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Plasma proinflammatory and anti-inflammatory cytokine and catecholamine concentrations as predictors of neurological outcome in acute stroke patients

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Abstract
Purpose. Proinflammatory and anti-inflammatory cytokines may play a pivotal role in cerebral inflammation, which is implicated in the development of brain injury. Systemic cytokine release is mediated by the sympathetic nervous system and catecholamines. The aim of this study was to investigate which parameters, among plasma levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), and tumor necrosis factor alpha (TNF-α) and the levels of the catecholamines, epinephrine and norepinephrine, contribute to the clinical outcome in acute stroke patients.
Methods. Thirty-seven acute stroke patients (ischemic, n = 19; hemorrhagic, n = 18) were enrolled. All of them were admitted to our hospital within 8 h after stroke onset. Neurological status was evaluated by a modified National Institute of Health Stroke Scale (mNIHSS) on admission and by a modified Rankin Scale (mRS) at 1 month. An mRS score of 3 or more at 1 month was considered to indicate poor outcome. Serum samples for the cytokine and catecholamine measurements were collected on admission. Plasma levels of IL-1β, IL-6, IL-10, and TNF-α were determined by an enzyme-linked immunosorbent assay (ELISA) method and epinephrine and norepinephrine concentrations were determined by high-performance liquid chromatography with electrochemical detection (HPLC-EC).
Results. In the ischemic stroke patients, poor outcome was noted in 9 (47%). There were no significant differences in cytokine or catecholamine concentrations between patients with poor and good outcomes, and there was no association between clinical outcome and cytokine and catecholamine concentrations. In the hemorrhagic stroke patients, poor outcome was noted in 10 (56%). IL-6 and IL-10 levels were higher in patients with poor outcome. On logistic regression analysis, higher values of IL-6 were significantly associated with clinical outcome at 1 month (odds ratio [OR], 1.25; 95% confidence interval [CI], 1.02–1.54).
Conclusion. In ischemic stroke, plasma cytokines and catecholamines were not predictors of neurological outcome at 1 month. In hemorrhagic stroke, high levels of IL-6 in the early phase indicated a poor neurological outcome.

Key words Cytokine · Catecholamine · Acute stroke

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Introduction

There are several lines of evidence suggesting that inflammation plays a pivotal role in the pathophysiology of acute stroke [1,2]. Inflammatory reactions appear to be initiated and modulated by cytokines, which are released by leukocytes, astrocytes, microglial cells, and endothelial cells [1–3]. Several studies have reported that proinflammatory cytokines, including interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) are elevated both in serum and cerebrospinal fluid (CSF) in the early phase of disease [4–6]. Some investigators have demonstrated that the magnitude of proinflammatory cytokine levels in humans was correlated with stroke severity or clinical outcome [7–9].

Interleukin-10 (IL-10), an anti-inflammatory cytokine, mainly released by lymphocytes and monocytes/macrophages, may provide a negative feedback mechanism to limit the production of inflammatory cytokines in stroke patients [3]. The IL-10 concentration increases transiently in the plasma and CSF of patients with acute stroke [10,11]. Low levels of IL-10 increased the risk of stroke and were associated with early worsening of neurological symptoms in acute ischemic stroke [12]. These studies suggest that IL-10 may have neuroprotective effects.

While the mechanism of cytokine release in patients with acute stroke is not clear, it is reportedly mediated

by sympathetic nervous system activity and catecholamines [13,14]. Elevation of plasma catecholamine concentrations has been observed after brain infarction or subarachnoid hemorrhage, revealing sympathetic nervous system and adrenal medullary overactivitiy [15–18]. Some investigators have shown that both locally produced proinflammatory cytokines in the brain and direct brainstem irritation can trigger strong sympathetic activation and release catecholamines leading to the systemic release of anti-inflammatory cytokines [3,13].

Acute stroke is characterized by brain tissue damage of variable extent and development. The acute phase, especially within 16 h from onset, is crucial as the therapeutic duration for hyperacute treatment that aims to restore perfusion and protect neuronal tissue [19]. Pro- and anti-inflammatory cytokines and catecholamines were considered to affect the pathophysiology of stroke development in the acute phase [1–3]. However, the impact of plasma cytokines and catecholamines in the acute phase on the clinical outcome in acute stroke patients has not been well evaluated.

In this study, we tested the hypothesis that the clinical outcome in acute stroke patients would be affected by higher levels of proinflammatory cytokines (IL-1β, IL-6, TNF-α), an anti-inflammatory cytokine (IL-10), and catecholamines (epinephrine, norepinephrine).

Patients and methods

Patients

Consecutive patients with acute stroke admitted to our intensive care unit between April 2004 and January 2005, within 8 h of stroke onset, were enrolled. Patients with evident infection, inflammatory disease, concurrent malignancy or autoimmune disease, or severe cardiac, renal, or hepatic insufficiency were excluded. Attending physicians carried out physical and neurological examinations of all patients on admission. Questions on a past history of hypertension, diabetes mellitus, hyperlipidemia, smoking, or previous atrial fibrillation were asked of the patients, if possible, or their relatives. The study was approved by the local ethics committee and prior informed consent was obtained from all study participants or their relatives.

Evaluation of neurological stroke severity and clinical outcome

Neurological stroke severity was determined with a modified National Institute of Health Stroke Scale (mNIHSS) within 8 h after stroke onset [20]. The mNIHSS has a score ranging from 0 (normal neurological status) to 31 (maximal neurological deficit). Clinical outcomes were assessed with a modified Rankin Scale (mRS), a global disability/handicap scale ranging from 0 (no symptoms) to 6 (death), at 1 month after stroke onset or at the time of discharge [21]. We defined poor outcome as an mRS score of 3 (not independent) to 6 (dead).

Measurement of the volume of brain infarction and hematoma

Brain computed tomography (CT) or brain magnetic resonance imaging (MRI) was performed on admission for all patients. The volume of the infarction and hematoma were calculated from standard MRI or CT scans with the use of the largest perpendicular diameters and slice thickness [22]. The measurements included all actual lesions, but excluded older lesion.

Laboratory procedure

On admission, blood samples were obtained through an antecubital venipuncture and collected in tubes containing ethylene diamine tetraacetic acid (EDTA). Samples were centrifuged at 3000 rpm for 10 min. The supernatant was aspirated and frozen immediately at −25 °C until assayed. Analyses of serum for IL-1β, IL-6, TNF-α, and IL-10 were performed by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (ENDOGEN Human ELISA Kit; Pierce endogen, Boston, MA, USA). The lower detection limits were as follows; IL-1β, <0.4 pg/ml; IL-6, <0.8 pg/ml; TNF-α, <1.6 pg/ml; and IL-10, <0.8 pg/ml. The intra- and interassay variabilities were lower than 10% for all assays used. Plasma levels of epinephrine and norepinephrine were measured by high-performance liquid chromatography with electrochemical detection (HPLC-EC) according to the manufacturer's instructions (Plasma Catecholamine Analysis Kit; ESA, Bedford, MA, USA).

Statistical analysis

For statistical analysis, the Mann-Whitney test was used to compare patients' characteristics and cytokine and catecholamine concentrations between two outcome groups. Logistic regression analysis was used to assess the association between clinical outcome and plasma cytokine and catecholamine concentrations. Parameters with a significance level of $P < 0.1$ were included as independent variables in a logistic regression analysis. Results were reported as odds ratios (ORs), and statistical significance was ascertained by the 95% confidence interval (CI). Plasma cytokine and catecholamine concentrations were not normally distributed; they were

expressed as medians (quartiles). Values for other results are expressed as means ± SD. Values were considered significant at $P < 0.05$.

Results

Thirty-seven patients were enrolled during the study period. Of these, 19 patients had suffered an ischemic stroke and 18, a hemorrhagic stroke. Baseline characteristics of the patients, showing differences between two outcome groups at entry, stroke types, and outcome measures are summarized in Table 1. Causes of ischemic stroke were atherosclerotic ($n = 9$), cardioembolic ($n = 5$), and lacunar ($n = 5$). Four patients were treated with intraarterial thrombolysis and 1 patient received cerebrovascular stenting. The causes of hemorrhagic stroke were subarachnoid hemorrhage (SAH; $n = 8$) and intracerebral hemorrhage (ICH; $n = 10$). Five of the 8 SAH patients received endovascular coiling and 2 patients received neurosurgical clipping. Two of the 10 patients with ICH were operated to evacuate hematoma. Mean time from the onset of stroke to admission was 3.4 ± 2.0 h (range, 1 to 8 h). One patient with hemorrhagic stroke died 6 days after admission. In the whole cohort of acute stroke patients, mNIHSS was higher in those with poor outcome than in those with good outcome. Infarct volume and hematoma volume were not significantly different between the two outcome groups. The mRS score was not significantly different between the ischemic and hemorrhagic stroke patients ($P = 0.07$).

Plasma levels of cytokines and catecholamines

The plasma levels of IL-1β, IL-6, TNF-α, IL-10, norepinephrine, and epinephrine on admission are shown in Table 2. In ischemic stroke patients, there were no significant differences in cytokine or catecholamine levels between patients with a poor outcome and those with a good outcome. In hemorrhagic stroke patients, IL-6 and IL-10 were significantly higher in patients with a poor outcome than in those with a good outcome (IL-6; $P = 0.003$; IL-10, $P = 0.04$). IL-6 and IL-10 levels were higher in hemorrhagic stroke patients than in ischemic stoke patients (IL-6, $P = 0.002$; IL-10, $P = 0.049$). In 31 of the 37 samples assayed for TNF-α (84%), TNF-α was undetectable. The highest concentration of TNF-α in the remaining 6 samples (16%) was 38 pg/ml. Levels of TNF-α, IL-1β, epinephrine, and norepinephrine did not differ between the poor and good outcome groups.

Association between clinical outcome and plasma cytokine and catecholamine levels

In patients with ischemic stroke, no parameters other than mNIHSS predicted poor outcome according to univariative logistic regression analysis. In contrast, in hemorrhagic stroke patients, higher values of IL-6 were associated with poor outcome at 1 month (Table 3). IL-1β, TNF-α, epinephrine, and norepinephrine showed no association with clinical outcome at 1 month (Table 3).

Discussion

In the present study, in ischemic stroke patients, poor clinical outcome was not associated with proinflammatory and anti-inflammatory cytokine levels or with catecholamine levels in serum. In hemorrhagic stroke patients, high values of IL-6 were significantly associated with poor clinical outcome at 1 month.

Proinflammatory cytokines (IL-1β, IL-6, and TNF-α) released after brain ischemia by activated brain resident and peripheral blood cells are important mediators of inflammatory responses, and these processes may play

Table 1. Patients' characteristics: differences between two outcome groups

	Good outcome (mRS, 0–2)	Poor outcome (mRS, 3–6)	*P* value
Ischemic stroke (*n*)	10	9	
Age[a]	65 ± 13.8	75 ± 5.2	0.40
Male/female (*n*)	6/4	5/4	0.79
mNIHSS[a]	3.9 ± 2.8	11.2 ± 5.3	0.01
Infarct volume (cm^3)[b]	1.5 (1, 4.5)	10 (2, 20)	0.38
Hemorrhagic stroke (*n*)	8	10	
Age[a]	68 ± 5.9	63 ± 8.8	0.16
Male/female	3/5	7/3	0.34
mNIHSS[a]	6.1 ± 3.4	22.5 ± 8.3	0.001
Hematoma volume (cm^3)[b]	20 (18, 27)	108 (30, 110)	0.1

mNIHSS, Modified National Institute Health Stroke Scale; mRS, modified Rankin Scale
[a] Means ± SD
[b] Median (quartile)

Table 2. Levels of plasma cytokines and catecholamines

	Good outcome (mRS, 0–2)	Poor outcome (mRS, 3–6)	*P* value
Ischemic stroke (*n*)	10	9	
IL-1β	0 (0, 0.4)	0.2 (0.1, 0.3)	0.62
IL-6	1.3 (0.1, 3.5)	1.4 (1.0, 3.6)	0.87
TNF-α	0 (0, 0)	0 (0, 0.1)	0.68
IL-10	3.0 (0.7, 22.6)	2.3 (1.9, 6.7)	0.87
Epinephrine	560 (202, 1212)	595 (551, 1492)	0.93
Norepinephrine	287 (183, 577)	273 (210, 1030)	0.91
Hemorrhagic stroke (*n*)	8	10	
IL-1β	0.3 (0, 1.5)	0.7 (0.1, 1.0)	0.68
IL-6	5.5 (1.5, 9.7)	24.1 (18.2, 33.8)	0.003
TNF-α	0 (0, 0)	0 (0, 0)	0.75
IL-10	3.3 (1.3, 16.2)	18.0 (13.6, 53.7)	0.04
Epinephrine	492 (311, 710)	556 (448, 709)	0.72
Norepinephrine	308 (120, 437)	398 (278, 1041)	0.28

All data values are shown as medians (quartile)
IL, interleukin; TNF-α, tumor necrosis factor-α

Table 3. Odds ratios of clinical outcome for baseline clinical characteristics and biochemical variables

Variables	OR (95% CI)	*P* value
Ischemic stroke (*n*)	19	
IL-1β	1.68 (0.39–7.23)	0.49
IL-6	1.09 (0.85–1.39)	0.51
TNF-α	1.14 (0.05–25.1)	0.93
IL-10	0.97 (0.86–1.10)	0.65
E	1.00 (0.99–1.01)	0.22
NE	1.00 (0.99–1.00)	0.50
mNIHSS	1.90 (1.11–3.22)	0.02
Infarct volume	1.10 (0.96–1.24)	0.15
Hemorrhagic stroke (*n*)	18	
IL-1β	1.23 (0.45–3.39)	0.69
IL-6	1.25 (1.02–1.54)	0.03
TNF-α	1.18 (0.40–3.46)	0.77
IL-10	1.05 (0.99–1.11)	0.12
E	1.00 (0.99–1.00)	0.47
NE	1.01 (0.99–1.01)	0.49
mNIHSS	1.51 (0.91–2.49)	0.11
Hematoma volume ($n = 10$)	1.05 (0.96–1.16)	0.27

mNIHSS, Modified National Institute of Health Stroke Scale; IL, interleukin; TNF-α, tumor necrosis factor-α; NE, norepinephrine; E, epinephrine; OR, odds ratio; CI, confidence interval

a pivotal role in the development of central nervous system injury following focal ischemia [1–3]. Several studies have reported elevations of proinflammatory cytokine levels in peripheral blood, as well as in CSF, in patients with ischemic stroke [4–6]. On the other hand, an anti-inflammatory cytokine (IL-10) may provide negative feedback mechanisms to limit the production of proinflammatory cytokines, resulting in a neuroprotective effect for brain injury following ischemic stroke [3]. Low levels of IL-10 may be associated with neurological worsening in acute ischemic stroke [12]. However, in the present study, proinflammatory and anti-inflammatory cytokines were not associated with neurological outcome. One possible explanation is that the brain infarct volumes were much smaller (median, 3 cm^3) than those in previous studies, resulting in less release of cytokines and catecholamines. Fassbender et al. [7] demonstrated that patients with brain infarct volumes of less than 5 cm^3 showed no significantly increased values of IL-6 compared with those with a brain infarct volume of more than 5 cm^3 ($P < 0.05$). Perini et al. [11] demonstrated that there were no significant increases in plasma IL-6 concentrations in patients with lacunar infarction (small deep lesions less than 15 mm in diameter) compared with levels in healthy control subjects. These findings suggest that normal IL-6 values may be indicative of no brain lesion or a very small brain lesion.

In our hemorrhagic stroke patients, IL-6 and IL-10 levels were higher in patients with a poor outcome. Higher IL-6 values were significantly associated with clinical outcome at 1 month. Dziedzic et al. [8] found that intracerebral hemorrhage triggered IL-6 and IL-10 release into blood. They also found that IL-6 and IL-10 were significantly correlated with neurological deficit (Glasgow Coma Scale; $P < 0.001$), and that IL-6 was correlated with hematoma volume ($P = 0.003$) [8]. The mechanisms of proinflammatory and anti-inflammatory cytokine release in hemorrhagic stroke patients remain unclear. In an animal model of brain trauma, systemic IL-6 and IL-10 release was mediated by the sympathetic nervous system and catecholamines [14]. Intracranial pressure is commonly elevated immediately after stroke onset in patients with severe hemorrhagic stroke, and high intracranial pressure also activates the sympathetic nervous system, and then IL-6 and IL-10 levels increase further [3,14]. On univariative logistic regression analysis, we found that high levels of IL-6 were significantly

associated with poor clinical outcome. However, we could not use a multifactorial model to address whether a high plasma concentration of IL-6 was related to clinical outcome independently of neurological severity or hematoma size, due to the limitation of our sample size and insufficient power.

In the present study, TNF-α and IL-1β increased in neither ischemic nor hemorrhagic stroke patients. McKeating et al. [23] demonstrated that, in 88% of samples assayed for TNF-α and 86% of samples assayed for IL-1β concentration, these cytokines were undetectable in patients with traumatic brain injury or SAH. Fassbender et al. [7] showed that IL-6 serum levels increased within the first 6 h after stroke; however, no changes in IL-1β and TNF-α levels were observed in that study. On the other hand, some studies have shown increased IL-1β or TNF-α serum levels after stroke [24,25]. The reasons for these conflicting results may be related to the short half-life of cytokines, possible high concentrations at the site of release and lower concentrations in peripheral blood, and to the different types of assays and different sensitivities of the detecting techniques used [7,26]. IL-6 and IL-10 may inhibit the production of IL-1β and TNF-α via negative feedback mechanisms; this might be one reason that we may have missed an early peak of production of IL-1β and TNF-α before admission [3,23,26].

Although catecholamines have been reported to modulate the release of proinflammatory and anti-inflammatory cytokines, we failed to find a relationship between plasma catecholamine and cytokine concentrations in acute stroke patients. Plasma catecholamine levels were not associated with clinical outcome. Benedict et al. [17] reported that SAH patients with a poor outcome showed higher catecholamine concentrations in the systemic circulation than those with a good outcome. Meyer et al. [18] reported that plasma catecholamine concentrations were higher in hemorrhagic stroke patients than in ischemic stroke patients. In the present study, the levels of epinephrine and norepinephrine did not differ between patients with hemorrhagic stroke and those with ischemic stroke. One reason for these conflicting results may be related the half-life of epinephrine and norepinephrine; the half-life of both epinephrine and norepinephrine is less than a few minutes [27]. In addition, various stimuli, arising from emotional distress or a pathologic condition, such as heart failure, could elicit different degrees of release of epinephrine and norepinephrine [28].

The major limitation of the present study is the lack of adequate statistical power to detect associations between clinical outcome and plasma cytokine and catecholamine concentrations. Also, the present study included a variety of stroke subtypes and treatments for acute stroke patients, making detailed analysis difficult. For these reasons, the present results should be confirmed in further larger studies.

In conclusion, we demonstrated that plasma IL-6 and IL-10 levels increased in patients with acute hemorrhagic stroke. Initial plasma levels of IL-6 may have a prognostic value for patients with acute hemorrhagic stroke. In patients with ischemic stroke, the levels of plasma cytokines and catecholamines did not predict clinical outcome.

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