Dynamics of inflammatory factors expression in ischemic brain tissue injury

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Abstract

Ischemic stroke is one of the most common cause of mortality and disability in the modern world. Still, therapeutic options remain modest. Aim of the study was to present dynamics of inflammatory factors expression (C reactive protein, procalcitonin, interleukin 10) in patients after ischemic stroke. Our study included 101 patients divided in thrombolised and nonthrombolised groups. Inflammatory factors concentration in serum was determinate at admission, 24, 48 hours and seven days after the initial onset, while neurological assessment was measured at the admission, 24 hours, seven days and three months after the initial onset using National Institute of Health Stroke Scale and Rankin Scale. Certain pattern was observed in dynamics of inflammatory factors: intensive increase in first and second day after the stroke, followed by decrease till day seven in both groups. Additionally, thrombolised group showed significant neurological improvement. Although well investigated, the role of inflammatory factors in the ischemic stroke still stays controversial. High association of C reactive protein and interleukin 10 values suggest potential prognostic role in patient's follow-up, while the role of procalcitonin values still remains unclear.

Introduction

Ischemic stroke is the third leading cause of mortality and the most common cause of disability.[1,2] Although numerous mechanisms are underlying the pathogenesis of the stroke, ischemic lesions and inflammations are responsible for its progression.[1,2] Penumbra is the site of therapeutic action and its size plays great role in planning the treatment.[3,4] After ischemic brain tissue injury reperfusion is necessary to stop the damage and establish normal brain function.[4] Several inflammatory markers have been studied previously; C reactive protein (CRP) levels are significantly elevated in patients with cerebral ischemia for seven days, then subsequently decreases but remained elevated.[5] Also, three to six months after injury, CRP levels remains higher than in the healthy population.[5] In addition, patients with elevated CRP values presented with increased mortality four years after stroke and increased incidence of recurrent stroke.[5,6] Number of studies consider the CRP values at the time of admission significant and independent of thrombolysis, while other studies deny prognostic value of CRP.[2,7-9] The basal values of procalcitonin (PCT) in healthy people are low, therefore, elevated PCT values can be caused by infections, trauma or cardiogenic shock.[10] Positive association between PCT value and outcome in patients with neurotrauma, subarachnoid hemorrhage or migraine was already presented.[11-13] Few studies tried to associate dynamics of PCT values with the size, development and prognosis of the ischemic stroke patients.[14] On the other side, neuroprotective role of interleukin 10 (IL-10) in brain ischemia is largely accepted and confirmed in animal experiments, as well as in humans researches.[15,16] The patients with decreased IL-10 concentration presented with a three times greater incidence of worsening neurological deficit within 48 hours after stroke.[17] Previous studies revealed that post-ischemic IL-10 production can positively affect the regeneration of brain tissue by reducing inflammation and acting protective on surviving cells in the area of ischemia.[18] Still, therapeutic options are modest. The only approved and effective drug is a recombinant tissue plasminogen activator (rt-PA), whose efficiency has been confirmed by numerous studies.[3,4,19,20] Rt-PA therapy, if performed three hours after the initial onset of symptoms, improves the outcome of patients with thromboembolic brain ischemia.[20] So far, ideal brain ischemia therapy has not yet been found.

The aim of this study was to examine the possibility of monitoring and comparing the dynamics of inflammatory markers in order to predict the success of the therapy, primarily thrombolysis and possibly, find a pattern based on which the future therapeutic procedures can be estimated.

Ethics approval and consent to participate

This study was carried out in accordance with the recommendations of Ethical Board of the University Hospital Dubrava, Zagreb, Croatia with written informed consent from patients in accordance with the Declaration of Helsinki. The study was approved by the Institutional Review Board of the University Hospital Dubrava, Zagreb, Croatia.

Materials and Methods

The study included 101 patients, 41 females and 60 males. Average age of patients was 71.04 ± 9.56 years. Diagnosis

of ischemic stroke was established both on the basis of clinical presentation and confirmed using multi slice computerized tomography (MSCT). All patients were given initial MSCT immediately upon arrival and control one within 24 hours after admission. Patients with transient ischemic attack, bleeding or other disease were excluded from the study. Using National Institutes of Health Stroke Scale (NIHSS) patients' clinical presentation and the success of the treatment were determined individually at the admission, 24 hours and seven days after initial onset. A clinical examination of each respondent was performed by an experienced neurologist. Further, based on applied therapy, patients were divided into two groups; thrombolised and non-thrombolised group. Out of total number of 101, 31 patient received thrombolytic therapy; alteplase was administered, dose 0.9 mg/kg, up to a maximum of 90 mg. Inflammatory factors concentration (CRP, PCT, IL-10) in serum was also determinate at admission, 24 hours, 48 hours and seven days after the initial onset. The patients evaluation was done using the modified Rankin's Scale (mRS) three months after the initial onset of symptoms. Data analysis was performed using the Statistical Package for Social Sciences (SPSS), statistics software package (*IBM Corporation, Armonk, New York, USA*). Data were analyzed using Student's T-test. The association between individual parameters was assessed by Pearson's linear correlation coefficient. The level of statistical significance was set at 95% ($P<0.05$).

Results

When comparing NIHSS score at the admission (day 0) and at day seven, statistically significant improvement in neurological presentation was observed in throm-

bolised group ($P<0,015$), while in non-thrombolised group, no statistical significance was observed. Significant difference in CRP values in-between thrombolised and non-thrombolised group of patients was not observed (Figure 1A). Both PCT and IL-10 values presented with similar pattern (Figure 1B,C). In both groups of patients intensive increase of CRP values, as well as PCT and IL-10 values, was observed in first and second day, while at day seven, all measured values decreased, but remained slightly higher than at the admission (day 0).

PCT values measurements at day one, 24 hours after admission, revealed significant difference in-between males and females ($P<0,046$).

Positive association was observed in between NIHSS score at admission and day seven with the CRP values. Higher NIHSS score was accompanied with increased CRP values ($P<0,001$). Positive association of CRP values was observed initially, on first and second day, while at day seven, the correlation was weaker (Table 1). Additionally, positive association was observed in between NIHSS score at admission and at day seven with the levels of PCT and IL-10 values ($P<0,01$) (Table 2). There was no significant association between NIHSS score at admission and IL-10 value at day seven, while positive association of IL-10 values and NIHSS score was observed at day seven (Table 3).

Associations of neurological outcome three months after stroke and inflammatory markers were performed in both thrombolised and non-thrombolised group of patients. In thrombolised group of patients, significant positive association for all inflammatory markers and neurological outcome was observed (Table 4). In non-thrombolised group of patients, similar results were observed (Table 4). In addition, initial NIHSS score at the admission and at

day seven showed association with the patients outcome three months after the initial onset ($r=0.686$, $P<0.01$).

Discussion

The role of inflammatory markers in the pathophysiology of the ischemic stroke remains controversial. Initial CRP values in our patients were higher in non-thrombolised group and significantly increased than previously reported.[21,22] As in literature, significant differences in the CRP values were observed between the first and second day.[23,24] CRP dynamics showed increased values two to three days, while decreased values were described seven to about ten days after ischemic stroke.[25,26] Audebert *et al.* confirms observed dynamics of CRP values.[25] In addition, the authors showed lack of CRP elevation in case of successful thrombolysis.[25] In our study, we showed a strong CRP values increase from initial values at patients admission to the second day in both groups, regardless of the performed thrombolysis.

Number of studies showed positive association between the increased initial CRP values with clinically worsen patients, as well as association with mortality within three months.[5,18,21] The CRP values was showed to be independent prognostic factor for worsen or mortal outcome. In patients with thrombolysis performed, the results are ambiguous. Several authors showed association between initial values and mortality, while in other studies association has not been confirmed.[9,27] We observed increased CRP values in a positive association with deteriorating neurological presentation among our patients. On contraire, no association between the CRP values and patients outcome was described in several studies.[9] In thrombolised group of patients no association between initial NIHSS score and CRP

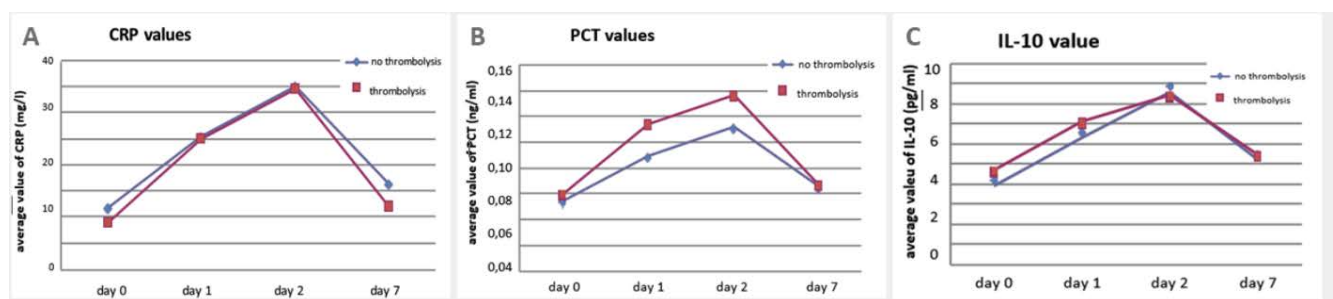**Figure 1. Dynamics of inflammatory factors expression in both groups measured at the admission, 24 hours, 48 hours and seven days after admission. All measured inflammatory markers, CRP (A), PCT (B) and IL-10 (C) showed similar pattern; intensive growth in first and second day, decreased values at day seven, but still slightly higher than at the admission. No statistically significant difference was observed in between groups.**

levels at day seven was observed.

The dynamics of PCT levels in serum of the patients with ischemic stroke have been poorly investigated. Association between increased PCT levels and poor neurological recovery in resuscitated patients after a heart attack was previously presented.[14,15] In addition, no association of initial PCT values with neurological outcome or mortality was described.[14] In our study, the initial PCT values in non-thrombolised group were decreased when comparing to thrombolised group of patients. Miyakis et al. observed the highest PCT values in the second and third day after ischemic stroke.[14] The PCT values dynamics follows the same pattern as the CRP values. The highest PCT values were observed on the second day,

Table 1. Associations of NIHSS score at admission and at day seven and CRP values at admission, 24 hours, 48 hours and seven days after admission in both groups of patients.

	Thrombolysis	NIHSS0	NIHSS7	CRP0	CRP1	CRP2	CRP7
NIHSS0	-	1	0,86 P=0,000	0,704 P=0,000	0,66 P=0,000	0,668 P=0,000	0,528 P=0,000
	+	1	0,308 P=0,134	0,788 P=0,000	0,554 P=0,001	0,496 P=0,006	0,095 P=0,675
NIHSS7	-	0,86 P=0,000	1	0,657 P=0,000	0,71 P=0,000	0,702 P=0,000	0,531 P=0,000
	+	0,308 P=0,134	1	0,544 P=0,005	0,814 P=0,000	0,77 P=0,000	0,545 P=0,009
CRP0	-	0,704 P=0,000	0,657 P=0,000	1	0,925 P=0,000	0,909 P=0,000	0,779 P=0,000
	+	0,788 P=0,000	0,544 P=0,005	1	0,767 P=0,000	0,749 P=0,000	0,335 P=0,128
CRP1	-	0,66 P=0,000	0,71 P=0,000	0,925 P=0,000	1	0,989 P=0,000	0,895 P=0,000
	+	0,554 P=0,001	0,814 P=0,000	0,767 P=0,000	1	0,987 P=0,000	0,767 P=0,000
CRP2	-	0,668 P=0,000	0,702 P=0,000	0,909 P=0,000	0,989 P=0,000	1	0,907 P=0,000
	+	0,496 P=0,006	0,77 P=0,000	0,749 P=0,000	0,987 P=0,000	1	0,878 P=0,000
CRP7	-	0,528 P=0,000	0,531 P=0,000	0,779 P=0,000	0,895 P=0,000	0,907 P=0,000	1
	+	0,095 P=0,675	0,545 P=0,009	0,335 P=0,128	0,767 P=0,000	0,878 P=0,006	1

Table 2. Associations of NIHSS score at admission and at day seven and PCT values at admission, 24 hours, 48 hours and seven days after admission in both groups of patients.

	Thrombolysis	NIHSS0	NIHSS7	PCT0	PCT1	PCT2	PCT7
NIHSS0	-	1	0,86 P=0,000	0,779 P=0,000	0,517 P=0,000	0,178 P=0,153	0,602 P=0,000
	+	1	0,308 P=0,134	0,684 P=0,000	0,015 P=0,937	0,006 P=0,974	0,268 P=0,228
NIHSS7	-	0,86 P=0,000	1	0,767 P=0,000	0,525 P=0,000	0,156 P=0,231	0,654 P=0,000
	+	0,308 P=0,134	1	0,638 P=0,001	0,001 P=0,995	-0,002 P=0,994	0,653 P=0,001
PCT0	-	0,779 P=0,000	0,767 P=0,000	1	0,514 P=0,000	0,37 P=0,002	0,758 P=0,000
	+	0,684 P=0,000	0,638 P=0,001	1	0,08 P=0,674	0,082 P=0,672	0,581 P=0,005
PCT1	-	0,517 P=0,000	0,525 P=0,000	0,514 P=0,000	1	0,189 P=0,129	0,414 P=0,001
	+	0,015 P=0,937	0,001 P=0,995	0,08 P=0,674	1	0,999 P=0,000	0,094 P=0,676
PCT2	-	0,178 P=0,153	0,156 P=0,231	0,37 P=0,002	0,189 P=0,129	1	0,342 P=0,006
	+	0,006 P=0,974	-0,002 P=0,994	0,082 P=0,672	0,999 P=0,000	1	0,097 P=0,669
PCT7	-	0,602 P=0,000	0,654 P=0,000	0,758 P=0,000	0,414 P=0,001	0,342 P=0,006	1
	+	0,268 P=0,228	0,653 P=0,001	0,581 P=0,005	0,094 P=0,676	0,097 P=0,602	1

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Table 3. Associations of NIHSS score at admission and at day seven and IL-10 values at admission, 24 hours, 48 hours and seven days after admission in both groups of patients.

	Thrombolysis	NIHSS0	NIHSS7	IL0	IL1	IL2	IL7
NIHSS0	-	1	0,86 P=0,000	0,76 P=0,000	0,712 P=0,000	0,654 P=0,000	0,582 P=0,000
	+	1	0,308 P=0,134	0,546 P=0,002	0,632 P=0,000	0,49 P=0,007	0,186 P=0,408
NIHSS7	-	0,86 P=0,000	1	0,682 P=0,000	0,655 P=0,000	0,671 P=0,231	0,585 P=0,000
	+	0,308 P=0,134	1	0,721 P=0,000	0,828 P=0,000	0,71 P=0,000	0,717 P=0,000
IL0	-	0,76 P=0,000	0,682 P=0,000	1	0,814 P=0,000	0,724 P=0,000	0,725 P=0,000
	+	0,546 P=0,002	0,721 P=0,000	1	0,829 P=0,000	0,682 P=0,000	0,651 P=0,001
IL1	-	0,712 P=0,000	0,665 P=0,000	0,814 P=0,000	1	0,915 P=0,000	0,76 P=0,000
	+	0,632 P=0,000	0,828 P=0,000	0,829 P=0,000	1	0,806 P=0,000	0,762 P=0,000
IL2	-	0,654 P=0,000	0,671 P=0,000	0,724 P=0,000	0,915 P=0,000	1	0,839 P=0,000
	+	0,49 P=0,007	0,71 P=0,000	0,682 P=0,000	0,806 P=0,000	1	0,675 P=0,001
IL7	-	0,582 P=0,000	0,585 P=0,000	0,725 P=0,000	0,76 P=0,000	0,839 P=0,000	1
	+	0,186 P=0,408	0,717 P=0,000	0,651 P=0,001	0,762 P=0,000	0,675 P=0,001	1

with slightly higher PCT values in thrombolised group of patients. Gender-dependent PCT values dynamics showed higher values in female in all measured points. In non-thrombolised group of patients, a high positive association between the initial and day seven PCT values with NIHSS score at the same time point was observed. In thrombolised group of patients, a positive association between initial PCT values and initial and day seven NIHSS score were presented. These changes could be explained by a thrombolysis. Based on our results, it can be assumed that patients with increased initial PCT values, prognosis is worsening, regardless of the thrombolysis performed.

Initial IL-10 values, as well as dynamics of IL-10 values in ischemic stroke patients were well investigated. In our patients, the initial IL-10 value was increased in non-thrombolised group, as previously reported.[15] IL-10 values increase after brain ischemia in first 24 hours after thrombolysis, and present highest values between the second and seventh day.[28,29] A negative association between IL-10 values in stroke patients and results of neurological presentation have been previously described in numerous studies, while several studies showed no association between IL-10 values and neurological outcome.[28]

Thrombolised group of patients present-

ed with initially increased NIHSS score, as previously reported.[21,27] Neurological outcome three months after ischemic stroke showed no statistically significant difference in between groups of patients. Therefore, the results imply efficiency of thrombolysis in patients with ischemic stroke. Positive association between initial and at day seven NIHSS score with mRS score three months after ischemic stroke was observed. Initial NIHSS and mRS score association decreased, positive association of NIHSS score at day seven increased, while association between initial and day seven NIHSS score disappears. Reducing the influence of initial neurological presentation on the outcome of treatment, and increasing the correlation with the neurological presentation on the seventh day after ischemic stroke could be considered as a contribution to the success of thrombolysis, as previously reported.[30]

Conclusions

Dynamics of inflammatory factors expression revealed similar pattern in both thrombolised and non-thrombolised groups of patients. We have showed that thrombolysis, as a therapeutic mode, doesn't affect dynamic of measured inflammatory markers. High correlation of the CRP and IL-10

Table 4. Associations of neurological outcome three months after ischemic stroke measured using mRS and inflammatory markers (CRP, PCT and IL-10) in both groups. In both groups of patients significant positive associations for all inflammatory markers and neurological outcome was observed.

Thrombolysis	mRS	
	-	+
CRP0	0,574 P=0,000	0,633 P=0,000
CRP1	0,652 P=0,000	0,777 P=0,000
CRP2	0,683 P=0,000	0,76 P=0,000
CRP7	0,52 P=0,000	0,584 P=0,004
PCT0	0,77 P=0,000	0,642 P=0,000
PCT1	0,566 P=0,000	0,124 P=0,515
PCT2	0,312 P=0,011	0,13 P=0,501
PCT7	0,688 P=0,000	0,654 P=0,001
IL0	0,666 P=0,000	0,803 P=0,000
IL1	0,699 P=0,000	0,902 P=0,000
IL2	0,73 P=0,000	0,803 P=0,000
IL7	0,643 P=0,000	0,685 P=0,000

values suggest potential prognostic role in patients follow up, while role of PCT values yet remains unclear. Further investigation in this field are required, in order to find and develop the most effective treatment option.

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