQR).

4.2.2. Cytokines associated with DCI and functional outcome

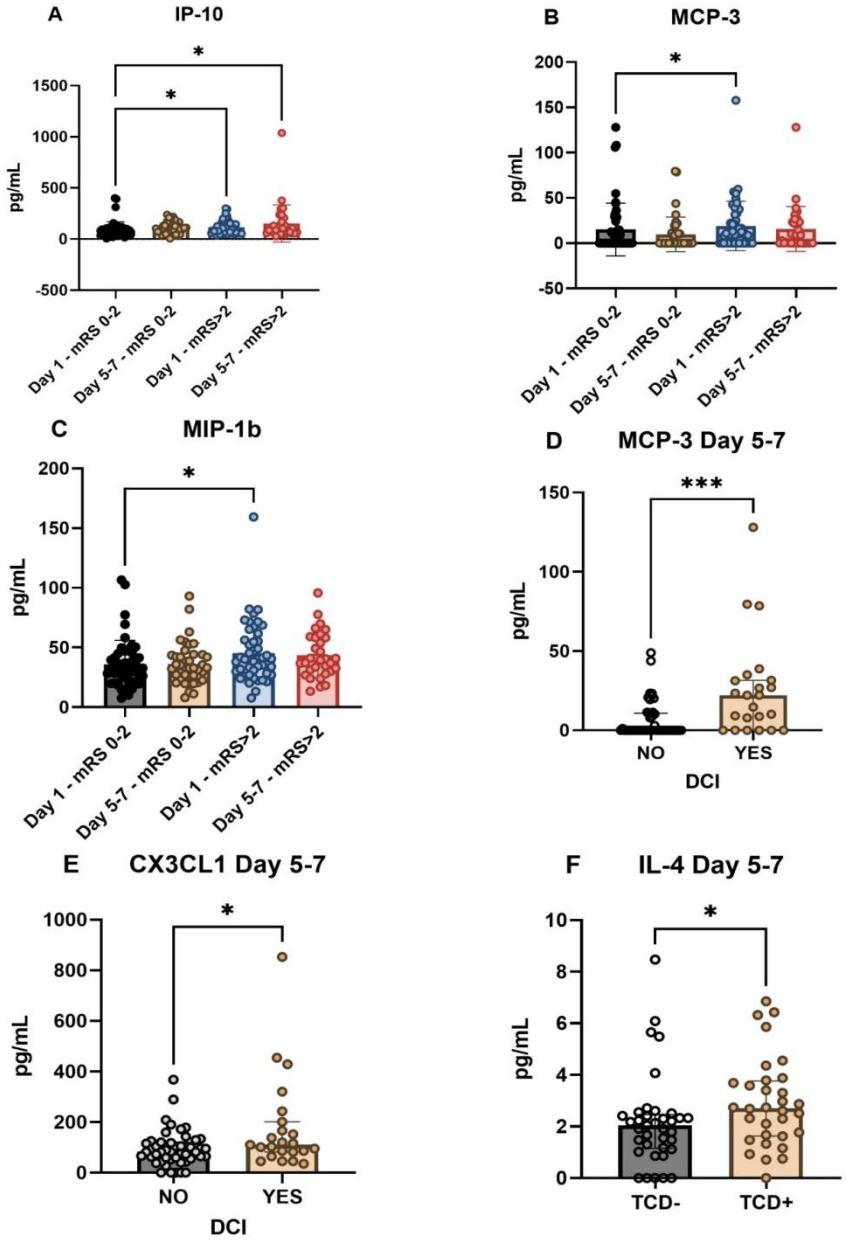
None of the cytokines tested on Day 1 were associated with DCI, whereas only cytokines measured on Day 1 (IP-10, MCP-3, MIP-1b) were associated with functional outcome (**Table 5**). CX3CL1 and MCP-3 measured on Days 5–7 were significantly higher in patients with DCI compared to those without DCI (CX3CL1: Day 5–7, without DCI: 82.6 pg/mL, IQR: 58–119 vs. Day 5–7, with DCI: 110.5 (82–201), p = 0.036 and MCP-3: Day 5–7, without DCI: 0 (0–11) vs. Day 5–7, with DCI: 22 (0–32), p < 0.001, **Figure 3**). Serum IP-10 levels in patients with poor outcomes were significantly higher than in patients with favorable outcomes at both time points (Day 1, favorable outcome: 74.7 pg/mL, IQR: 43–97 vs. Day 1, unfavorable outcome: 100, 68–146, p = 0.005 and Day 1, favorable outcome: 74.7 pg/mL, IQR: 43–97 vs. Day 5–7, unfavorable outcome: 98.8, 65–157, p = 0.004). For MCP-3 and MIP-1b, the serum concentrations measured on Day 1 showed significantly higher levels in patients with unfavorable outcome compared with the group with favorable Day 30 outcome (MCP-3: Day 1, favorable: 0 pg/mL, IQR: 0–15 vs. Day 1, unfavorable: 11.8, 0–25, p = 0.045 and MIP-1b: Day 1, favorable: 31.8 pg/mL, 23–42 vs. Day 1, unfavorable: 40, 28–56, p = 0.025, **Figure 3**).

Table 5. Cytokines associated with DCI during hospitalization and functional outcome on Day 30.

	DCI during hospitalization		**mRS score at Day 30**	
	DCI - (*n*=78 [71%]) vs. DCI + (*n*=32 [29%])		**Unfavorable outcome (mRS≥3, *n*=54 [48.2%]) vs. Favorable outcome (mRS≤2, *n*=58 [51.8%])**	
Cytokines	**Day 1**	**Day 5-7**	**Day 1**	**Day 5-7**
Eotaxin	-	-	-	-
FGF-2	-	-	-	-
FLT-3L	-	-	-	-
CX3CL1	-	H*	-	-
IL-1b	-	-	-	-
IL-4	-	-	-	-
IP-10	-	-	H*	-
MCP-3	-	H**	H*	-
MIP-1b	-	-	H*	-
Total	0	2	3	0

Abbreviations: DCI, delayed cerebral ischemia; mRS, modified-Rankin scale; FGF-2, fibroblast growth factor-2; FLT-3L, Fmsrelated tyrosine kinase 3 ligand; CX3CL1, chemokine ligand 1, also known as fractalkine; IL-1b, interleukin-1b; IL-4, interleukin-4; IP-10, interferon gamma-induced protein 10, also known as C-X-C motif chemokine ligand 10 (CXCL10); MCP-3, Monocyte chemotactic protein-3; MIP-1b, macrophage inflammatory protein 1-beta; H, high level; * p < 0.05; ** p < 0.001.

Figure 3. Characteristics of serum biomarker levels in different clinical subgroups in patients with aSAH. Correlation of the functional outcome with the investigated biomarkers, in the case of IP-10 (**A**), MCP-3 (**B**) and MIP-1b (**C**). Correlation of MCP-3 (**D**) and CX3CL1 (**E**) measured at T2 with DCI. Association of IL-4 (**F**) with TCD positivity.



Abbreviations: DCI, delayed cerebral ischemia; aSAH, aneurysmal subarachnoid hemorrhage; TCD, transcranial Doppler ultrasound; CX3CL1, chemokine ligand 1, also known as fractalkine; IL-4, interleukin-4; IP-10, interferon gamma-induced protein 10, also known as C-X-C motif chemokine ligand 10 (CXCL10); MCP-3, Monocyte chemotactic protein-3; MIP-1b, macrophage inflammatory protein 1-beta. *Note:* The functional outcome was examined 30 days after admission and characterized on the modified Rankin scale (mRS). Biomarker sampling times: Day 1, 24 h after aSAH, Day 5–7, 5–7 days after aSAH. * denotes $p < 0.05$, *** denotes $p < 0.001$.

In order to clarify how the increase of MCP-3 and CX3CL1 seen in DCI is related to the time of DCI, we performed an additional analysis. The average time of onset of DCI in our cohort was 6 ± 3.2 days (mean ± SD). We grouped the DCI cases based on the sampling dates: the cases before T2 were in Group A, while the cases after T2 were in Group B, **Table 6**.

Table 6. The association of MCP-3 and CX3CL1 levels measured at T2 with the time of DCI detection.

	No DCI (N=78)	**Group A**	**Group B**	***p*-value (between A-B)**
		DCI prior toT2 (N=7)	**DCI followingT2 N=25)**	
MCP-3 T2, pg/mL, median (IQR)	0 (0-11)	22 (8-27)	18 (0-32)	0.857
CX3CL1 T2, pg/mL, median (IQR)	83 (58-119)	116 (103-138)	106 (65-243)	0.691

Abbreviations: DCI, delayed cerebral ischemia; MCP-3, Monocyte chemotactic protein-3; CX3CL1, chemokine ligand 1, also known as fractalkine. *Note:* T1, serum sample at Day 1 following aSAH; T2, serum sample at Days 5-7 followingaSAH.

4.2.3. Clinical variables associated with DCI and Day 30 functional outcome

We found no significant association between admission WFNS and Fischer scores and the development of DCI in aSAH patients. Similarly, there was no association between demographic (female, age) and clinical risk factors (hypertension, diabetes, smoking) and the emergence of DCI during hospital stay (**Table 7**). The admission GCS score was significantly lower in the DCI group than in the non-DCI group (DCI: 9, IQR: 5–14 vs. no DCI: 14, 10–15, p = 0.02). Decompressive craniectomy was required more frequently in the DCI group, but there was no difference between the two groups in terms of EVD use. Other factors related to DCI and Day 30 functional outcome are shown in **Table 7-8**. We found that regardless of whether the patient had an infection or not during hospitalization, the serum level of MCP-3 was significantly higher in the DCI group than in the non-DCI group. In contrast, CX3CL1 concentration measured at T2 did not show a significant difference in the two groups of DCI, regardless of the presence of an infection (**Table 9**).

Table 7. Comparison of clinical and biochemical characteristics between patients with and without DCI in patients with aneurysmal subarachnoid hemorrhage.

Variable	DCI		*p*-value
	DCI (*n* = 32)	**No-DCI (*n* = 78)**	
Age (years, mean±SD)	54.8±11	57.9±14	0.223
Female, N (%)	17 (53)	50 (64)	0.284
Hypertension, *n* (%)	11 (34.4)	37 (47.4)	0.210
Diabetes mellitus, *n* (%)	3 (9.4)	8 (10.3)	0.889
Smoking, *n* (%)	2 (6.3)	10 (12.8)	0.315
WFNS, median (IQR)	3 (1–5)	2 (1–4)	0.412
modified Fischer grade, median (IQR)	3 (2–4)	3 (2–4)	1.000
Glasgow coma scale, median (IQR)	9 (5–14)	14 (10–15)	0.02
Neutrophile-lymphocyte ratio, median (IQR)	7 (5–10)	5 (3–11)	0.092
C-reactive protein, median (IQR)	24 (5–75)	9.5 (3–43)	0.104
Creatinine, median (IQR)	61 (50–72)	60 (50–72)	0.744
Extraventricular drainage, *n* (%)	18 (56.3)	33 (42.3)	0.183
Mechanical ventilation, *n* (%)	19 (59.4)	29 (37.2)	0.033
Decompressive craniotomy, *n* (%)	8 (25)	5 (6.4)	0.006
Angiographic vasospasm, *n* (%)	27 (84.4)	1 (1.5)	<0.001
Transcranial Doppler positivity, N (%)	30 (96.8)	11 (16.4)	<0.001
Ischemic lesion on MRI, N (%)	16 (50)	0 (0)	<0.001

Abbreviations: IQR, interquartile range; DCI, delayed cerebral ischemia; WFNS, World Federation of Neurological Surgeons; MRI, magnetic resonance imaging. *Note:* The categorical variables are presented as frequency and percentage, and the continuous variables are presented as mean ± standard deviation or median (percentile 25–75). The significances of inter-group differences were assessed using chi-square test or Fisher exact test for categorical data as well as Student t test or Mann–Whitney U test for continuous variables.

Table 8. Comparison of clinical and biochemical characteristics between patients with unfavorable vs. favorable outcome (Day 30) in patients with aneurysmal subarachnoid hemorrhage.

Variable	Functional Outcome at Day 30		*p*-Value
	Unfavorable (*n* = 54)	**Favorable (*n* = 58)**	
Age (years, mean±SD)	61.8 ± 12	52.6 ± 12	<0.001
Female, N (%)	29 (53.7)	40 (69)	0.097
Hypertension, *n* (%)	28 (51.9)	21 (36.2)	0.095
Diabetes, *n* (%)	10 (18.5)	1 (1.7)	0.003
Smoking, *n* (%)	4 (7.4)	8 (13.8)	0.275
WFNS, median (IQR)	4 (3–5)	1 (1–2)	<0.001
modified Fischer grade, median (IQR)	4 (3–4)	2 (1–3)	<0.001
Glasgow coma scale, median (IQR)	6 (3–12)	14 (13–15)	<0.001
Neutrophile-lymphocyte ratio, median (IQR)	7 (4–12)	5.3 (3–8)	0.054
C-reactive protein, median (IQR)	41 (9–89)	6.8 (3–17)	<0.001
Creatinine, median (IQR)	63 (50–76)	59 (50–67)	0.122
Extraventricular drainage, *n* (%)	41 (75.9)	12 (20.7)	<0.001
Mechanical ventilation, *n* (%)	43 (79.6)	7 (12.1)	<0.001
Decompressive craniotomy, *n* (%)	11 (20.4)	3 (5.2)	0.015
Angiographic vasospasm, *n* (%)	22 (52.4)	6 (10.5)	<0.001
Transcranial Doppler positivity, N (%)	23 (54.8)	18 (32.1)	0.025
Ischemic lesion on MRI, N (%)	16 (35.6)	0 (0)	<0.001

Abbreviations: IQR, interquartile range; DCI, delayed cerebral ischemia; WFNS, World Federation of Neurological Surgeons; MRI, magnetic resonance imaging. *Note:* Favorable outcome = modified Rankin score 0–2, unfavorable = 3–6. The categorical variables are presented as frequency and percentage, and the continuous variables are presented as mean ± standard deviation or median (percentile 25–75). The significances of inter-group differences were assessed using chi-square test or Fisher exact test for categorical data as well as Student t test or Mann–Whitney U test for continuous variables.

Table 9. Correlation between the occurrence of infection the appearance of DCI and biomarker values in aSAH patients.

	No Infection			**Infection during Hospitalization**		
	No DCI	**DCI**	***p*-Value**	**No DCI**	**DCI**	***p*-Value**
MCP-3 T2, pg/mL, median (IQR)	0 (0–11)	12 (0–32)	0.025	0 (0–8)	23 (9–27)	0.004
CX3CL1 T2, pg/mL, median (IQR)	82 (53–118)	102 (46–201)	0.221	94 (60–179)	116 (95–166)	0.152

Abbreviations: IQR, interquartile range; DCI, delayed cerebral ischemia; MCP-3, Monocyte chemotactic protein-3; CX3CL1, chemokine ligand 1, also known as fractalkine. *Note:* T2, serum sample at Day 5–7 after aSAH.

4.2.4. Correlations between biomarkers in aSAH patients

Correlations for all measured serum biomarkers at both measurement time points were examined. The Spearman r coefficient of correlation between all these parameters is presented as a heat-map in **Figure 4**. The heat-map confirmed a positive and strong correlation between IL-1b and FGF-2, CX3CL1 and MCP-3, as well as between MCP-3 and FGF-2 at T1 time point. For biomarkers measured at T2, only the correlation between MCP-3 and CX3CL1 remained strong. For more correlations see **Figure 4**.

Figure 4. Correlation between different serum biomarkers in patients with aSAH.

	Eotaxin T1	FGF-2 T1	FLT-3L T1	CX3CL 1 T1	IL-1b T1	IL-4 T1	IP-10 T1	MCP-3 T1	MIP-1b T1	Eotaxin T2	FGF-2 T2	FLT-3L T2	CX3CL 1 T2	IL-1b T2	IL-4 T2	IP-10 T2	MCP-3 T2	MIP-1b T2
Eotaxin T1	1,000	0,233	0,237	0,060	0,233	0,153	0,095	0,116	0,185	0,654	0,278	0,118	0,144	0,233	0,220	-0,122	0,208	0,039
FGF-2 T1	0,233	1,000	0,295	0,461	0,694	0,346	-0,086	0,524	0,151	0,259	0,724	0,367	0,257	0,580	0,219	-0,337	0,247	-0,116
FLT-3L T1	0,237	0,295	1,000	0,241	0,268	0,330	0,193	0,226	0,218	0,323	0,197	0,607	0,075	0,342	0,036	0,021	0,088	0,052
CX3CL1 T1	0,060	0,461	0,241	1,000	0,356	0,516	0,239	0,778	0,242	0,069	0,174	0,013	0,857	0,226	0,352	0,226	0,424	0,145
IL-1b T1	0,233	0,694	0,268	0,356	1,000	0,276	0,039	0,438	0,239	0,224	0,530	0,214	0,206	0,833	0,200	-0,181	0,210	0,102
IL- 4 T1	0,153	0,346	0,330	0,516	0,276	1,000	0,261	0,435	0,235	0,163	0,134	0,084	0,395	0,180	0,694	0,064	0,223	0,121
IP-10 T1	0,095	-0,086	0,193	0,239	0,039	0,261	1,000	0,216	0,411	-0,184	-0,243	-0,071	0,085	-0,126	0,046	0,519	-0,076	0,371
MCP-3 T1	0,116	0,524	0,226	0,778	0,438	0,435	0,216	1,000	0,210	0,054	0,298	0,044	0,615	0,260	0,348	0,002	0,687	0,128
MIP-1b T1	0,185	0,151	0,218	0,242	0,239	0,235	0,411	0,210	1,000	0,038	0,017	0,055	0,073	0,140	0,013	0,177	-0,039	0,643
Eotaxin T2	0,654	0,259	0,323	0,069	0,224	0,163	-0,184	0,054	0,038	1,000	0,194	0,242	0,095	0,037	0,196	-0,112	0,053	-0,058
FGF-2 T2	0,278	0,724	0,197	0,174	0,530	0,134	-0,243	0,298	0,017	0,194	1,000	0,182	0,415	0,486	0,247	-0,413	0,445	-0,121
FLT-3L T2	0,118	0,367	0,607	0,013	0,214	0,084	-0,071	0,044	0,055	0,242	0,182	1,000	-0,019	0,203	0,071	0,044	0,018	0,149
CX3CL1 T2	0,144	0,257	0,075	0,857	0,206	0,395	0,085	0,615	0,073	0,095	0,415	-0,019	1,000	0,237	0,443	0,133	0,637	-0,022
IL-1b T2	0,233	0,580	0,342	0,226	0,833	0,180	-0,126	0,260	0,140	0,037	0,486	0,203	0,237	1,000	0,248	-0,213	0,325	0,035
IL-4 T2	0,220	0,219	0,036	0,352	0,200	0,694	0,046	0,348	0,013	0,196	0,247	0,071	0,443	0,248	1,000	0,008	0,430	0,107
IP-10 T2	-0,122	-0,337	0,021	0,226	-0,181	0,064	0,519	0,002	0,177	-0,112	-0,413	0,044	0,133	-0,213	0,008	1,000	-0,134	0,328
MCP-3 T2	0,208	0,247	0,088	0,424	0,210	0,223	-0,076	0,687	-0,039	0,053	0,445	0,018	0,637	0,325	0,430	-0,134	1,000	-0,096
MIP-1b T2	0,039	-0,116	0,052	0,145	0,102	0,121	0,371	0,128	0,643	-0,058	-0,121	0,149	-0,022	0,035	0,107	0,328	-0,096	1,000

Abbreviations: FGF-2, fibroblast growth factor-2; FLT-3L, Fms-related tyrosine kinase 3 ligand; CX3CL1, chemokine ligand 1, also known as fractalkine; IL-1b, interleukin-1b; IL-4, interleukin-4; IP-10, interferon gamma-induced protein 10, also known as C-X-C motif chemokine ligand 10 (CXCL10); MCP-3, Monocyte chemotactic protein-3; MIP-1b, macrophage inflammatory protein 1-beta; aSAH, aneurysmal subarachnoid hemorrhage.

Note: red indicates that the two parameters were positively correlated, and blue indicates that the two parameters were negatively correlated; the darker the color, the stronger the correlation. T1, serum sample at Day 1 after aSAH; T2, serum sample at Day 5–7 after aSAH. Statistical method: Spearman.

The binary logistic regression analysis identified serum Day 5–7 MCP-3 levels as an independent predictor for DCI status, **Table 10**. Serum level of FGF-2 showed a strong negative correlation with serum level of IP-10 in patients with favorable outcome, while this correlation disappeared in the case of the group with an unfavorable outcome (**Figure 5**).

Table 10. Binary logistic regression model of independent predictors of DCI status after aSAH.

	B	**Wald**	**Sig.**	**Exp(B)**
MCP-3 T2	0.045	5.221	0.022	1.046
GCS on admission	−0.031	−0.062	0.803	0.97
Mechanical Ventilation	−0.954	0.638	0.424	0.385
Sex	−0.974	2.496	0.114	0.378
Age	−0.026	1.062	0.303	0.974
Constant	1.593	0.922	0.337	4.917

Abbreviations: GCS, Glasgow coma scale; aSAH, aneurysmal subarachnoid hemorrhage; MCP-3, Monocyte chemotactic protein-3; DCI, delayed cerebral ischemia. *Note:* T2, sample time: Day 5–7 after aSAH.

Figure 5. Correlation between serum biomarkers in different clinical subgroups (A): favorable, n = 58; (B): unfavorable, n = 58).

A	FGF-2 T1	CX3CL1 T1	IL-1b T1	IP-10 T1	MCP-3 T1	FGF-2 T2	CX3CL1 T2	IL-1b T2	IP-10 T2	MCP-3 T2
FGF-2 T1	1,000	0,523	0,684	-0,253	0,613	0,867	0,306	0,467	-0,420	0,320
CX3CL1 T1	0,523	1,000	0,369	0,137	0,730	0,121	0,872	0,195	0,049	0,564
IL-1b T1	0,684	0,369	1,000	-0,126	0,481	0,569	0,216	0,849	-0,345	0,211
IP-10 T1	-0,253	0,137	-0,126	1,000	0,090	-0,570	-0,034	-0,260	0,485	-0,158
MCP-3 T1	0,613	0,730	0,481	0,090	1,000	0,124	0,461	0,179	-0,165	0,729
FGF-2 T2	0,867	0,121	0,569	-0,570	0,124	1,000	0,480	0,495	-0,517	0,475
CX3CL1 T2	0,306	0,872	0,216	-0,034	0,461	0,480	1,000	0,235	0,011	0,697
IL-1b T2	0,467	0,195	0,849	-0,260	0,179	0,495	0,235	1,000	-0,366	0,295
IP-10 T2	-0,420	0,049	-0,345	0,485	-0,165	-0,517	0,011	-0,366	1,000	-0,164
MCP-3 T2	0,320	0,564	0,211	-0,158	0,729	0,475	0,697	0,295	-0,164	1,000

B	FGF-2 T1	CX3CL1 T1	IL-1b T1	IP-10 T1	MCP-3 T1	FGF-2 T2	CX3CL1 T2	IL-1b T2	IP-10 T2	MCP-3 T2
FGF-2 T1	1,000	0,409	0,722	0,068	0,465	0,708	0,260	0,712	-0,210	0,242
CX3CL1 T1	0,409	1,000	0,346	0,345	0,841	0,247	0,866	0,337	0,293	0,350
IL-1b T1	0,722	0,346	1,000	0,194	0,388	0,559	0,215	0,817	0,042	0,250
IP-10 T1	0,068	0,345	0,194	1,000	0,236	-0,006	0,194	-0,017	0,579	-0,115
MCP-3 T1	0,465	0,841	0,388	0,236	1,000	0,464	0,781	0,431	0,150	0,648
FGF-2 T2	0,708	0,247	0,559	-0,006	0,464	1,000	0,326	0,532	-0,300	0,374
CX3CL1 T2	0,260	0,866	0,215	0,194	0,781	0,326	1,000	0,291	0,246	0,559
IL-1b T2	0,712	0,337	0,817	-0,017	0,431	0,532	0,291	1,000	-0,050	0,396
IP-10 T2	-0,210	0,293	0,042	0,579	0,150	-0,300	0,246	-0,050	1,000	-0,108
MCP-3 T2	0,242	0,350	0,250	-0,115	0,648	0,374	0,559	0,396	-0,108	1,000

Abbreviations: T1, serum sample at Day 1 after aSAH; T2, serum sample at Day 5–7 after aSAH. FGF-2, fibroblast growth factor-2; FLT-3L, Fms-related tyrosine kinase 3 ligand; CX3CL1, chemokine ligand 1, also known as fractalkine; IL-1b, interleukin-1b; IL-4, interleukin4; IP-10, interferon gamma-induced protein 10, also known as C-X-C motif chemokine ligand 10 (CXCL10); MCP-3, Monocyte chemotactic protein-3; MIP-1b, macrophage inflammatory protein 1-beta.

Note: Unfavorable, mRS = 3–6 on Day 30; favorable, mRS = 0–2 on Day 30. Red indicates that the two parameters were positively correlated, and blue indicates that the two parameters were negatively correlated. The darker the color, the stronger the correlation. Statistical method: Spearman.

V. Conclusion

5.1. Ischemic stroke

As a novelty, we investigated the matricellular protein periostin in humans in the hyperacute phase of acute IS. The major findings were the following:

(i) Our results show that serum periostin level measured in the hyperacute phase of IS was significantly higher in patients with IS compared to healthy controls. Furthermore, the admission serum periostin level was significantly higher significantly higher in patients with unfavorable outcome at 90-day follow-up.

(ii) The NIHSS, indicating the severity of IS, was positively, while the ASPECT score reflecting the collateral circulation, was negatively correlated with the systemic concentration of periostin. Taken together, these may suggest that matricellular proteins regulating myogenesis/angiogenesis and vessel formation during regeneration processes may affect the collateral circulation that determines salvageable penumbral brain tissue.

(iii) Several studies have proven that the ASPECT score is a sensitive marker of the ischemic core and thus indirectly of the collateral network. In our study, we found that periostin level measured on admission was independently associated with ASPECT score < 6 calculated on admission CT scan. Thus, serum periostin level at the time of admission may indirectly indicate the quality of the collateral network in the acute phase of IS. It follows that periostin level measured within 6 hours after stroke may contribute to the indication for EVT.

In our study we found a strong positive correlation between atrial fibrillation and periostin level. Furthermore, CRP, NLR and WBC showed a close correlation with admission periostin levels, indicating a possible link between early immune response and post-ischemic brain injury. Elevated CRP levels following stroke were associated with poor functional outcome and mortality.

5.2. Aneurysmal subarachnoid hemorrhage

We created a biomarker profile to investigate the relationship of biomarkers to DCI and the functional outcome and their relationship to each other. Our findings are the following:

(i) Our results show that serum MCP-3 and CX3CL1 levels measured on Day 5-7 (T2) following aSAH, were significantly higher in patients with DCI. On the one hand, this suggests that high MCP-3 levels indicate marked inflammatory activity, which is part of the pathogenesis of DCI. On the other hand, the elevated level of CX3CL1 in the late phase of aSAH which also coincides with the time of microglia polarization of the M2 phenotype, may contribute to the pathogenesis of DCI through its effect on microglia.

(ii) Elevated IP-10, MCP-3 and MIP-1b levels measured on Day 1 (T1) were correlated with Day 30 adverse outcome.

(iii) Serum level of IL-4 measured on Day 5-7 was significantly higher in TCD-positive patients than in TCD-negative ones. Our study supports the assumption that patients with DCI and a putatively larger inflammatory response mount an even greater compensatory anti-inflammatory response reflected by IL-4 elevation. However, since serum IL-4 level showed a correlation with TCD positivity and not with DCI, the increase in velocity detected with TCD in the arteries is a part of the development of DCI, but not the sole mechanism.

In addition, a strong positive correlation was observed between IL-1b and FGF-2, a CX3CL1 and MCP-3, as well as between MCP-3 and FGF-2 on Day 1. On Day 5-7, the correlation only remained strong between MCP-3 and CX3CL1. Serum level of FGF-2 showed a strong negative correlation with serum level of IP-10 in patients with favorable 30-Day outcome.

Periostin may be a useful laboratory marker to identify patients with a poor collateral network. If randomized controlled studies with a higher number of cases confirm our results, measuring periostin serum levels could be developed into a point-of-care diagnostic test, which facilitates the prehospital selection of patients suitable for neurointervention. Additionally, as our results suggest, several biomarkers are associated with DCI, but their pathophysiological role remains unknown. Some of the markers found in our study may hold promise in predicting disease and could be potential therapeutic targets for personalized treatment strategies in the future.

VI. Own findings

1. The systemic matricellular protein periostin is an early prognostic marker in patients with IS.
2. The serum periostin level at the time of admission may indirectly indicate the quality of the collateral network in the acute phase of IS.
3. Early elevation in serum concentration of IP-10, MCP-3, and MIP-1b shows association with 30-day poor outcome in patients with aSAH.
4. MCP-3 and CX3CL1 levels measured at 5–7 post-SAH days predict DCI.

VII. List of publications and presentations

List of publications

Spantler D., Molnar T., Simon D., Berki T., Buki A., Schwarcz A., Csecsei P. Biomarker associations in delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage, *Int. J. of Molecular Sciences*, 2022, 23, 8789. https://doi.org/10.3390/ijms23158789

IF: 6,208

Spantler D., Csecsei P., Borocz K., Berki T., Zavori L., Schwarcz A., Lenzser G., Molnar T. Serum Periostin May Help to Identify Patients with Poor Collaterals in the Hyperacute Phase of Ischemic Stroke. *Diagnostics* 2022, 12, 1942. https://doi.org/10.3390/diagnostics12081942

IF: 3,992

List of presentations

Osijek student congress (OSCON) 2022

Presentation title: Comparison of external ventricular drain related infections before vs during the COVID-19 Pandemic

Magyar Aneszteziológiai és Intenzív Terápiás Társaság (MAITT) congress 2023

Presentation title: Periostin és a kollaterális hálózat kapcsolata ischaemiás stroke hiperakut fázisában (MAITT Szabad előadások I. díj)

MedPECS congress 2023

Presentation title: Association between serum periostin level and poor collaterals in the hyperacute phase of ischemic stroke

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