RESEARCH ARTICLE

Acute Phase IL-10 Plasma Concentration Associates with the High Risk Sources of Cardiogenic Stroke

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Abstract

Background

Etiological assessment of stroke is essential for accurate treatment decisions and for secondary prevention of recurrence. There is evidence that interleukin-10 (IL-10) associates with ischemic stroke. The aim of this prospective study was to assess the levels of IL-10 in ischemic stroke with unknown or suspected cardiogenic etiology, and evaluate the correlation between IL-10 plasma concentration and the number of diagnosed high risk sources for cardioembolism.

Methods

A total of 141 patients (97 males; mean age 61±11 years) with acute ischemic stroke with unknown etiology or suspected cardiogenic etiology other than known atrial fibrillation (AF) underwent imaging investigations to assess high risk sources for cardioembolic stroke established by the European Association of Echocardiography (EAE). IL-10 was measured on admission to the hospital and on a three month follow-up visit.

Results

Acute phase IL-10 concentration was higher in patients with EAE high risk sources, and correlated with their number (p<0.01). In patients with no risk sources (n = 104), the mean IL-10 concentration was 2.7±3.1 ng/L (range 0.3–16.3 ng/L), with one risk source (n = 26) 3.7±5.5 ng/L (0.3–23.6 ng/L), with two risk sources (n = 10) 7.0±10.0 ng/L (1.29–34.8 ng/L) and with three risk sources (n = 1) 37.2 ng/L. IL-10 level was not significantly associated with cerebral infarct volume, presence of previous or recent myocardial infarction, carotid/

vertebral artery atherosclerosis, paroxysmal AF registered on 24-hour ECG Holter monitoring or given intravenous thrombolytic treatment.

Conclusion

IL-10 plasma concentration correlates independently with the number of EAE cardioembolic risk sources in patients with acute stroke. IL-10 may have potential to improve differential diagnostics of stroke with unknown etiology.

Introduction

Stroke is the third highest cause of mortality in industrialized countries, accounting for 10% of all deaths, and is the leading cause of long-term disability worldwide [1,2]. The currently recognized mechanisms of ischemic stroke and transient ischemic attack (TIA) are thrombosis, embolism, or decreased perfusion [3]. Brain embolism can be of arterial or cardiac origin, with atrial fibrillation (AF) being by far the most common cause of cardioembolic stroke/TIA [4,5]. Exclusion of hemorrhage and accurate etiological assessment are vital for appropriate treatment and secondary prevention of stroke. Despite current imaging techniques and modern diagnostic work-up, approximately 30–40% of patients with ischemic strokes/TIAs remain without a well-defined etiology [6]. A cardiac origin for ischemic stroke has traditionally been suspected on the basis of characteristic clinical symptoms and, more recently, by imaging findings established by the European Association of Echocardiography (EAE) [7–9].

Interleukin-10 (IL-10) is an anti-inflammatory cytokine [10]. IL-10 concentrations have been found to be elevated in the cerebrospinal fluid (CSF) in patients with acute ischemic stroke [11]. It has been suggested that increased IL-10 concentration has a neuroprotective function [12,13]. High serum concentration of IL-10 predicts the presence of salvageable ischemic tissue after ischemic stroke [14,15]. In rodent models of stroke, systemically administrated IL-10 reduces the cerebral infarction volume after stroke [13]. In addition, the number of peripheral blood mononuclear cells (PBMC) secreting IL-10 has been shown to be elevated in patients with ischemic stroke and cerebral hemorrhage [16]. We prospectively studied patients with acute ischemic stroke with unknown etiology or suspected cardiogenic etiology other than known AF. The purpose of the study was to assess the plasma levels of IL-10, and evaluate whether IL-10 plasma concentration is associated with the number of EAE cardioembolic risk sources [17].

Materials and Methods

The study was approved by the University Hospital Research Ethics Board. Prior to participation in the study, written informed consent was obtained from the patient or the patient's legally authorized representative.

Study Design and Population

Patients with acute stroke/TIA admitted to university hospital of Eastern Finland were evaluated as candidates for this EMBODETECT study. The neurologists involved in this study recruited 162 patients with unknown etiology or suspected cardiogenic etiology other than known AF, as described previously [7].

Of the 162 patients initially recruited, 21 were excluded; eight patients lacked the IL-10 analyses in the acute phase, cardiac CT (cCT) image quality was not appropriate for analyses in ten patients due to technical error, and three patients decided not to participate after giving informed consent. Altogether 141 patients underwent IL-10 plasma concentration analyses in the acute phase. All patients were invited to a follow-up visit three months later, but only 69 (49%) turned up. According to medical records none of the patients died within 3 months.

CT Imaging of the Heart, Carotid/Vertebral Arteries and Brain

Profound imaging investigations including combined examination of the heart, aorta, and cervicocranial arteries with CT (CACC-CT) and echocardiography were performed to define the etiology of stroke according to the the EAE high risk source categories for cardioembolism.

In patients *without* cardioembolic high risk sources, the etiology of stroke was categorized according to the TOAST criteria [9]. The volume of the infarcted cerebral tissue was quantified.

All 141 stroke/TIA patients underwent the contrast-enhanced CT scan (Somatom Sensation 16 and Somatom Definition AS; Siemens Medical Solutions, Forchheim, Germany) of the aortic arch, cervical arteries and intracranial arteries, immediately followed by scanning of the ascending aorta and heart. Cardiac imaging was performed during mid-diastole in all study subjects. For volume measurements of infarcted cerebral tissue, 4.5–5 mm transversal slices were reconstructed in the brain CT in the subacute phase (> two days). Infarction volume was calculated by using Simpson's method [18]. Carotid and vertebral artery stenoses were evaluated from CT angiography (CTA). TEE and CT were used to diagnose aortic arch atheromas. Atheroma plaques with thickness ≥4 mm, ulceration ≥2 mm or aortic atherosclerosis with intimal thickening <4 mm were regarded as high risk sources.

Additional Imaging with Echocardiography and Magnetic Resonance Imaging

Transthoracic (TTE) and transesophageal echocardiography (TEE) were performed for all patients (Vivid 7 cardiovascular ultrasound system, GE Medical Systems, Buckinghamshire, UK) by several cardiologists as part of their clinical routine [8]. Contrast-enhanced cardiac magnetic resonance imaging (MRI) was performed according to the protocols of the Society of Cardiovascular Magnetic Resonance using a 1.5 T scanner and 12-element phased-array surface coil (Siemens Avanto, Erlangen, Germany), to confirm suspected structural abnormalities in cCT (n = 18) or in patients with discrepant findings in cCT and echocardiography (n = 12). Ambulatory 24-hour Holter ECG was performed to evaluate the presence of paroxysmal AF for all patients.

IL-10 measurement

Blood samples for IL-10 analyses were taken 3±2 days after hospitalization (range 0–10 days), representing the acute phase, and also at 3 months, representing the chronic phase, by medical laboratory technologists. Plasma samples were separated with centrifugation and stored frozen at -70°C until analyzed. IL-10 concentrations were measured from EDTA plasma samples with sandwich-type high-sensitivity ELISA (Quantikine, R&D Systems, Minneapolis, MN, USA). The sensitivity of the assays was 0.09 ng/L.

Statistical Analyses

Continuous variables with normal distribution are presented as mean±SD, and categorical variables as absolute values and percentages. Based on the Kolmogorov-Smirnov test, Student's t-

test for normally distributed and the Mann-Whitney U test for abnormally distributed non-parametric values, these tests were used to compare significance in IL-10 concentration between dichotomous groups.

The Kruskal-Wallis test was used when the difference in continuous variable (IL-10 concentration) was tested with ordinal variable with three or more groups (the number of cardioembolic risk sources). Spearman's correlation coefficient was used to investigate the associations between continuous background characteristics and absolute IL-10 plasma concentration, and the chi-squared test was used test to investigate nominal variables. The ANCOVA test was used to test the effect of patient characteristics on the correlation between IL-10 concentration and the number of cardioembolic risk sources. Statistical significance was set at $p < 0.05$ and high statistical significance at $p < 0.01$. The Bonferroni correction to control the familywise error rate was used for multiple group comparisons (evaluation of statistical significance in 13 EAE high risk sources) [17]; statistical significance was adjusted to $p \leq 0.003$. Data were analyzed using SPSS for Windows (version 19, 1989–2010 SPSS Inc., Chicago, USA).

Results

Patient Characteristics and Stroke Etiologies

Patient characteristics, prevalence of common risk factors and different stroke etiologies are presented in Table 1. IL-10 plasma concentrations were evaluated in 141 patients (97 males; mean age 61±11 years; range: 32–84 years) in the acute phase and in 69 patients (49 males;

Table 1. The Correlation Between Interleukin(IL)-10 Plasma Concentration and Patient Characteristics of 141 Acute and 69 Chronic Phase Stroke Patients.

	Variable	Acute phase	Sig.	Chronic phase	Sig.
Characteristics	Age, y	60.5±10.6	ns.	60.3±11.3	ns.
	Males, n (%)	97 (68.8)	ns.	49 (71.0)	ns.
	Body mass index, kg/m^2	28.0±4.5	0.008	27.4±4.1	ns.
	Body surface area, m^2	2.0±0.2	ns.	1.9±0.2	ns.
	Caucasian race, n (%)	141 (100.0)	ns.	69 (100.0)	ns.
	Hypertension, n (%)	80 (56.7)	ns.	36 (52.2)	ns.
	Hyperlipidemia, n (%)	56 (39.7)	ns.	28 (19.9)	ns.
	Diabetes, n (%)	19 (13.5)	ns.	2 (2.9)	ns.
	Smokers, n (%)	38 (27.0)	ns.	18 (26.1)	ns.
	Stroke, n (%)	103 (73.0)	ns.	48 (69.6)	ns.
	Transient ischemic attack, n (%)	38 (27.0)	ns.	21 (30.4)	ns.
Medication during hospitalization	Aspirin, n (%)	45 (31.9)	ns.	24 (34.8)	ns.
	Warfarin, n (%)	9 (6.4)	ns.	5 (7.2)	ns.
	Clopidogrel, n (%)	3 (2.1)	0.014	1 (1.4)	ns.
	Dipyridamole, n (%)	5 (3.5)	ns.	2 (2.9)	ns.
	Statin, n (%)	40 (28.4)	ns.	18 (26.1)	ns.
IL-10 sample collection	Sample retrieval after hospitalization, days	3±2	ns.	102±13	ns.
Stroke/TIA etiology	Cardioembolic risk source ($\geq$1)	39 (28%)	0.006	11 (16%)	ns.
	Large-artery atherosclerosis	24 (17%)	ns.	5 (7%)	ns.
	Cardioembolic source combined with large-artery atherosclerosis	9 (6%)	ns.	5 (7%)	ns.
	Small-vessel occlusion	11 (8%)	ns.	4 (6%)	ns.
	Cryptogenic	78 (55%)	0.034	44 (64%)	ns.

Sig. = significance; ns. = no significance at level $p < 0.05$

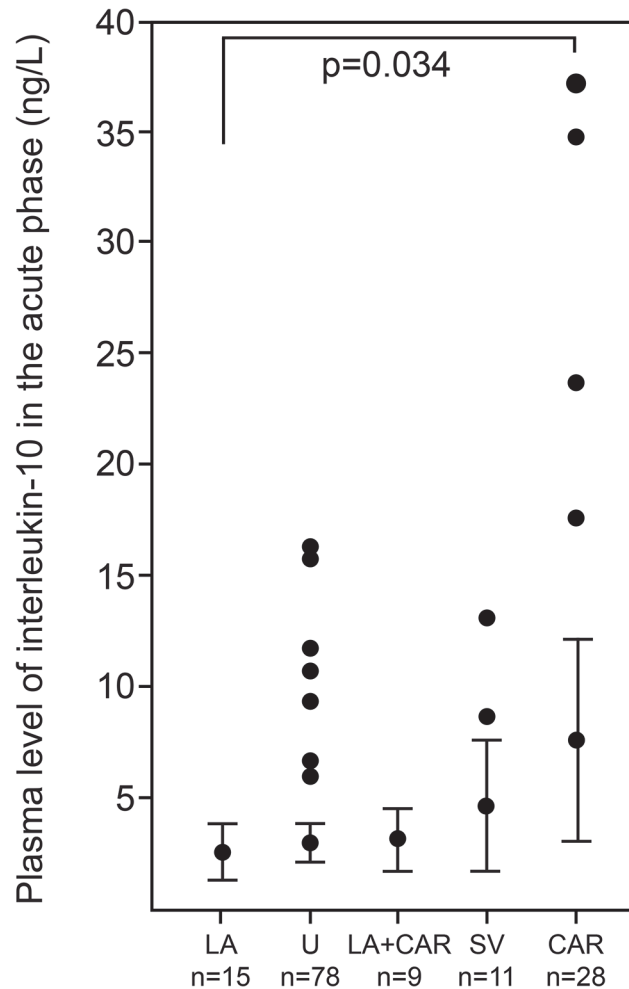doi:10.1371/journal.pone.0120910.t001

Fig 1. Acute Phase Interleukin(IL)-10 Plasma Concentration (ng/L) Presented with 95% Confidence Intervals in Different Stroke/Transient Ischemic Attack (TIA) Etiologies. IL-10 concentration in 28 patients (20%) with cardioembolic risk sources for stroke/TIA (CAR) had a mean IL-10 plasma concentration of 6.5±9.9 ng/L. Stroke/TIA with a large-artery atherosclerosis etiology (LA) was found in 15 patients (11%), with a mean IL-10 plasma concentration of 2.2±1.8 ng/L. Nine patients (6%) were characterized with both cardiogenic and atherosclerotic etiologies (LA+CAR), and had a mean IL-10 concentration of 2.7±1.8 ng/L. Small-vessel occlusion (SV) was found in 11 patients (8%) with a mean IL-10 plasma concentration of 4.0 ±3.8 ng/L; in the remaining 78 patients (55%) with undetermined etiology (U), the IL-10 plasma concentration was 2.6±3.2 ng/L. IL-10 concentration in stroke/TIA patients with major risk sources for cardioembolism was significantly higher (p = 0.034) than in patients with large-artery carotid/vertebral atherosclerosis and without risk sources for cardioembolism.

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mean age 60±11 years; range 32–84 years) in the chronic phase. BMI was only background characteritic which had significant association to IL-10 plasma concentration.

The mean IL-10 plasma concentration was 3.4±5.3 ng/L (range: 0.3–37.2 ng/L) in the acute phase and 2.5±2.6 ng/L (range 0.3–15.5 ng/L) in the chronic phase (ns). Neither acute nor chronic phase IL-10 plasma concentrations were associated with prior or recent (<6 months) ischemic MI, vertebral/carotid stenosis, given intravenous thrombolytic treatment, or infarction volume in cerebral tissue. Plasma IL-10 concentration in the acute phase was significantly higher (p<0.034) in patients with cardioembolic risk source/sources (≥1) compared to those with atherosclerotic etiology (Fig 1), the significance remained also after the IL-10 plasma
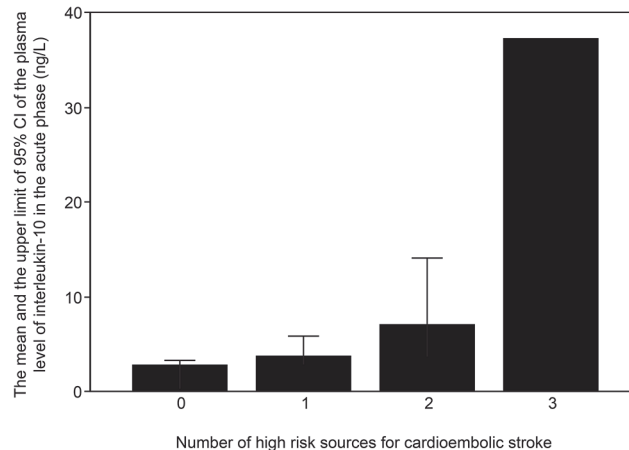
Fig 2. Acute Phase Interleukin (IL)-10 Plasma Concentration (ng/L) in 141 Patients with Cardioembolic Stroke According to the Number of High Risk Source Findings Presented by the European Association of Echocardiography (EAE). In patients with no EAE high risk factors, the mean IL-10 concentration was 2.7±3.1 ng/L (n = 104; range: 0.3–16.3 ng/L); with one risk factor it was 3.7±5.5 ng/L (n = 26; range: 0.3–23.6 ng/L), with two risk factors 7.0±10.0 ng/L (n = 10; range: 1.29–34.8 ng/L) and with three risk factors the IL-10 measurement was 37.2 ng/L (n = 1).

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concentration was adjusted for BMI. Between other etiologies, no significant differences were observed.

IL-10 Concentration in Patients with Cardioembolic Stroke/TIA

A statistically significant association was observed between IL-10 plasma concentration and the number of detected EAE high risk sources (p = 0.023). The association between IL-10 concentration and the number of EAE high risk sources remained significant after excluding the single case with three risk factors (p = 0.036), and also after adjustment for body mass index (BMI).

In patients with no risk factors, IL-10 concentration in the acute phase was 2.7±3.1 ng/L (n = 104; range: 0.3–16.3 ng/L), with one risk factor 3.7±5.5 ng/L (n = 26; range: 0.3–23.6 ng/L), with two risk factors 7.0±10.0 ng/L (n = 10; range: 1.29–34.8 ng/L) and with three risk factors 37.2 ng/L (n = 1) (Fig 2). No statistical significance was found between IL-10 and the number of EAE high risk sources in the chronic phase.

IL-10 levels in patients with or without each single EAE high risk sources are presented in Table 2. Mean level of IL-10 was high when previous MI, left ventricular aneurysm, intracardiac thrombus or aortic arch atheromatous plaque was present. However, the number of patients was small and several patients had multiple high risk sources. After Bonferroni correction, none of the individual risk sources correlated significantly with IL-10 concentration. Previously known AF was an exclusion criteria of the study, but patients who were found to have paroxysmal AF in 24-h Holter recording during hospital stay (n = 20) were not excluded. There was no significant difference in IL-10 plasma concentrations between patients with or without AF.

Discussion

The main finding of the current study was that plasma IL-10 concentration in stroke patients with major risk sources for cardioembolism (highly probable cardiogenic etiology) was significantly higher than in patients with large-artery carotid/vertebral atherosclerosis and without major risk sources for cardioembolism (highly probable non-cardiogenic etiology) (Fig 1).

Table 2. Interleukin-10 Plasma Concentration in Risk Source Groups Defined by the European Association of Echocardiography (EAE).

EAE high risk sources	At hospitalization						After 3 months					
	Present		Absent		Significance		Present		Absent		Significance	
	N (%)	Mean ± SD (ng/L)	N (%)	Mean ± SD (ng/L)	Unadjusted	Bonferroni Adjusted	N (%)	Mean ± SD (ng/L)	N (%)	Mean ± SD (ng/L)	Unadjusted	Bonferroni Adjusted
Atrial fibrillation	20 (14.2)	3.0±2.6	121 (85.8)	3.5±5.6	ns.	ns.	9 (13.0)	1.5±1.2	60 (87.0)	2.7±2.7	ns.	ns.
Previous myocardial infarction	13 (9.2)	7.8±10.7	128 (90.7)	3.0±4.3	ns.	ns.	6 (8.7)	2.5±2.1	63 (91.3)	2.5±2.7	ns.	ns.
Recent myocardial infarction (left ventricular aneurysm)	1 (0.7)	37.2	140 (99.3)	3.2±4.5	n/a	n/a	0 (0.0)	-	69 (100.0)	2.5±2.6	n/a	n/a
Cardiomyopathies	0 (0.0)	-	141 (100.0)	3.4±5.3	n/a	n/a	0 (0.0)	-	69 (100.0)	2.5±2.6	n/a	n/a
Intracardiac thrombus	6 (4.3)	13.7± 17.4	135 (95.7)	3.0±3.6	ns.	ns.	0 (0.0)	-	69 (100.0)	2.5±2.6	n/a	n/a
Intracardiac tumours	2 (1.4)	3.0±2.5	139 (98.6)	3.5±5.3	n/a	n/a	0 (0.0)	-	69 (100.0)	2.5±2.6	n/a	n/a
Fibroelastoma	1 (0.7)	0.6±0.0	140 (99.3)	3.5±5.3	n/a	n/a	0 (0.0)	-	69 (100.0)	2.5±2.6	n/a	n/a
Marantic vegetations	0 (0.0)	-	141 (100.0)	3.4±5.3	n/a	n/a	0 (0.0)	-	69 (100.0)	2.5±2.6	n/a	n/a
Rheumatic valve disease (mitral stenosis)	0 (0.0)	-	141 (100.0)	3.4±5.3	n/a	n/a	0 (0.0)	-	69 (100)	2.5±2.6	n/a	n/a
Aortic arch atheromatous plaques	6 (4.3)	9.8± 13.6	135 (95.7)	3.2±4.5	0.023	ns.	1 (1.4)	0.8±0.0	68 (98.6)	2.5±2.6	ns.	ns.
Endocarditis	0 (0.0)	-	141 (100.0)	3.4±5.3	n/a	n/a	0 (0.0)	-	69 (100)	2.5±2.6	n/a	n/a
Mechanical valve prosthesis	0 (0.0)	-	141 (100.0)	3.4±5.3	n/a	n/a	0 (0.0)	-	69 (100)	2.5±2.6	n/a	n/a

SD = standard deviation; ns. = no significance at level $p<0.05$ or <0.003 for unadjusted and adjusted significances, respectively; n/a = not applicable.

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Table 3. Interleukin-10 Plasma Concentration in Different Stroke Patient Categories Defined by the Total Number of the European Association of Echocardiography High Risk Sources.

Number of high risk sources	At hospitalization		After three months	
	N (%)	Mean±SD (ng/L)	N (%)	Mean±SD (ng/L)
0	104 (73.8)	2.7±3.1	54 (78.3)	2.7±2.8
1	26 (18.4)	3.7±5.5	14 (20.3)	2.0±1.7
2	10 (7.1)	7.0±10.0	1 (1.4)	1.7±1.2
3	1 (0.7)	37.2±0.0	0 (0.0)	0.0±0.0

SD = standard deviation.

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Moreover, plasma IL-10 level was associated with the number of cardioembolic risk sources; the higher the number, the higher the measured acute phase IL-10 concentration (Table 3). The association of IL-10 concentrations could be seen only at the acute phase of stroke; three months later there was no difference in IL-10 levels between the categories. This suggests that IL-10 secretion is related to acute cardiogenic thrombus, either to its formation, resolution or to the acute consequences of its embolism, and might have potential to improve the differential diagnostics of stroke/TIA etiology.

Previously, Emsley et al. have reported that patients with acute ischemic stroke and significant large artery atherosclerosis have significantly lower concentrations of plasma IL-10 than stroke patients without significant atherosclerosis [19]. Our results are in line with their observation. However, in the study of Emsley [19] major sources of cardioembolism, and their association with IL-10 were not evaluated. To the best of our knowledge, the association between the presence of cardioembolic risk sources and IL-10 levels has neither been reported elsewhere before. In our study, the patients were thoroughly investigated by cardiac imaging, and patients with ≥ 1 detected visible cardioembolic sources (left chamber aneurysm, thrombus detected by TTE, >4 mm aortic arch atheromatous plaque and previous myocardial infarction with abnormal left ventricle wall motion) had the highest plasma IL-10 concentrations.

AF is the most common etiology for cardioembolic stroke [4,5]. Our study was aimed to assess patients with unknown or suspected (but not obvious) cardiogenic stroke etiology. Therefore, previously known atrial fibrillation was an exclusion criteria. However, patients who were diagnosed to have paroxysmal AF (PAF) in 24-h Holter recording during hospital stay (n = 20) were not excluded. This subgroup of patients was relatively small (14.2% of the whole population) and selected, and does not represent the whole spectrum of AF. Thereby, strong conclusions of the association between AF and IL-10 plasma concentration cannot be drawn. Interestingly, we found mean IL-10 concentration low in this PAF population.

In an animal model, both intraventricularly and systemically administered IL-10 have been shown to reduce cerebral infarction volume [13], and IL-10 has been suggested to be neuroprotective [13–15, 20]. High levels of serum IL-10 have been suggested to facilitate the selection of ischemic stroke patients with salvageable brain tissue for systemic thrombolysis [14]. However, no association with the IL-10 serum concentration and the cerebral infarction volume was found in our study.

IL-10, an anti-inflammatory cytokine produced primarily by T-cells and monocytes, is suggested to play a role in vascular protection, although the exact mechanisms remain unclear. IL-10 has been shown to reduce atherogenesis via inhibition of the LDL/Ox-LDL dependent monocyte-endothelial interaction [21–23]. In addition, low production of IL-10 has been associated with several cardiovascular risk factors, such as metabolic syndrome, insulin resistance and type 2 diabetes mellitus [24,25], and overweight-related sleep apnea [26]. Decreased IL-10

levels are found at the onset of acute coronary syndrome and increased concentrations have been associated with improved prognosis [27–32]. Interestingly, IL-10 knockout mice have been reported to develop vascular and cardiac dysfunction [33]. In addition, epidemiological data indicate that individuals with elevated IL-10 plasma levels have a reduced risk of stroke [34], and that subjects with a history of stroke have lower levels of IL-10 than those without previous stroke [24].

As the increased level of IL-10 seems to be vascular- and cardioprotective according to the previous literature, IL-10 is not likely to be the predisposing factor for the stroke in our cardioembolic subgroup, either. More likely, the acutely elevated IL-10 might play a role in the auto-regulatory or protective feedback mechanism during thrombus generation. This presumption is supported by several previous findings. Earlier, Downing et al. (1998) have demonstrated in an animal model that emerging high quantities of IL-10 secreted by monocytes and neutrophils consequently to mechanical stasis-induced venous thrombosis regulate the resultant vein wall inflammatory response [10]. Moreover, IL-10 has been shown to reduce procoagulant and proaggregatory activity in various *in vitro* and *in vivo* models [22,30,35–40]. The main limitation of this study was the relatively small number of patients with more than two EAE risk sources for cardioembolic stroke. The study population decreased even further over the three month follow-up due to low attendance to follow-up visits. The treatment of the patients was not standardized, but was tailored individually according to the current guidelines. Therefore, there is a possibility that antithrombotic treatment or other medication might have interfered with the results. Eleven patients (7.8%) were treated with recombinant tissue plasminogen activator (r-tPA). The IL-10 levels of this small group did not differ from the rest of the population, suggesting that plasma IL-10 concentration was not affected by the administration of r-tPA.

In conclusion, we found significant difference in IL-10 concentration between patients with cardioembolic sources and large-artery atherosclerosis in acute-phase of stroke. IL-10 plasma concentration was strongly associated with the number of cardioembolic high risk sources. The results merit further studies in larger stroke populations, also including AF patient groups. According to our preliminary results, IL-10 measurement may potentially improve the diagnostics of stroke etiology.

Author Contributions

Conceived and designed the experiments: AM MT PS MH PJ RV KP PM. Performed the experiments: OA AM MT. Analyzed the data: OA AM MT RV KP PM. Contributed reagents/materials/analysis tools: PS MH PJ RV KP PM. Wrote the paper: OA AM MT PS MH PJ RV KP PM.

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