Predicting neurological deficit severity due to subarachnoid haemorrhage: soluble CD40 ligand and platelet-derived growth factor-BB

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ABSTRACT

Background: Several biological markers can predict outcomes in patients with subarachnoid haemorrhage (SAH), but markers to predict neurological deficit severity in patients with SAH and poor neurological condition have not yet been established. Soluble CD40 ligand (sCD40L) and platelet-derived growth factor (PDGF) are related to the systemic inflammatory response.

Objective: In a prospective study, to investigate the relationship between clinical outcomes and blood test results in patients with SAH and severe neurological deficits.

Methods: We studied 17 patients with Hunt and Hess Grade IV and Fisher Class III neurological deficits who had undergone aneurysmal clipping within 48 hours of onset of SAH. We measured their levels of sCD40L, PDGF-AA, PDGF-AB, PDGF-BB and C-reactive protein (CRP), their white blood cell (WBC) and platelet counts and their body temperature. Blood tests were performed at an early time point (Day 0, the day of the SAH before craniotomy) and at a late time point (Day 10). The modified Rankin Scale (mRS) score of the patients was assessed at Day 60.

Results: Seven patients (41%) were classified as mRS 0–2 (good outcome) and 10 (59%) as mRS 3–5 (poor outcome). The blood levels of sCD40L ($P = 0.05$), PDGF-BB ($P = 0.02$) and CRP ($P = 0.02$), WBC count ($P = 0.005$) and body temperature ($P = 0.01$) at the late time point were significantly higher in patients with poor outcomes than in patients with good outcomes.

Conclusion: Our data suggest that sCD40L, PDGF-BB, WBC count, CRP and body temperature can predict the neurological outcome in patients with SAH and poor neurological condition.

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Aneurysmal subarachnoid haemorrhage (SAH) is a severe disorder with high mortality and morbidity rates.[1] Rebleeding of a ruptured aneurysm and delayed onset of ischaemic neurological deficits (DIND) due to cerebral vasospasm are significant determinants of outcome after SAH.[2] Even in the absence of those complications, many patients with SAH develop severe neurological deficits (such as Hunt and Hess Grade IV or V SAH severity[3]). Several biological markers, including white blood cell (WBC) and platelet count; haematocrit; glucose, D-dimer and C-reactive protein (CRP) levels; and body temperature,[4-8] can predict outcomes in patients with SAH. Equivalent markers to predict neurological outcomes in patients with SAH and poor neurological condition have not yet been established.

CD40 ligand (CD40L) is a transmembrane glycoprotein that belongs to the tumour necrosis factor- family.[9] It can be cleaved from the cell surface, releasing soluble CD40L (sCD40L), which is biologically active.[10] Circulating sCD40L is mainly derived from platelets,[11] and levels of circulating sCD40L are elevated in acute cerebral ischaemia, acute coronary syndrome, traumatic brain injury and SAH.[12-15] A recent study determined that plasma sCD40L levels are related to long-term clinical outcomes after SAH and to the severity of SAH.[15]

In addition, platelet-derived growth factor (PDGF) is widely expressed when there is inflammation and tissue injury.[16] PDGF is a homodimer or heterodimer with A, B, C or D chains (PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC or PDGF-DD)[17,18] and is stored in the platelet alpha-granule.[19] It is expressed at low or almost undetectable levels in normal cells, and its generation is tightly regulated. PDGF is released on platelet degranulation (such as in response to thrombin and inflammatory stimuli).[20]

We investigated the relationships between clinical outcomes and blood test results (including CD40L, PDGF-AA, PDGF-AB and PDGF-BB levels) and body temperature in patients with SAH with Hunt and Hess Grade IV neurological deficits. Our investigation was based on data suggesting that PDGF and CD40L levels are related to the systemic inflammatory response.

Methods

Patient population

Between January 2009 and December 2014, 17 patients were enrolled in our prospective study (six men and 11

women, with a mean age of 53 years [SD, 9.5 years]). The inclusion criteria were:

- SAH without intracerebral haematoma, as assessed by computed tomography on admission and defined as Fisher Class III[21]
- Hunt and Hess Grade IV SAH
- ruptured aneurysm in the anterior circulation.

The Hunt and Hess grade is the most widely used SAH grading system. It describes the severity of neurological deficits and assists in choosing the most appropriate treatment option. The scale has five grades, with Grade IV assigned to patients who are stuporous, who have more severe focal deficits and/or who have early decerebrate rigidity.

The exclusion criteria were:

- history of intracranial disorders, including ruptured arteriovenous malformation or aneurysmal SAH
- any chronic neurological disease
- systemic disease, such as cancer, sepsis, chronic inflammatory disease, diabetes mellitus, renal failure or heart disease
- surgical treatment for any kind of disease within 4 weeks before admission.

Day 0 was defined as the onset of SAH. All patients underwent multislice computed tomography angiography (CTA) or cerebral angiography with arterial catheterisation, and underwent aneurysmal clipping within 48 hours of onset of SAH.

Post-operative management

We adjusted the rate of intravenous fluid infusion to maintain the central venous pressure in the range of 5–10 cmH_2O. We maintained the mean arterial blood pressure at a level about 15% higher than the original levels. Dobutamine and/or dopamine were infused intravenously as needed, to secure therapeutic hypertension. We did not use hyperosmolar fluids (mannitol and glycerol) or blood products. When the serum glucose level increased to > 11.1 mmol/L, we initiated insulin therapy.

When DIND developed, we administered ozagrel sodium (a thromboxane synthetase inhibitor) intravenously at a rate of 80 mg/day, and edaravone (a free radical scavenger) intravenously at a rate of 60 mg/day. We did not perform transluminal angioplasty or administer drugs intra-arterially. For all patients, we used cisternal or ventricle drainage from the time of surgery until 12–14 days after the onset of SAH. When communicating hydrocephalus developed post-operatively, we initiated ventriculoperitoneal or lumboperitoneal shunting.

All protocols were reviewed and approved by the institutional ethics committee, and informed consent was obtained from all patients or their next of kin.

Clinical evaluation

The neurological status of patients was assessed post-operatively every 3–24 hours until 60 days after the onset of SAH. DIND after SAH was defined as the onset of confusion or disorientation, a decline in the level of consciousness, or any focal neurological deficit occurring 3–14 days after the onset of SAH without computed tomography, laboratory or clinical evidence of other causes (such as rebleeding, hydrocephalus, electrolyte disturbances, surgical morbidity, hypoxia or seizures).

All patients underwent repeat multislice CTA[22] on Day 8 or Day 9, and we evaluated arterial narrowing due to cerebral vasospasm using the Schneck and Kricheff scale[23] as follows: none; mild (up to 30% reduction in lumen diameter); moderate (31%–60% reduction); or marked (at least 60% reduction). We defined moderate or marked arterial narrowing as angiographic vasospasm.

Blood sampling

We collected blood samples for all patients by venepuncture at Day 0 and at Day 10. We obtained initial samples before craniotomy. Each 5 mL sample was centrifuged at 1500 rpm for 10 minutes, and the resulting supernatant was collected and stored at –80°C until it was assayed. We assessed serum levels of the following blood parameters with commercially available enzyme-linked immunosorbent assay (ELISA) kits:

- sCD40L (Human soluble CD40 Ligand ELISA kit, PromoCell)
- serum PDGF-AA (Quantikine kit, R&D Systems)
- serum PDGF-AB (Quantikine kit, R&D Systems)
- serum PDGF-BB (Quantikine kit, R&D Systems).

Statistical analysis

Data are expressed as means with SDs. We compared patient characteristics (age, sex, aneurysm location and diameter, history of acute inflammatory disease [such as pneumonia], chronic hydrocephalus and DIND) at the early and late time points (Day 0 and Day 10) between patients with modified Rankin scale (mRS)[24] scores of 0–2 (good outcome) and 3–5 (poor outcome) using the Mann–Whitney *U* test or the χ^2 test. We compared blood levels of sCD40L, PDGF-AA, PDGF-AB, PDGF-BB and CRP; platelet and WBC counts; and body temperature at the early and late time points between patients with good outcome and patients with poor outcome, using the Mann–Whitney *U* test. Blood levels of sCD40L, PDGF-BB and CRP; platelet and WBC counts; and body temperature were compared between the early and late time points and stratified by outcomes using the paired *t* test.

Results

Of the 17 patients studied, seven (41%) had good outcomes at 60 days after the onset of SAH, and 10 (59%) had poor outcomes for the same time frame. Table 1 shows some baseline patient and disease characteristics for patients with good outcomes and patients with poor outcomes. There were no significant differences in other variables when comparing the groups.

Table 2 shows blood test results and body temperatures for patients with good outcomes and poor outcomes. The mean serum concentrations of sCD40L and PDGF-BB, WBC count, CRP level and body temperature at the late time point were significantly higher in patients with poor outcomes than in those with good outcomes.

Table 3 shows a comparison of blood test results and body temperature between early and late time points and stratified by outcome. Serum concentrations of sCD40L increased significantly from the early to the late time point among all patients and in patients with poor outcomes. Serum concentrations of PDGF-BB increased significantly from the early to late time point in patients with poor outcomes. The WBC count decreased significantly from the early to late time point for patients with good outcomes. Plasma concentrations of CRP increased significantly from the early to late time point, regardless of outcome. The platelet count increased significantly from the early to late time point, regardless of outcome, and body temperature increased significantly from the early to late time point among all patients and for patients with poor outcomes.

Table 1. Baseline characteristics of patients with mRS score 0–2 (good outcome) (*n* = 7) and mRS score 3–5 (poor outcome) (*n* = 10)

	mRS score		
Characterisitic	0–2	3–5	P
Mean age, years (SD)	53.7 (5.1)	53.3 (12.1)	0.46
Women, *n*	5	6	0.52
Aneurysm location, *n*			0.87
AcomA	2	2	
ICA	3	4	
MCA	2	4	
Mean aneurysm diameter, mm (SD)	8.0 (2.3)	6.9 (2.4)	0.27
Concomitant condition			
Acute inflammatory disease, *n*	1	1	0.67
Chronic hydrocephalus, *n*	2	4	0.52
DIND from cerebral vasospasm, *n*	1	3	0.45

mRS = modified Rankin scale. AcomA = anterior communicating artery. ICA = internal carotid artery. MCA = middle cerebral artery. DIND = delayed ischaemic neurological deficit.

Discussion

We found no significant differences in some patient and disease characteristics (age, sex, location and diameter of aneurysm, concomitant medical conditions) when comparing patients with good outcome and poor outcome. However, there were significant increases in blood levels of sCD40L, PDGF-BB, WBC and CRP and an elevation in

Table 2. Blood test results and body temperatures of patients with mRS score 0–2 (good outcome) (*n* = 7) and mRS score 3–5 (poor outcome) (*n* = 10)

	mRS score		
Variable, mean (SD)	0–2	3–5	*P*
Early sCD40L (ng/mL)	6.0 (2.7)	5.4 (1.7)	0.77
Late sCD40L (ng/mL)	6.5 (4.6)	9.7 (4.1)	0.05
Early PDGF-AA (ng/mL)	2.7 (1.1)	2.7 (1.0)	0.99
Late PDGF-AA (ng/mL)	2.6 (1.8)	3.6 (1.2)	0.17
Early PDGF-AB (ng/mL)	16.3 (7.8)	15.9 (7.5)	0.49
Late PDGF-AB (ng/mL)	13.2 (8.6)	18.2 (7.4)	0.08
Early PDGF-BB (ng/mL)	4.5 (2.4)	4.7 (2.5)	0.33
Late PDGF-BB (ng/mL)	3.5 (1.9)	5.9 (2.6)	0.02
Early Plt (10^4/μL)	26.8 (3.8)	30.0 (7.6)	0.56
Late Plt (10^4/μL)	34.7 (4.2)	38.6 (10.6)	0.33
Early WBC (10^3/μL)	9405.0 (2366.7)	12 186.7 (3619.5)	0.08
Late WBC (10^3/μL)	7190.0 (1848.9)	12 866.7 (6718.2)	0.005
Early CRP (mg/dL)	0.3 (0.2)	1.2 (2.9)	0.83
Late CRP (mg/dL)	1.5 (1.3)	4.7 (4.5)	0.02
Early BT (°C)	36.9 (0.2)	37.1 (0.4)	0.26
Late BT (°C)	37.0 (0.3)	37.7 (0.5)	0.01

mRS = modified Rankin scale. sCD40L = soluble CD40 ligand. PDGF = platelet-derived growth factor. Plt = platelet. WBC = white blood cells. CRP = C-reactive protein. BT = body temperature.

Table 3. Comparison (*P*) of blood test results and body temperatures, by time point (early v late) and mRS score (0–2 [good outcome] [*n* = 7] and 3–5 [poor outcome] [*n* = 10])

		mRS score	
Comparison, early v late	All patients	0–2	3–5
sCD40L level	0.002	0.26	0.001
PDGF-BB level	0.2	0.15	0.04
White blood cell count	0.28	0.04	0.42
C-reactive protein	0.002	0.02	0.005
Platelet count	0.0004	0.01	0.01
Body temperature	0.001	0.26	0.01

mRS = modified Rankin scale. sCD40L = soluble CD40 ligand. PDGF = platelet-derived growth factor.

body temperature at the late time point in patients with SAH with poor outcomes when compared with patients with SAH with good outcomes. We found no differences in blood levels of PDGF-AA, PDGF-AB and platelets at the early or late time points when comparing patients with good outcomes and patients with poor outcomes. Serum concentrations of sCD40L increased significantly from the early to the late time point among all patients and in those with poor outcomes, but not in those with good outcomes. Previous studies have found that sCD40L levels are elevated in the peripheral blood after brain injury, including ischaemic stroke,[12] traumatic brain injury[14] and SAH.[15]

A recent study showed that serum sCD40L levels were higher in patients with SAH than in healthy controls, and that there was an association between serum sCD40L levels, SAH severity and long-term outcomes, including 6-month mortality and morbidity.[15] The role of sCD40L in SAH remains unclear, but it is possible that its pro-inflammatory effects[10] could contribute to the pathophysiology of SAH.

PDGF-BB levels also significantly increased from the early to the late time point in patients with poor outcomes. PDGF-BB is secreted from platelets and inflammatory cells, and upregulation of PDGF is induced by a variety of stimuli, including hypoxia and ischaemia.[25] PDGF-BB production is enhanced by the arterial stretching injury that occurs during the formation of a thick haematoma and by brain oedema.[16] Further, PDGF-BB upregulation is beneficial for the repair of the damaged brain and also for neuronal survival, and the potent neuroprotective effect of PDGF-BB was shown in a focal ischaemia model.[26] Therefore, when patients with SAH and poor neurological condition develop early diffuse brain damage, sCD40L and PDGF-BB may be upregulated and released into the blood circulation from activated platelets and/or inflammatory cells. This notion is consistent with the increase in platelet and WBC counts that we saw.

In our study, the WBC count decreased significantly from the early to the late time point in patients with good outcomes, but the CRP level increased significantly from the early to the late time point for patients with poor outcomes. Body temperature increased significantly from the early to the late time point for patients with poor outcomes. Increased WBC count, CRP level and body temperature, as a systemic inflammatory response without infection, can accompany SAH, and can together be used as a marker of a poor outcome after SAH.[4,7,8] We did not measure concentrations of cytokines other than CD40L and PDGF, but it has been shown that activation of early-phase cytokine cascades in conjunction with the biological effects of complement activation may contribute to the systemic inflammatory response after SAH.[8]

Our study was limited by its small size and the fact that it was only a preliminary study. These results require confirmation in a large-scale trial, and the correlations between variables should be examined. However, our data suggest that sCD40L, PDGF-BB and CRP levels, WBC count and body temperature may be prognostic biomarkers in patients with SAH with severe neurological deficits.

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Competing interests

None declared.

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