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Interleukin-12 in acute ischemic stroke patients

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Abstract

Cytokines are important mediators of stroke-induced immunological/inflammatory reaction which contributes to brain infarct progression as well as to the disease severity and outcome.
The aim of the study was to evaluate the levels of the proinflammatory and immunomodulatory cytokine interleukin-12 (IL-12) in serum of acute ischemic stroke patients, and to investigate the relation between these levels and demographic, laboratory, neuroimaging, and clinical data.
The study comprised 23 first-ever ischemic stroke patients and 15 age- and sex-matched controls. Blood sampling for the determination of IL-12 and such peripheral markers of inflammation as erythrocyte sedimentation rate (ESR) and leukocyte count, together with cranial CT were performed within 24 h of stroke, while neurological and functional deficits were estimated, respectively, with the Scandinavian Stroke Scale (SSS) and Barthel Index (BI) within the same period and two weeks later.
Stroke patients displayed significantly higher serum IL-12 levels in comparison with controls.
The serum IL-12 levels in stroke patients correlated significantly with the ESR values, the volume of early brain CT hypodense areas, and with the SSS and BI scores calculated within both studied times.
The results indirectly suggest that IL-12 may play a role in the pathophysiology of ischemic stroke.

Key words: *stroke, inflammation, cytokines, interleukin-12.*

Introduction

Ischemic stroke is one of the leading causes of death or permanent disability, and its severity and outcome partly depends on the extent of brain lesion. Current therapeutic strategies show limited efficacy in reducing acute ischemic brain damage even when reperfusion is achieved with the best-approved thrombolytic treatment [15].

The central and peripheral immunological/inflammatory reaction is an important phenomenon of the pathophysiological response to ischemic stroke and contributes to brain infarct progression [6,15]. Accordingly, the stroke-induced immunological/inflammatory reaction is of particular interest as its suppression at cellular and/or molecular levels significantly reduces the size of brain damage and neurological impairment in animal models of stroke [6].

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In cerebral ischemia, the reaction cellular components, i.e. brain resident and peripheral blood cells are activated and gain antigen-presenting capacity through the expression of MHC molecules [6,11,13,16,32].

Cytokines contribute to the expression of MHC antigens and the reaction of other molecular components, including chemokines and adhesion molecules [6,7,15]. Consequently, cytokine-mediated activation and recruitment of leukocytes facilitate leukocyte migration into the ischemic brain area [6,11,15]. The brain-invading leukocytes contribute to tissue injury in stroke [4,5]. The infiltrate consists of different cellular subsets of cytotoxic properties, including the phagocytic component of neutrophils and monocytes, and the lymphocytic component of T and NK cells [4,5].

Experimental models of stroke have shown that an intracerebroventricular injection of proinflammatory cytokines enlarges the infarct volume and brain edema after middle cerebral artery occlusion in rats, whereas the administration of the anticytokine antibodies reduces ischemic brain injury [15]. Several studies indicate that the magnitude of the proinflammatory cytokines response in the CNS or in peripheral blood correlates with the brain infarct size, stroke severity and outcome in ischemic stroke patients [17,39,48,49].

Interleukin-12 (IL-12) is a cytokine at the interface of inflammation and immunity which is produced primarily by antigen-presenting cells [41]. The cytokine was originally discovered as a product of human B-lymphoblastoid cell lines; however, phagocytes rather than B cells appear to be the predominant producers of IL-12 [36].

IL-12 synthesis in phagocytic cells is induced by a variety of invading pathogens or its products in a T-cell-independent pathway or a T-cell-dependent one, the latter mediated through an interaction of CD40 ligand on activated T cells with CD40 receptor on IL-12 producers [36].

The major target cells of IL-12 action are NK and T cells stimulated for production of cytokines, proliferation, and cytotoxic activity [40].

IL-12 produced early during infections or an immune response exerts proinflammatory functions and is a cofactor of the polarization of T cells response towards cell-mediated immunity [1,40]. These functions of IL-12 imply its important role in resistance to infections; however, because of the same activities, the cytokine may play a deleterious role in tissue damage during inflammation [40].

It is therefore intriguing whether IL-12 may be involved in the pathophysiology of acute cerebral ischemia. Here, we present IL-12 levels in the serum of ischemic stroke patients within 24 h after the onset of the disease and compare the results with those of a control group. Moreover, we demonstrate the behaviour of serum IL-12 levels in stroke patients in relation to demographic, laboratory, neuroimaging, and clinical data.

Material and methods

Patients

The study involved 23 patients with first-ever acute ischemic stroke and 15 controls diagnosed with tension headache. Stroke patients were admitted between the 6th and the 20th h /median=12th h/after the onset of symptoms. After admission, serum samples were collected from each patient within the next 30 min, cranial CT and evaluation of neurological stroke severity and functional disability was determined within the next 2 hours.

The patients had complete ischemic stroke defined as clinical symptoms persisting for >24 h [8]. To avoid the enrollment of patients with concurrent diseases or conditions interfering with inflammatory mediators expression, the following exclusion criteria for stroke and control subjects were applied: history or coincidence of any central nervous system diseases, hyperthermia, presence of infections, other inflammatory, autoimmune, hematological and malignant diseases, major renal or hepatic failure, intoxications, malnutrition, addiction, deep vein thrombosis, tissue injury related conditions within the past year, immunosuppression or treatment with anti-inflammatory drugs within the last six months. In addition, as acute stroke patients are prone to infections, especially of the chest and urinary tract [12], we included only patients without signs of infections on chest x-ray and urine tests performed after admission and repeated during the observational interval of two weeks after the stroke onset.

The study was conducted according to the principles established in the Declaration of Helsinki and was approved by the Local Ethics Committee. Both stroke and control patients gave their informed consent prior to their inclusion into the study.

Laboratory procedure

Blood samples from stroke patients were collected within 24 h from the onset of the disease symptoms. Blood samples from tension headache patients served as a control group. The samples were allowed to clot at room temperature for 30 min, and after being centrifuged for 10 min, the serum was immediately frozen and stored at -80^{0}C. IL-12 levels in serum samples were determined with ELISA method using human IL-12 immunoassay (R & D Systems, USA).

Parts of the blood samples served for the determination of peripheral markers of inflammation such as erythrocyte sedimentation rate (ESR) with the use of a manual Westergren method on a Quick ESR Measurement Kit (MedLab, Poland) and leukocyte count using an automated hematology analyzer AHA Sysmex K 4500 (ICN, Japan).

Evaluation of the volume of early brain CT hypodense areas

Early hemispheric brain CT hypodense areas recorded within 24 h after the onset of stroke are considered early CT signs of hemispheric brain infarction [45].

Cranial CT was performed using a CT ProSpeed SX Advantage (General Electric Medical Systems, Japan). CT brain scans were obtained parallel to orbitomeatal line using 10 mm (supratentorial) and 5 mm (infratentorial) slice thickness. The volume (given in ccm) of early CT hypodense areas was calculated according to the formula based on length x depth x height (in mm) hypodense area measurements [38]. CT scans were reviewed by an experienced neuroradiologist blinded to the clinical and laboratory data. Each ischemic stroke patient - except one with radiologically invisible changes – presented a single early CT hypodense area localized in the middle or anterior cerebral artery territory contralaterally to the side of neurological signs. Brain CT did not reveal any other changes. Measurements of the hypodense areas and calculations of their volume were performed twice with the difference not exceeding 5%. In all control subjects cranial CT was also performed which revealed no pathological changes.

Evaluation of neurological stroke severity and stroke-related functional disability

The neurological stroke severity was determined with the Scandinavian Stroke Scale (SSS) scores [37]. SSS scoring within 24 h after the disease onset is related to early neurological stroke severity, while SSS scoring at the 2nd week after the beginning of symptoms is considered short-term neurological stroke outcome.

The functional disability of stroke patients was determined with the Barthel Index (BI) scores [29].

BI scoring within 24 h after the disease onset is related to early functional disability, while BI scoring at the 2nd week after the beginning of symptoms is considered short-term functional stroke outcome.

Statistical analysis

As the Shapiro-Wilk test revealed that the data on serum IL-12 levels in both stroke patients and controls were abnormally distributed, further statistical analyses concerning the cytokine levels were performed with nonparametric tests, including the Mann-Whitney *U* test applied to compare serum IL-12 levels of stroke patients with control values.

For a comparison of the data inserted in Table I, the Student *t* or Mann-Whitney *U* tests were used as appropriate, except for comparison within the stroke group serum IL-12 levels in patients with separate stroke risk factors, where the Kruskal-Wallis test was adapted, and comparisons of the SSS and the BI scores calculated in patients within 24 h of stroke with those determined two weeks later, where the ANOVA Friedmann test was applied.

The Spearman rank-order correlation test was used to examine correlations between serum IL-12 levels and the data in Table I. The results are presented as mean ±SD. $P<0.05$ was considered statistically significant.

Results

Serum IL-12 levels in patients within 24 h after the onset of stroke and controls

The level of IL-12 in serum of the stroke patients was 16.73±7.45 pg/ml and was significantly higher ($p<0.0001$) in comparison with the controls in which the level of IL-12 in serum was 8.23±0.87 pg/ml.

Table I. Characterization of stroke patients and controls (values as mean (SD) unless otherwise indicated)

Characterization	Stroke (n=23)	Controls (n=15)
demographic data		
age, years	72.2 (10.8)	70.1 (8.6)
sex (F/M), n (%)	17/6 (74/26)	11/4 (73/27)
stroke risk factors, n (%)		
hypertension	12 (52)	0 (0)
smoking	5 (22)	0 (0)
diabetes mellitus	4 (17)	0 (0)
atrial fibrillation	2 (9)	0 (0)
laboratory data		
ESR, mm/h[a]	26.8 (11.7)	7.6 (4.8)**
leukocyte count, 10^9/l[a]	8.1 (2.4)	5.4 (1.1)*
neuroimaging data		
volume of early brain CT hypodense areas (ccm)	10.0 (10.7)	
clinical data		
Scandinavian Stroke Scale[b]		
within 24 h	31	
at 2nd week	45*	
Barthel Index[b]		
within 24 h	25	
at 2nd week	65*	

[a] evaluated within 24 h
[b] scores expressed by median
**p<0.001 **p<0.00001*

Serum IL-12 levels in stroke patients and controls in relation to the demographic, laboratory, neuroimaging, and clinical data distribution inserted in Table I:

i) Serum IL-12 levels in stroke patients and controls in relation to the demographic data

Serum IL-12 levels in the stroke patients and controls were not related to age or sex.

Serum IL-12 levels in the stroke patients referred to the following individual stroke risk factors: hypertension, smoking, diabetes mellitus, and atrial fibrillation were 16.94±7.52 pg/ml, 16.02±6.16 pg/ml, 15.24±8.53 pg/ml, and 15.86±7.09 pg/ml, respectively, and they did not differ significantly between each other.

ii) Serum IL-12 levels in stroke patients and controls in relation to the laboratory data

Serum IL-12 levels in the stroke patients correlated positively with ESR values (r=0.81; p<0.00001) but the cytokine serum levels did not correlate with ESR values in controls.

Serum IL-12 levels in the stroke patients and controls did not correlate with the leukocyte count.

iii) Serum IL-12 levels in stroke patients in relation to the volume of early brain CT hypodense areas

Serum IL-12 levels in the stroke patients correlated positively with the volume of early brain CT hypodense areas (r=0.97; p<0.000001) (Fig. 1).

iv) Serum IL-12 levels in stroke patients in relation to the SSS scores calculated within 24 h after the onset of stroke /SSS-1/ and at the 2nd week after the onset of stroke (SSS-2)

Serum IL-12 levels in the stroke patients correlated inversely with the SSS-1 scores (r=-0.77; p<0.0001) and with the SSS-2 scores (r=-0.78; p<0.0001) (Fig. 2).

v) Serum IL-12 levels in stroke patients in relation to the BI scores calculated within 24 h after the onset of stroke (BI-1) and at the 2nd week after the onset of stroke (BI-2)

Serum IL-12 levels in the stroke patients also correlated inversely with the BI-1 scores (r=-0.83;

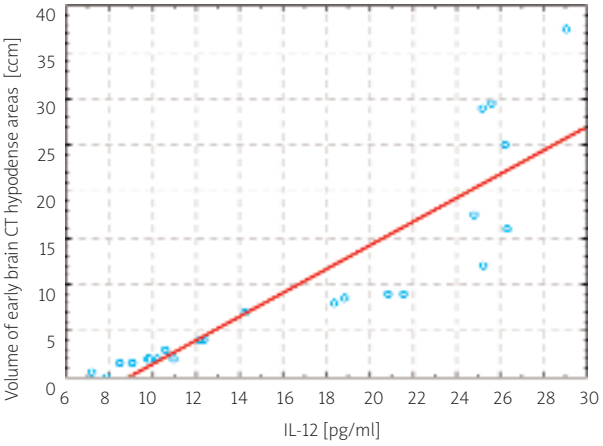

Fig. 1. The correlation (r) between serum IL-12 levels [pg/ml] in stroke patients and the volume of early brain CT hypodense areas [ccm]

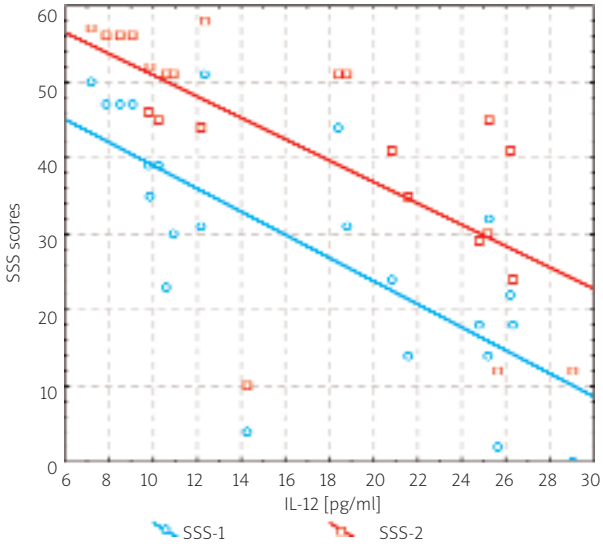

Fig. 2. The correlation (r) between serum IL-12 levels [pg/ml] in stroke patients and SSS scores within 24 h (SSS-1) and at 2nd week (SSS-2)

p<0.00001) and with the BI-2 scores (r=-0.84; p<0.00001) (Fig. 3).

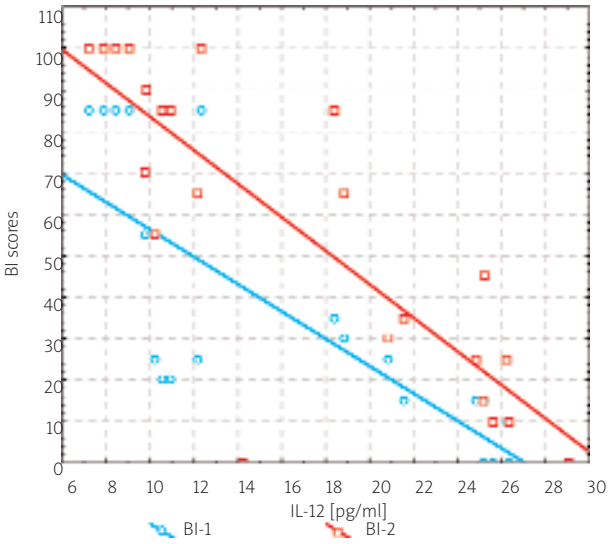

Fig. 3. The correlation (r) between serum IL-12 levels [pg/ml] in stroke patients and BI scores within 24 h (BI-1) and at 2nd week (BI-2)

Discussion

Cytokines are commonly released in response to tissue injury [13]. Thus, an increase in serum IL-12 levels in patients with acute cerebral infarction is in line with the reported rapid elevations in IL-12 concentrations in the serum of patients with such tissue injuries as acute myocardial infarction [50] and severe brain trauma [2].

The finding of IL-12 increment in the serum of stroke patients seems to be consecutive to the cytokine local and/or systemic immune response to ischemic brain damage. Numerous authors indicate serum cytokine aberrations in human stroke to be a result of the molecules local production by stroke-activated brain resident cells and ischemic brain-infiltrated leukocytes and/or of the cytokines systemic expression by circulating leukocytes [6,13,15,18,24].

Cellular origins of local IL-12 production in stroke remains unknown, but monocytes/macrophages lineage and its morphological and functional CNS counterpart, i.e. microglial cells, are the cytokine important producers following cerebral ischemia [1,19,20,27]. Thus, IL-12 increase in the serum of stroke patients may result from the cytokine leakage from the infarcted brain region or from CSF to the systemic compartment. This is supported by previous studies indicating that local production of cytokines in stroke could lead to their concentration gradient over the blood-brain barrier, which partly can be detected systemically [15,23].

On the other hand, stroke-activated brain endothelial cells secrete cytokines that might

activate peripheral blood mononuclear cells, resulting in cytokines systemic expression [19,24].

Kouwenhoven et al. [25] demonstrated elevated numbers of IL-12-secreting monocytes and mononuclear cells isolated from the peripheral blood of acute ischemic stroke patients. In this context, an increase in IL-12 levels in the serum of stroke patients may also result from the cytokine production in peripheral blood cells.

Cytokines participate as autocrine or paracrine mediators in the development and progression of atherosclerosis [28], the common pathologic entity underlying the majority of vascular disease, including cerebral and cardiac ischemia [15]. IL-12 protein has been found in macrophages within atherosclerotic plaques [26,42], and some authors, but not others [50] have reported increased serum IL-12 concentrations in coronary atherosclerotic patients [46]. Bearing in mind that the stroke patients under study presented stroke risk factors associated with atherogenesis and atherosclerosis [35], it is possible that these factors contributed to the cytokine measurements in our patients.

A positive correlation between serum IL-12 levels in acute ischemic stroke patients and the ESR values may suggest that the magnitude of IL-12 response influences the intensity of acute phase response to brain damage. Indeed, acute phase response appears an important mechanism of host reaction to tissue injury, and is characterized by cytokine-induced hepatic synthesis of proinflammatory and procoagulant proteins which promote erythrocyte aggregation and falling, resulting in ESR being the measure of acute phase response [34]. Several studies demonstrate correlations between plasma levels of proinflammatory cytokines or its receptors and ESR values or acute phase proteins in acute stroke [3,14,44].

We found a significant correlation between serum IL-12 levels and the volume of early brain CT hypodense areas in patients within 24 h after the onset of stroke. CT hypodense areas recorded in cerebral hemispheres within 24 h of stroke represent early ischemic brain injury along with perilesional swelling [45]. Numerous authors indicate that cytokine-induced inflammation occurs within hours of stroke event and involves leukocyte-mediated proedematic and cytotoxic effects on the ischemic brain [3,4,6].

IL-12 may potentially promote exacerbation of ischemic brain damage with the cytokine ability to activate immunocompetent cells and to regulate its function towards the inflammatory response [41]. IL-12 augments production and action of several proinflammatory cytokines and chemokines [9,10,16], increases expression of endothelial cell adhesion molecules [31,47], and is a potent chemoattractant for different leukocyte subsets, including monocytes and neutrophils [21,33]. Recently, it has been shown that IL-12 gene therapy in a murine carcinoma model is associated with increased tissue infiltration by NK and T cells, and apoptosis [22]. In addition to the cytotoxicity, apoptosis is one of the important mechanisms whereby the ischemic brain-invading inflammatory cells contribute to neuronal death [30,43]. Considering the above data, the relationship between serum IL-12 levels and the size of acute ischemic lesions in stroke patients may indirectly suggest that the cytokine could potentially participate in the mechanisms associated with brain infarct evolution.

We also found a significant correlation between serum IL-12 levels in stroke patients and the neurological stroke severity and functional disability calculated both within 24 h of stroke and at the 2nd week after the disease onset. This finding indirectly indicates that magnitude of the initial IL-12 response may influence degree of early neurological stroke severity and functional disability of stroke patients and may also predispose to worse short-term stroke outcome. This supports the suggestion of the cytokine involvement in mechanisms contributing to brain infarct progression, the process affecting stroke severity and outcome [4,6].

However, we would like to mark that since the effect of IL-12 administration on experimental stroke as well as the effect of the molecule inhibition on experimental or clinical stroke has not been studied yet, the role of this proinflammatory cytokine in cerebral ischemia cannot be clearly defined at present. We therefore consider the results of this study as a preliminary report encouraging to further studies on IL-12 in brain ischemia. Nevertheless, the results presented an early increase in IL-12 serum levels in ischemic stroke patients as well as the relationship between the cytokine level and the intensity of acute phase response, the size of early brain damage, and the degree of neurological stroke severity and functional disability indirectly suggest that IL-12 may play a role in the pathophysiology of cerebral ischemia.

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