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ORIGINAL COMMUNICATION

Serum levels of cytokines and C-reactive protein in acute ischemic stroke patients, and their relationship to stroke lateralization, type, and infarct volume

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Abstract There is increasing evidence that inflammation plays an important role in the progression of acute ischemic stroke (AIS). The primary aims of this study were to examine the serum levels of 13 cytokines, C-reactive protein (CRP), glucose, and hemoglobin in AIS patients, and their relationship to stroke lateralization, type, and infarct volume. Forty-five patients with AIS were evaluated. Blood samples were taken within 72 h, and volumetric analyses performed within 1–7 days after AIS onset. Cytokines were measured in serum from all patients and from 40 control subjects using Luminex Bio-Plex XMap technology. The levels of interleukin (IL)-1ra ($p < 0.001$), IL-6 ($p < 0.001$), IL-8 ($p < 0.001$), IL-9 ($p = 0.038$), IL-10 ($p = 0.001$), IL-12 ($p = 0.001$), IL-18 ($p < 0.001$), and GRO-α (CXCL1) ($p = 0.017$) were significantly higher in the AIS patients than in the controls. The IL-8 level was significantly correlated with age in the patient group ($r = 0.52$, $p < 0.001$). None of the variables were found to be associated with stroke lateralization. Infarct volume was significantly positively correlated with CRP level ($r = 0.47$, $p = 0.005$). Patients with radiologically confirmed infarctions had significantly elevated serum levels of GRO-α ($p = 0.023$). The cytokine profile of the AIS patients supports not only earlier findings of a proinflammatory response but also early activation of endogenous immunosuppressive mechanisms. Novel findings of this study are elevated serum levels of IL-9 and GRO-α. Elevated GRO-α in AIS patients with radiologically confirmed infarctions suggests that GRO-α is specific for stroke of known etiology. Our results indicate that CRP plays an important role in the progression of cerebral tissue injury.

Keywords Acute ischemic stroke · Inflammation · Cytokines · Proinflammatory · Anti-inflammatory

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Background

There is increasing evidence that inflammation plays an important role in acute ischemic stroke (AIS), indicating important interactions between the nervous and immune systems [1]. Cerebral ischemia induces a robust inflammatory reaction that involves several cell types. Many recent studies have focused on the inflammatory reaction after the ischemic episode, identifying the roles of important inflammatory signaling molecules, particularly cytokines [2]. Cytokines are up-regulated in the brain after stroke, and are expressed not only in immunological cells but also in glial cells and neurons [3].

The most studied cytokines related to stroke are interleukin (IL)-1β, IL-6, IL-10, and tumor necrosis factor-α (TNF-α). The proinflammatory cytokines TNF-α, IL-1β, and IL-6 are secreted in the ischemic region by activated immune cells, which drive the inflammatory process and accelerate additional inflammatory processes by inducing the expression of inflammatory molecules. These molecules recruit more circulating leukocytes, which infiltrate the ischemic region and lead to further loss of neuronal cells and brain tissue, thereby possibly enlarging the cerebral infarct area [4, 5]. Whether postischemic inflammatory responses are deleterious or beneficial to brain recovery is presently a matter of debate [6]. Several studies have examined whether the inflammatory response following AIS is related to infarct volume [7–14] and stroke subtype [15]. The results are inconsistent, thus the roles of the cytokines involved are still unclear.

Various known cytokines have been studied individually, but no study has evaluated numerous different cytokines in a single patient group using one methodology. The aim of this study was to measure the serum levels of 13 cytokines in patients with AIS to improve the understanding of the complex interplay between the pro- and anti-inflammatory cytokines involved. We also examined the relation of serum levels of hemoglobin, C-reactive protein (CRP), and glucose to stroke lateralization, diagnostic subtype, and infarct volume.

Methods

Patients

The cohort comprised 45 patients included in a longitudinal study of first-ever stroke patients admitted to Buskerud Hospital, a regional hospital in Norway. The recruiting procedure is described in detail elsewhere [16]. In short, the inclusion criteria were clinical presentation of first-ever stroke, age 18 years or older, and exhibiting sufficient cognitive functioning to participate. Stroke was defined as the presence of rapidly developing focal neurological signs or symptoms of vascular origin that persisted for >24 h. The exclusion criteria were no serum samples collected, uncertain diagnosis of AIS, presence of cancer, receiving thrombolysis therapy, and uncertain symptom onset.

Clinical and instrumental evaluation

A general medical history was collected, and physical and neurological examinations, standard laboratory tests, 12-lead ECG, and cerebral CT were performed in all patients on admission. Patients without clear radiological findings on admission, but with persistent clinical symptoms indicative of AIS after 2–4 days were submitted to a secondary CT or MRI scan. The type of AIS was classified according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification [17]: cardioembolic infarct (CEI), large-artery atherosclerosis (LAAS), lacunar infarct (LAC), stroke of undetermined etiology (UDE), and stroke of other determined etiology. Lateralization of the stroke was determined by clinical findings and imaging results, or on a clinical basis only in patients without conclusive CT or MRI findings. Location of the stroke, supra- or infratentorial respectively, was determined by radiological findings.

The functional disability of patients in the acute phase was measured using the Barthel Index-20 scale (BI-20) [18]. The BI-20 has proven to be the most reliable disability scale for stroke; it correlates strongly with the immediate poststroke condition [19].

Imaging and volumetric analysis

The imaging used for measurement of infarct volume was performed within 1–2 days, 3–5 days, and 7 days after stroke onset for 11, 22, and one of the 34 patients, respectively. Infarct volumes were measured on either MRI (24/34 cases) or CT (10/34). On MRI, diffusion sequences were used in the axial plane, combined with sagittal T2 turbo spin-echo and/or coronal fluid-attenuated inversion recovery imaging. An on-screen measuring technique was used. On CT, measurements were made on axial slices in combination with thickness data.

Control subjects

Control serum samples were prepared by collecting blood from 40 healthy donors (The Bloodbank, Oslo University Hospital, Ullevål, Oslo, Norway) with an even female/male distribution. We achieved a biobank similar to that of the material collected from our stroke patients by selecting donors aged 50–70 years. None of the donors were using immunosuppressive medication.

Blood collection and serum preparation

Blood samples were taken at the same time point for all blood components measured; at <24, 24–48, and 48–72 h after stroke onset in 35, 7, and three of the 45 patients, respectively. For cytokine measurements blood was collected in 7.5-ml serum S-Monovette gel tubes (Sarstedt, Nümbrecht, Germany), and stored for 30–120 min at room temperature prior to centrifugation at 1,400$\times g$ for 12 min. Serum samples were frozen at −70°C.

CRP, glucose, and hemoglobin measurements

Serum levels of CRP, glucose, and hemoglobin were all determined with clinically validated assays. Determination

of CRP was performed using a standard latex immunoassay; CRP Vario (Abbott Diagnostics). Glucose was determined using Architect cSystems (Abbott Diagnostics). Finally, Hemoglobin was determined using Advia 120 Hematology system (Siemens Health Care Diagnostics).

Cytokine measurement

Cytokine levels were measured using a Luminex IS 100 instrument (Bio-Rad, Hercules, CA). Based on a screening of representative serum samples using the Bio-Plex human cytokine 27-plex assay (catalog no. 171A11127, Bio-Rad), the following custom plex was purchased to screen all samples: Bio-Plex Pro^{TM} Human Cytokine Group I; IL-1β, IL-1ra, IL-2, IL-4, IL-6, IL-8, IL-9, IL-10, IL-12, TNF-α, and interferon-γ (IFN-γ); Bio-Plex Pro^{TM} Human Cytokine Group II; and GRO-α (CXCL1) and IL-18 (Bio-Rad). All samples were thawed on ice, vortexed, spun down at 14,000$\times g$ for 10 min at 4°C, and then diluted at 1:4. Longitudinal controls were used to assess the interassay variation. The coefficient of variation for controls ranged from 9 to 19% on the exponential phase of the standard curve.

Statistical analysis

The two-tailed Mann–Whitney test was used to compare the median values of cytokine variables in two groups. The independent-samples t test was used to compare means. The level of statistical significance was set at $p < 0.05$. If more than 50% of the values of a cytokine variable were not detectable in at least one of the groups, the variable was dichotomized as detectable (yes/no) and analyzed using Pearson's Chi-square test.

Since the distributions of most of the cytokine variables were markedly skewed, the results are presented as medians and interquartile ranges. When age was found to be a confounder for a difference in cytokine variables between the groups, linear regression analysis was used to adjust for age. The Spearman correlation coefficient was used to quantify the association between two continuous variables. All statistical analyses were performed using the SPSS statistical package (version 15, SPSS, Chicago, IL).

Results

All clinical data of the AIS patients are presented in Table 1. The gender distribution did not differ significantly between the AIS and control groups, but the mean age was significantly higher in the former (67.7 vs. 59.1 years, respectively; $p < 0.001$). Twenty-two patients had right-sided, 21 left-sided and 2 bilateral ischemic stroke. The infarct volume measured in the 34 patients with a radiologically confirmed infarction ranged from 0.5 to 140 cm^3, with a median value of 2 cm^3 (1, 17).

Serum levels of cytokines in the AIS and control subjects are also presented in Table 1. The levels of the following cytokines were significantly higher in the AIS patients than in the controls: IL-1ra ($p < 0.001$), IL-6 ($p < 0.001$), IL-8 ($p < 0.001$), IL-9 ($p = 0.038$), IL-10 ($p = 0.001$), IL-12 ($p = 0.001$), IL-18 ($p < 0.001$), and GRO-α ($p = 0.017$). IL-8 was significantly correlated with age in the patient group ($r = 0.52$, $p < 0.001$), but not in the control group. The difference in IL-8 between the groups remained significant after adjusting for age ($p < 0.001$).

To get an indication of the stability of the cytokines, secondary blood samples were taken from 33 of the patients, between 2 and 5 days after stroke onset. Comparing the acute-phase cytokine levels and these secondary cytokine levels revealed that the only cytokines that changed significantly were IL-18 and GRO-α. The median level of IL-18 decreased from 1,820 to 1,630 μg/ml ($p = 0.011$), whilst that of GRO-α decreased from 1,207 to 868 μg/ml ($p = 0.006$). This indicates that most of the investigated cytokines are relatively stable for many hours, or even days, after stroke onset. This is also supported by several previous studies, as discussed later.

The BI-20 score was significantly negatively correlated with the levels of IL-2 ($r = –0.354$, $p = 0.02$), IL-6 ($r = –0.334$, $p = 0.028$), IL-12 ($r = 0.38$, $p = –0.012$), and IFN-γ ($r = –0.37$, $p = 0.014$). No differences were found between left-sided and right-sided infractions for any of the immunological markers, hemoglobin, glucose, or BI-20 score. The group with infratentorial findings was too small ($n = 4$) to allow for detailed statistical analysis on location.

Infarct volume was significantly positively correlated with CRP level ($r = 0.47$, $p = 0.005$). No correlation was found between infarct volume and any of the immunological markers, hemoglobin, glucose, or BI-20 score.

TOAST variables were dichotomized in radiologically confirmed infarctions (merged CEI, LAAS, and LAC) and radiologically unconfirmed infarctions (UDE); data presented in Table 2. Patients with radiologically confirmed infarctions had significantly elevated serum levels of GRO-α (1,613 vs. 888 μg/ml, $p = 0.023$). This group also had significantly lower age (56 vs. 74 years, $p = 0.037$) and significantly lower prevalence of coronary disease (9 vs. 36%), $p = 0.028$). Age and presence of coronary disease was not correlated with GRO-α; thus, the differences in serum level of GRO-α cannot be explained by the difference in these variables between the two groups. We did not find any differences between these two groups in regard to any of the other immunological markers, hemoglobin, glucose, or BI-20 score.

Table 1 Clinical variables of the acute ischemic stroke (AIS) patients and cytokine serum levels of these and control subjects

Characteristic	Stroke patients ($n = 45$)	Control subject ($n = 40$)	p
Male, n (%)	27 (60)	20 (50)	0.14
Mean age, years ± SD[e]	67.7 ± 11.8	59.1 ± 5.7	**<0.001**
Right-sided ischemic stroke, n (%)	22 (49%)		
Radiologically confirmed infarction, n (%)	34 (53%)		
Supratentorial infarction, n (% of 34)	30 (88%)		
TOAST			
CEI	5 (11%)		
LAAS	10 (22%)		
LAAC	19 (42%)		
UDE	11 (24%)		
Medication			
Prednisolone	3 (7%)		
NSAIDs	3 (7%)		
Co-morbidity			
Hypertension	28 (62%)		
Atrial fibrillation	6 (13%)		
Coronary disease	7 (16%)		
Diabetes	4 (9%)		
Rheumatism	2 (4%)		
Psoriatic arthritis	3 (7%)		
Bechterew's disease	1		
Hyperthyroidism	1		
Mean values, ±SD			
Hemoglobin (g/dl)	14.3 ± 1.3		
Glucose (mmol/l)	7.0 ± 2.3[a]		
Median values, interquartile range			
BI-20	20 (17, 20)[b]		
Infarct volume (cm^3)	2 (1, 17)		
CRP (mg/l)	3 (2.0, 6.5)		
Median values, interquartile range[c]			
Cytokines			
IL-1 ra (μg/ml)	1,170 (840, 1,573)	492 (386, 662)	**<0.001**
IL-8 (μg/ml)	200 (123, 251)	98 (73, 130)	**<0.001**
IL-18 (μg/ml)	1,767 (1,405, 2,262)	1,187 (899, 1,689)	**<0.001**
GRO-α	1,493 (431, 1,821)	691 (483, 1,351)	**0.017**
Dichotomized values (% detectable of total)[d]			
Cytokines			
IL-1β	10 (22%)	8 (20%)	0.80
IL-2	2 (4%)	1 (3%)	0.63
IL-4	9 (22%)	4 (10%)	0.19
IL-6	42 (93%)	15 (38%)	**<0.001**
IL-9	18 (41%)	8 (20%)	**0.038**
IL-10	24 (56%)	8 (21%)	**0.001**
IL-12	24 (57%)	9 (23%)	**0.001**
IFN-γ	12 (27%)	8 (20%)	0.47
TNF-α	27 (60%)	18 (45%)	0.17

Significant findings ($p < 0.05$) are marked with bold-faced types

[a] One missing case

[b] Two missing cases

[c] Two-tailed Mann–Whitney test was used for comparison of the groups

[d] Pearson Chi-square test was used for comparison of the groups

[e] Independent sample t-test was used for comparison of the groups

Table 2 Clinical variables and serum levels of cytokine in patients with and without radiologically confirmed infarctions

Variable	Patients with radiologically confirmed infarctions (merged CEI, LAAS and LAC) ($n = 34$)	Patients without radiologically confirmed infarctions (UDE) ($n = 11$)	p
Male, n (%)	20 (74)	7 (64)	
Mean age, years ± SD[c]	65 ± 12.2	74.1 ± 8.1	**0.037**
Right-sided ischemic stroke, n (%)	16 (47%)	6 (55%)	0.34
Medication			
Prednisolone	2 (6%)	1 (9%)	
NSAIDs	3 (9%)	None	
Co-morbidity			
Hypertension	21 (62%)	7 (63%)	0.91
Atrial fibrillation	5 (15%)	1 (9%)	0.63
Coronary disease	3 (9%)	4 (36%)	**0.028**
Diabetes	2 (6%)	2 (18%)	0.21
Rheumatism	2 (6%)	None	0.41
Psoriatic arthritis	1 (3%)	2 (18%)	0.14
Bechterew's disease	1 (3%)	None	0.57
Hyperthyroidism	None	1 (9%)	0.24
Mean values, ±SD[c]			
Hemoglobin (g/dl)	14.3 ± 1.2	14.3 ± 1.6	0.96
Glucose (mmol/l)	6.8 ± 2.3	7.7 ± 2.4[a]	0.29
Median values, interquartile range[d]			
BI-20	20 (19, 20)[b]	18 (16, 20)	0.06
Infarct volume (cm^3)	2 (1, 16.8)	–	–
CRP (mg/l)	2.5 (2.0, 6.3)	4.0 (2.0, 7.0)	0.99
Median values, interquartile range[d]			
Cytokines			
IL-1 ra (μg/ml)	1,206 (917, 1,618)	843 (718, 1,561)	0.26
IL-8 (μg/ml)	192 (122, 228)	267 (123, 367)	0.11
IL-18 (μg/ml)	1,807 (1,474, 2,255)	1,649 (1,227, 2,290)	0.49
GRO-α	1,613 (520, 2,192)	888 (0, 1,214)	**0.023**
Dichotomized values[e] (% detectable of total)			
Cytokines			
IL-1β	8 (24%)	2 (18%)	0.83
IL-2	1 (3%)	1 (9%)	0.79
IL-4	8 (24%)	1 (10%)	0.65
IL-6	31 (91%)	11 (100%)	0.09
IL-9	16 (47%)	2 (20%)	0.30
IL-10	18 (53%)	6 (55%)	0.65
IL-12	20 (59%)	7 (64%)	0.78
IFN-γ	8 (24%)	4 (36%)	0.56
TNF-α	20 (59%)	7 (64%)	0.81

Significant findings ($p < 0.05$) are marked with bold-faced types

[a] One missing case

[b] Two missing cases

[c] The independent sample t-test was used to compare means

[d] The two-tailed Mann–Whitney test was used to compare median values

[e] The two-tailed Mann–Whitney test was used to compare median values for the continuously values

Discussion

Our finding of significantly elevated circulatory IL-6 in the AIS patients is consistent with the findings of numerous previous studies [7–10, 20–25]. Although IL-6 is predominantly viewed as a proinflammatory cytokine, it has been suggested that it has two roles in cerebral ischemia: (1) as an inflammatory mediator during the acute phase and (2) as a neurotrophic mediator between the subacute and prolonged phases [26].

TNF-α and IL-1β were not elevated in the AIS group, which is in accordance with two previous studies [7, 27], but conflicts with others [15, 23, 28]. Based on the findings that TNF-α and IL-1β are detectable as early as 1 h after the onset of ischemia [26], and IL-6 is up-regulated by IL-1β and TNF-α [29], it is reasonable to assume that the levels of TNF-α and IL-1β had already peaked when we made our measurements. Our findings of elevated levels of IL-6 and IL-1ra, but not of TNF-α and IL-β, support earlier findings that IL-6 suppresses the effects of TNF-α and IL-1β both by inhibiting their production and by stimulating the production of their respective circulating antagonists: soluble TNF-α receptor and IL-1 receptor antagonist [30, 31]. Previous studies have also found elevated IL-1ra levels in stroke patients [20, 27]. Consistent with our findings, Beamer and coworkers found that both IL-1ra and IL-6 levels are elevated in stroke patients [32].

We found only one previous study of circulating IL-12 in AIS patients [33]. Consistent with our study, they found an early increase in IL-12 serum level. Our finding of significantly elevated IL-18 levels in the AIS group is supported by two previous studies [34, 35]. These results suggest that both IL-12 and IL-18 are involved in stroke-induced inflammation. Serum levels of IFN-γ were not elevated in the AIS group. We have not found any previous studies on circulating IFN-γ in AIS patients.

Studies of IL-8 in AIS patients are also scarce. In accordance with the present study, two other studies found a significant elevation of plasma IL-8 in AIS patients [36, 37], while another study did not [38]. We found a significant correlation between serum IL-8 and age in the patient group. The difference in IL-8 between the groups remained significant after adjusting for age ($p < 0.001$). This indicates that IL-8 may be a more prominent cytokine in older patients. It is interesting to ask whether IL-8 may contribute to that older age predicts poorer functional outcome [39, 40] and mortality [41] after stroke. More studies are needed to verify the role of IL-8 in older stroke patients.

We found that levels of GRO-α (also known as CXCL1), a proinflammatory chemokine similar to IL-8, were significantly elevated in the AIS group. We were unable to find any other study measuring circulating GRO-α in AIS patients; however, elevated cerebrospinal fluid levels of this cytokine in AIS patients have been noted [42]. The ability of GRO-α to interact with other cytokines and adhesion molecules expressed after stroke so as to potentially promote leukocyte migration into the ischemic brain has been thoroughly reviewed [42].

We found a significant elevation in IL-9 in the AIS group. To the best of our knowledge, our study is the first to have measured IL-9 in AIS patients, thus the role for IL-9 in the progression of AIS is unclear. However, it has been shown that the IL-9/IL-9 receptor signaling pathway represents a novel endogenous antiapoptotic mechanism for cortical neurons [43]. It may therefore be hypothesized that IL-9 may protect against stress-induced neuronal damage.

IL-10, but not IL-4, was significantly elevated in the AIS patients. One previous study supports our findings regarding IL-4 [44]. A significant decrease in IL-10 concentration at 24 h followed by significant increases at 72 and 144 h has recently been found [45]. Elevated levels of the anti-inflammatory cytokines IL-10 and IL-1ra suggest early activation of endogenous immunosuppressive mechanisms after stroke.

There have been large variations between similar studies exploring relevant confounding factors such as medication and comorbidities. To study a broader, more typical stroke population, we chose not to exclude patients who were using anti-inflammatory drugs, or those having any comorbidity, except for cancer. The significant differences between the groups remained significant (except for IL-9) after adjusting for the medication or comorbidity (except for hypertension) present in 20 patients (data not shown). Hypertension was not excluded, because if we did, only 11 patients remained in the stroke group, which is too low of a number for an appropriate statistical analysis.

No association was found between any of the studied variables and stroke lateralization in our study. Relevant research on whether the site of the lesion plays a role in regulating alterations in the immune response in stroke is limited. To the best of our knowledge, no other studies have related stroke laterality to serum levels of cytokines in AIS patients.

We found that infarct volume was significantly positively correlated with CRP levels ($r = 0.47$, $p = 0.005$). This is expected to be of clinical relevance, as a considerable difference in median volume score was found between patients with CRP above and below the median CRP value (2.5 g/l); 11 vs. 1 cm^3, respectively. The findings of previous studies on the correlation between CRP and cerebral infarct volume are conflicting; two are in accordance with our findings [11, 38], one failed to find a correlation [24], and one found a higher plasma CRP only for patients with larger infarcts [46]. We did not find any correlations between infarct volume and the levels of cytokines. Previous studies have produced conflicting

findings also regarding IL-6 levels and infarct volume. Several have shown positive correlations between infarct volume and IL-6 level in serum or plasma [7, 9–11], while Sotgiu and coworkers found serum IL-6 levels to be significantly negatively correlated with infarct volume [14], and two other studies found no association between serum IL-6 concentration and infarct volume [8, 21].

The release of inflammatory markers and lesion progression after a stroke are time dependent. The differences between the aforementioned studies may be attributable to large variations in timings of blood sampling and measurements of infarct volume. We used a wide time window for both. There are large variations between the aforementioned studies regarding the timings of blood sampling and volumetric measurements, and reasonable explanations for the divergent findings cannot be found. Our finding that CRP was significantly positively correlated with infarct volume, whereas the IL-6 was not, may reflect that IL-6 is more sensitive to measurement timing. More studies are needed, and in particular those designed to elucidate the release of inflammatory markers in AIS and their role in the progression of cerebral tissue injury. One aim of such studies should be to clarify whether a reduction in some central inflammatory markers could be beneficial to stroke patients.

Another aspect of blood samples timing is the question of the stability of cytokines in blood. In our study, for 35 of the 45 patients, blood samples were taken within 24 h after stroke onset (T_{24}), 7 were taken 24–48 h ($T_{24–48}$) after stroke onset and finally, 3 were taken between 48 and 72 h ($T_{48–72}$) after stroke onset. The $T_{24–48}$ ($n = 7$) and $T_{48–72}$ ($n = 3$) groups were considered too small to allow for detailed statistical analysis. However, we found the same significant differences between the stroke patients and controls analyzing the T_{24} sample alone, the $T_{24} + T_{24–48}$ sample, and finally, as presented in the paper, the $T_{24} + T_{24–48} + T_{48–72}$ sample. Thus significant elevated cytokine levels were found, in accordance with earlier studies as discussed above, also in this "time-broad-sample". This indicates that elevated cytokine levels may last longer than earlier supposed. This is supported by several studies; serum levels of IL-6 has been found elevated until 90 days after the stroke [8, 10, 21], and IL-8 has been found elevated in plasma during the first week after ischemic stroke [47]. In our opinion, our results are strengthened by the fact that including these late samples did not affect the significant finding.

It is furthermore likely that the distribution of stroke subtypes influences the differential findings discussed. For CRP, this is supported by the finding of higher plasma CRP only in patients with larger infarcts [46]. Regarding IL-6, CEI subtype has been found to exhibit significantly higher plasma levels of IL-6 (and TNF-α and IL1-β), whereas LAC subtype exhibited significantly lower plasma levels of these cytokines [15]. In our study, the numbers of patients in the different subtype groups were low, and the distribution was skewed. Some of the TOAST groups were too small to allow for more detailed statistical analysis, which is also a limitation of our study. However, we did find that the serum level of GRO-α was significantly higher in patients with radiologically confirmed infarctions (pooled subtypes CEI, LAAS, and LAC) than in patients without (subtype UDE), where the latter had nearly normal levels of GRO-α. These findings are novel and may indicate that GRO-α may be the only cytokine measured that is specific for strokes of known etiology. This aspect of GRO-α is interesting and should be studied further in aim to accomplish more knowledge about the pathophysiological mechanisms in stroke of known etiology distinct to cryptogenic stroke.

The mean age was significantly higher in the group of patients without radiologically confirmed infarctions as compared to those with (74 vs. 56 years, $p = 0.037$). A higher age among patients with UDE was also noted in a population-based study on 531 stroke patients [48].

Another limitation of this study is that stroke severity was not measured by using i.e., National Institutes of Health Stroke Scale (NIHSS) or the Scandinavian Stroke Scale. The BI-20 score was measured in the acute phase to assess early functional disability, and was found to be significantly negatively correlated with levels of IL-2, IL-6, IL-12, and IFN-γ. An association between IL-6 and IL-12 and acute clinical outcome has also been noted by others, using the NIHSS [25, 49] and the BI-20 [33], respectively.

Conclusions

This is the first study to examine the serum levels of 13 cytokines in a single AIS population using one methodology; eight were found to be elevated. The findings of elevated levels of IL-9 and GRO-α in AIS patients are novel. Our study supports the assertion that inflammatory signaling molecules are of importance in AIS; an association, assumed to be of clinical relevance, was found between infarct volume and CRP level. Patients with radiologically confirmed infarctions had significantly elevated GRO-α, indicating that this cytokine may be the only one specific for strokes of known etiology.

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Conflict of interest The authors declare no conflict of interest.

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