Serum Levels of Monocyte Chemoattractant Protein-1 Correlate with Poor Clinical Grades in Cerebral Aneurysms

Abdolkarim Rahmanian[1], Navideh Mohebali[1], Ali Haghnegahdar[1*], Eskandar Kamali Sarvestani[2], Ali Razmkon[1], Juri Kivelev[3], Fahim Baghban[1]

[1]Department of Neurosurgery, [2]Department of Immunology, Shiraz Medical School, Shiraz University of Medical Sciences, Shiraz, Iran, [3]Department of Neurosurgery, Helsinki University Central Hospital, Topeliuksenkatu, Finland

ABSTRACT

Background: Ruptured cerebral aneurysms (ICAs) are the most common non-traumatic cause of subarachnoid hemorrhage (SAH) that is associated with life threatening complications such as Vasospasm, Infarction, and Hydrocephalus (HCP). The active participation of macrophage/monocyte-mediated inflammatory response in the pathogenesis of cerebral aneurysm as labeled with Monocyte ChemoattractantProtein-1 (MCP-1) is suggested. **Objective:** To measure the serum level of MCP-1 in ruptured CAs in different time intervals**. Methods:** We measured the serum levels of MCP-1 in SAH patients who had CAs and compared it with that of MCP-1 in two control groups: including patients with SAH without CAs, and the normal population of blood donors. We also measured the MCP-1 levels in patients with CAs one week afterward to evaluate the effect of treatment. Serum level of MCP-1 was measured by a commercial ELISA assay. **Results:** Mean serum MCP-1 level in patients with SAH and CAs was 188.2168 Pg/ml and 331.3982 Pg/ml in the normal population. There was no statistically significant difference between serum levels of MCP-1 on the first (mean=188.2168 Pg/ml) and 7th days after SAH onset (mean=171.8450 Pg/ml) (p=0.739). Serum level of MCP-1 increased significantly as Glasgow Coma Scale decreased (p=0.078) and Hunt and Hess score increased (p=0.089).**Conclusion:** Our results did not show an increasing MCP-1 serum level in patients with aneurysmal SAH. There was a relationship between poor clinical grade and MCP-1 levels in patients with CAs. MCP-1 may be a local inflammatory marker for cerebral aneurysms without systemic manifestation.

Iran.J.Immunol. VOL.12 NO.4 December 2015

302

----
*Corresponding author: Dr. Ali Haghnegahdar, Department of Neurosurgery, Nemazee Hospital, Shiraz University of Medical Sciences, Shiraz, Iran, Tel: (+) 98 9173148711, Fax: (+)98-71-36474259, e- mail: ali.razmkon@gmail.com

INTRODUCTION

Subarachnoid hemorrhage (SAH) accounts for 5% of strokes. The aneurysm rupture is the most common cause of spontaneous SAH (1).After SAH, cerebral arteries may undergo spasm resulting in poor cerebral perfusion and ischemia, which in turn may cause infarction. Circulation of cerebrospinal fluid may be impeded as blood becomes organized into scar tissue that blocks the subarachnoid space causing hydrocephalus. The reported mortality after SAH is about 40% within the first 30 days. Among those who survive, less than 25% have a good functional outcome.

Histological studies suggest that cerebral aneurysm walls rupture as a consequence of matrix degeneration and de-cellularization, which may be the result of direct vascular stress (2). The presence of macrophages, T cells, B cells, immunoglobulin antibodies, and activated complement in cerebral aneurysm walls, especially in the ruptured aneurysms, indicates that active innate and adaptive immunologic reactions are associated with aneurysm formation and rupture (2). Up-regulated expression of the pro-inflammatory chemokine monocyte chemoatacticprotein-I (MCP-I) in the aneurysm wall has also been observed (2). Even though MCP-l mRNA has not been detectable in normal arteries, it was often observed in the intima of the aneurysmal walls and appeared to be expressed in the cytoplasm of the fibroblast cells that are assembled with monocyte-like cells. The extent of inflammatory cell infiltration and complement activation appeared to be higher in the ruptured rather than unruptured aneurysms (2).

Elevation of serum MCP-1 was seen in hemodialysis patients as well (3). According to previous studies, arole for MCP-1 in neurological injury was assessed, implying that it may act as a biomarker of poor outcome in the sera of patients with vasospasm following aneurysmal subarachnoid hemorrhage (4).Although these data imply that inflammation is involved in the process of aneurysm progression or rupture, it is still unknown whether inflammation is the primary process causing the initiation and progression of disease or it is a secondary reaction accompanied by disease process. At present, treatment modalities for cerebral aneurysms are confined to surgical obliteration of aneurysms by clipping or coiling. If we could disclose more detailed mechanisms of inflammatory reactions involved in the progression of cerebral aneurysms, specific anti-inflammatory treatments would be the first choice for the patients with cerebral aneurysms in future (1). A number of articles evaluate the role of MCP-1 in CAs but serum level of MCP-1 has not been evaluated in any of them. Because monitoring the serum level is more convenient, less expensive and safer than tissue sampling and it is assessable before the rupture of aneurysms, we decided to evaluate the serum level of MCP-1 in CAs. The main hypothesis of this study was to find if serum levels of MCP-1 are significantly higher in ruptured aneurysms, so it can be used as a less-invasive screening test in diagnosis, or may be used as a target for future innovative treatments.

MATERIALS AND METHODS

Subjects. This is a case control study for evaluating the serum level of monocyte chemoattractantprotein-1 (MCP-1) in ruptured cerebral aneurysms. Measuring of MCP-

Iran.J.Immunol. VOL.12 NO.4December 2015

303

1 was done in 3 groups for 4 times. The samples were obtained from patients who referred to Shiraz Namazi hospital from May 2011 to May 2013 with definite diagnosis of subarachnoid hemorrhage (SAH). SAH was established with brain CT scan. Following admission, CT Angiography of the brain was done for all of them. Patients who had CAs were selected as the case group (labeled as group A) and patients who had no evidence of CAs were selected as the first control group (labeled as group B). Group B included patients with non-aneurysmal causes of SAH, most commonly labeled as benign peri-mesecephalic SAH. Samples of the 3rd group were taken from voluntary blood donors in the blood bank center (labeled as group C). Another blood sample was taken from patients in group A to be compared with the first sample in order to evaluate the role of therapeutic management in the serum level; this dataset was labeled as group D (Table 1).

Table 1. Characteristics of the groups in the study.

	Type of Group	Time of Sampling	SAH	ICAs	Number
A	case group	First 48 hr from onset	+	+	22
B	control group 1	First 48 hr from onset	+	_	22
C	control group 2	-	_	_	22
D	case group	1 week after first sample (after surgery)	+	+	22

Inclusion Criteria. Patients in this study were selected based on the following inclusion criteria: 1) patients who were alive with a Glasgow Coma Scale (GCS) score higher than 3 and with present stem reflexes, 2) time between the onset of SAH and serum sampling was not longer than 48 hours, 3) there was no need for any emergency neurosurgical intervention before serum sampling.

Exclusion Criteria. Exclusion criteria were: 1) patients with any history of inflammatory, rheumatoid and tumoral disease, 2) those who deseased before aneurysmal clipping operation, 3) patients with traumatic SAH, 4) those with other causes of SAH except CAs and prepontine hemorrhage such as toxins, blood disease, neoplasm, infections, 5) patients with a history of anticoagulant therapy, and 6) patients with normal CTA that had aneurysm in the follow up Conventional Angiography. In both groups, consent form was taken from the patient or first degree relatives.

Sampling and Data Collection. Data were recorded in data collection forms including: 1) demographic information such as age and sex, 2) SAH information such as the time of onset, time of admission, GCS Score, Fisher Grading, Hunt and Hess (H&H) grading, 3) aneurysm information such as location, size, number, shape, and 4) operation information such as date, duration, bleeding. Groups A and B were the same in management except in the plan for surgery in group A. We did not rule out CAs in group B completely until the follow up angiography was done during the first 14 days. We managed all SAH patients to see if they have CAs in the follow up angiography, therefore, all of therapeutic managements were similar to that of documented CAs in group A. About 5 ml blood sample was taken from each patient in the clot tube and sent to the Autoimmune Diseases Research Center in Shiraz University of Medical Sciences

within 3 hours. We allowed the samples to clot for 30 minutes at room temperature; then, centrifugation of the samples was done for 15 minutes at 1000 xg; and, after separation of the serum, the samples were stored at ≤ -20°C until MCP-1was measured. Group C was matched in demographic information with group A. Group C was considered as the second control group. Screening of group C for the history of previous CAs or other inflammatory and tumoral disease was performed (Table 1). The samples in this study were selected based on the MCP-1 plate; we could check MCP-1 on 96-well plates, 8 of which were used for standardization. After completion of the sampling, the serum level of MCP-1 was measured by Enzyme Linked Immuno Sorbent Assay (ELISA) (Human CCL2/MCP-1 Quantikine R&D Systems kit, USA) according to manufacturer's recommendations (5). The sensitivity of the test was 10 Pg/ml.

Statistical Analysis: The comparison of data was done between groups using the Statistical Package for Social Sciences, version 18.0 (SPSS Inc., Chicago, IL, USA). Comparisons of serum level between groups were made using One-way ANOVA test. Paired-sample *t-test*, one-sample *t-test*, Fisher test, Kruskall-Wallis and Mann-Whitney tests were applied for the analysis of data. All the tests were performed with confidence interval (CI) of 95% and pvalue≤0.05 was considered as significant.

RESULTS

We gathered the data from 3 groups with a total number of 66 and with 22 patients in each group (34 male and 32 female). Mean age was 53.83 with p=0.549. Distribution of age and sex in the 3 groups was almost the same. Patients in groups A and B were evaluated for clinical and radiological findings. Three parameters were assessed: H&H grade, Fisher Grading and GCS score (Table 2).

Table 2. Results of statistical analysis of age, GCS, H&H, Fisher, admission day and operation between groups.

	Group A	Group B	Group C	P-value
Age	55 ± 11.2	51.6 ± 11	54.9 ± 12.2	0.549
GCS	12.1 ± 3.6	13.6 ± 2.1	-	0.078
H&H	2.1 ± 1.3	1.5 ± 0.9	-	0.089
Fisher	3 ± 1	2.4 ± 0.9	-	0.052

H&H, Fisher and GCS were compared between groups A and B using one-way ANOVA test. Mean H&H was higher in group B compared with group A with no significant relationship (p=0.089). Mean GCS was higher in group A compared with group B again with no significant relationship (p=0.078).

In group A, 2 patients had 2 CAs and 1 had 3 CAs for whom clipping was done in one procedure but in 2 different surgical positions. With summation of multiple and simple CAs, we had 25 CAs that distribution was as below (Figure1).
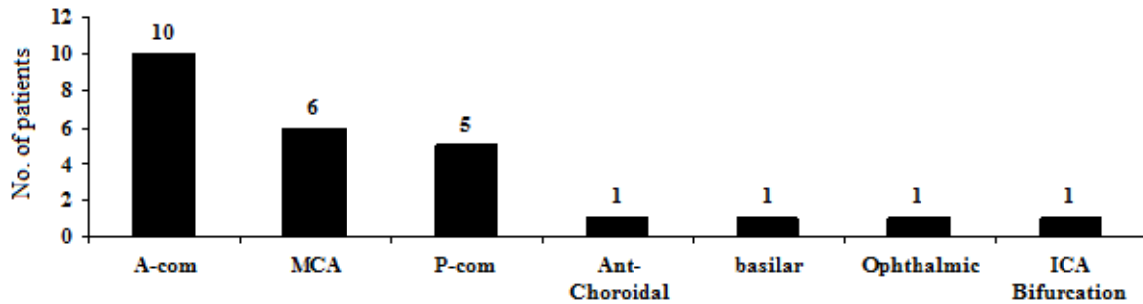
Figure 1. Distribution of 25 specific aneurysms in 22 patients in group A.

Distribution of CAs location was broad and most of them were Anterior communicating artery (A-Com) and middle cerebral artery (MCA) and posterior communicating artery (P-Com). We analyzed MCP-1 level in these 3 locations with Kruskall-Wallis test and there was no statistical relationship between them (p=0.350).

Figure 2 shows the results of serum sampling in the four groups. MCP-1 serum level was not significantly higher in group A than group B (p>0.05). It was also unexpectedly lower in group A and B than group C. Mean MCP-1 level decreased after one weak but there was no significant statistical relationship between them (p=0.930).
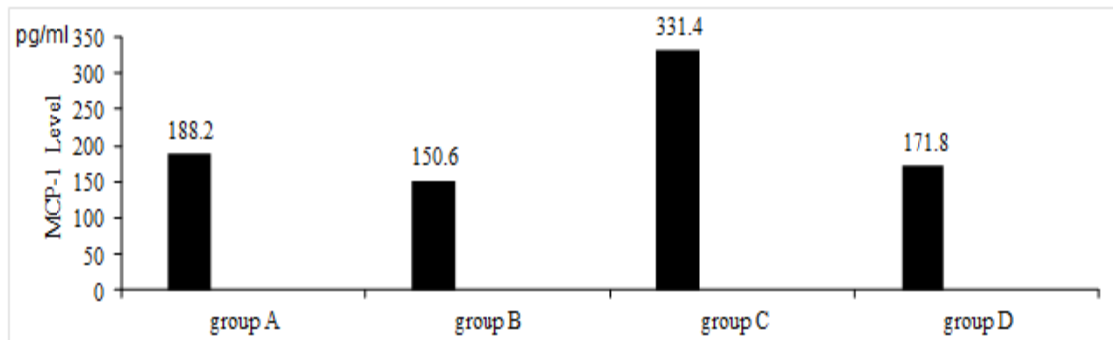

Figure 2. Distribution of MCP-1 level in 4 studied groups.

The relationship between MCP-1 level and clinical and radiological grade of patients was evaluated by linear Pearson Correlation. In group A, the correlation between GCS and MCP-1 had p=001 and in group B, p value was 0.717, so there was a significant relationship between GCS and MCP-1 in the case group. This means that if GCS was better, the MCP-1 level was lower but this result was not seen in SAH without CAs. In group A, correlation between H&H and Fisher scale with MCP-1 level was also analyzed with Pearson Correlation test. This table indicates that MCP-1 level was higher in patients with poor grade and no significant relationship existed between them. Fisher scale was analyzed in the same manner; MCP-1 levels in group A & B and in group A mean MCP-1 level were higher in patients with poor Fisher scale but in both

Iran.J.Immunol. VOL.12 NO.4 December 2015

306

groups, there was no significant relationship between Fisher scale and MCP-1 level (Table 3).

Table 3. Correlation of GCS and H&H and Fisher with MCP-1 level.

Group		HH	Fisher	GCS
MCP-1 level in group A	Pearson Correlation	0.599	0.161	-0.647
	Sig. (2-tailed)	0.003	0.473	0.001
	N	22	22	22
MCP-1 level in group B	Pearson Correlation	-0.075	-0.119	0.082
	Sig. (2-tailed)	0.741	0.599	0.717
	N	22	22	22

Based on the results, there was no relationship between age and MCP-1 with Pearson correlation test (p=0.968); also, no relationship was found between sex and MCP-1 level using Mann-Whitney test (p=0.482).

DISCUSSION

About 85% of cases of subarachnoid hemorrhage are caused by rupture of saccular aneurysms. Three to 18% of patients suffering from SAH die before hospitalization and mortality of hospitalized SAH patients depends on factors such as initial hemorrhage re-bleeding and medical complications. Current evidence on the pathogenesis of cerebral aneurysms suggests that arterial wall proteolysis by matrix metalloproteinases, apoptosis, and chronic inflammation play a key role in disease progression. Up-regulated expression of the proinflammatory chemokine monocyte chemoattractant protein-I (MCP-I) in the aneurysm wall has also been observed. Even though MCP-l mRNA was not detectable in normal arteries, it was often observed in the intima of aneurysmal walls and appeared to be expressed in the cytoplasm of the fibroblast cells that assembled with monocyte-like cells (1).

The role of MCP-1 in the inflammatory processes in the body and its inhibition for reduction of inflammatory response and decrease of the severity of disease was demonstrated in many studies. For example, in an animal model for atherosclerosis (6), experimental autoimmune encephalomyelitis (7), peripheral endotoxin insult (8), lethal endotoxemia (9), acute septic peritonitis (10) and cerebral aneurysm (11). Many inflammatory pathways and mediators are involved in aneurysm formation. MCP-1, Ets-1 and NFκB have been suggested to have a critical role in CAs formation by the induction of an inflammatory response (12).

The monocyte chemoattractant protein-1 (MCP-1/CCL2) is a member of the C-C chemokine family, and a potent chemotactic factor for monocytes. MCP-1 regulates the migration and infiltration of the monocytes, memory T lymphocytes, and natural killer (NK) cells. It is worth mentioning that MCP-1 is among the most studied members of

Iran.J.Immunol. VOL.12 NO.4December 2015

307

the chemokine family, and has been shown to be a potential intervention point for the treatment of various diseases, including multiple sclerosis, rheumatoid arthritis, atherosclerosis , and insulin-resistant diabetes (13).In approving the role of MCP-1 in inflammatory process, regression of CAs with blocking MCP-1 was seen (14) or decrease of serum level of MCP-1 with treatment of inflammatory disease (15),elevation of circulating level of MCP-1 in another focal disease (16) were shown. Elevation of circulating levels of MCP-1 in other diseases such as Ischemic Stroke, Myocardial Infarction (16) and hemodialysis patients was shown. According to the previous studies, the role of MCP-1 in neurological injury was assessed and implied that it may act as a biomarker of poor outcome in the serum of patients with vasospasm following aneurysmal subarachnoid hemorrhage .However, for approving the MCP-1 role in inflammatory process of CAs, regression of CAs with blocking MCP-1 was seen (11) and dramatic decrease of serum level of MCP-1 was shown with treatment of diseases such as Kawasaki (15). The activity of coils coated with MCP-1in promoting inflammatory intra-aneurysmal tissue healing was assessed and MCP-1 releasing coils promoted significantly greater aneurysm tissue in-growth than bare platinum or PLGA-only coils (17).

According to the role of MCP-1 in the aneurysm formation and its high level in aneurysmal tissue, we decided to measure serum MCP-1 level in patients with ruptured CAs and correlate it with two control groups: first with SAH without CAs and second with the general population. Also, we measured MCP-1 level in CAs patients after one week and compared it with the level on admission and before surgery. Distribution of age and sex between groups was the same. There was no relationship between age and sex with MCP-1 level (p=0.968 and 0.482). Most of the patients were admitted within2 days after SAH onset and most of them were operated within 3 days after SAH onset. Most of the CAs were A-com aneurysm (40%). There was no relationship between MCP-1 level and location of CAs (p=0.350).

MCP-1 level in groups A and B was lower than that of group C (control group) (p<0.001) but this relationship is opposite to what we expected. We expected that serum MCP-1 level in CAs patients, according to high tissue level, to be higher than in patients without CAs. In another study in type 2 diabetic patients, serum level of MCP-1 wasnot elevated and no correlation was seen between local and systemic level. Mean MCP-1 level was higher in SAH with CAs than SAH without CAs but the difference was not significant (p=0.409). It means that we could not find any relationship between increasing of serum MCP-1 level and CAs mean.MCP-1 level was decreased after one week of medical and surgical treatment but again not statistically significant. A significant relationship may be acquired with increase of sample size in groups A and B. GCS score was lower in group A than group B. H&H and Fisher score was higher in patients in group A as compared to group B. This means that neurological condition in patients with only SAH was better than patients with SAH and CAs. In patients with CAs, MCP-1 level was higher in patients with lower GCS scores and poor H&H grade (p=0.001 and 0.003), indicating that MCP-1 is higher in patients with worsened neurological condition that may show the role of MCP-1 in severity of patients' condition. The same significant result was not seen between Fisher score and MCP-1 level but the mean level of MCP-1 was higher in higher Fisher score probably owing to the small sample size.

One of our patients in group A had 3 aneurysms; the results of statistical analyses were not reliable and needed a larger number of cases. Because the types of aneurysms were

Iran.J.Immunol. VOL.12 NO.4 December 2015

308

broad and we had only one patient in some aneurysm location, we could notcorrelate demographic and clinical and radiological information with aneurysm location.

According to these results, we suggest that elevation of MCP-1 was a local inflammatory process, and its serum level did not correlate with that of local process; we can not use MCP-1 serum level as a marker of CAs in diagnosis and evaluation of CAs patients. One limitation of the study is the small number of the groups to reach statistical significance. Performing the study on both tissue and serum of the patients simultaneously, as well as CSF may also be different options in reaching promising results. Another limitation of the study was the universal administration of corticosteroids in all patients with SAH after admission to hospital. Some studies have noted the effect of corticosteroids on decreasing MCP-1 levels in human subjects (18). Therefore, it seems logical to measure the serum level of the marker before administration of such drugs in future studies.

In conclusion, our results did not show an increasedMCP-1 serum level in patients with aneurysmal SAH. A larger study, including CSF data with more controlled conditions before use of any drug such as corticosteroids may be necessary to better testsuch hypothesis. The relationship between poor clinical grade and MCP-1 level may suggest that MCP-1 is a local inflammatory marker for cerebral aneurysms without systemic manifestation.

ACKNOWLEDGEMENTS

REFERENCES

1. Winn HR, GavinW. The natural history of cerebral aneurysm. In: Winn HR (editor). Youmans Neurological Surgery. 6th ed. Philadelphia: 2011: 3737-3747.
2. AokiT, KataokaH, MorimotoM, NozakiK, HashimotoN.Macrophagederived matrix metalloproteinase-2 and -9 promote the progression of cerebral aneurysms in rats. Stroke. 2007;38:162-169
3. Papayianni A, Alexopoulos E, Giamalis P, Gionanlis L, Belechri AM, Koukoudis P, et al. Circulating levels of ICAM-1, VCAM-1, and MCP-1 are increased in haemodialysis patients: association with inflammation, dyslipidaemia, and vascular events. Nephrol Dial Transplant. 2002; 17:435-441.
4. Kim GH1, Kellner CP, HahnDK, Desantis BM, MusabbirM, Starke RM, et al: Monocyte chemoattractant protein-1 predicts outcome and vasospasm following aneurysmal subarachnoid hemorrhage. J Neurosurg, 2008; 109:38-43.
5. Quantikine® ELISA, Human CCL2/MCP-1 Immunoassay. Catalog Number DCP00. WWW.RnDSystems.com
6. Daly C, RollinsBJ. Monocyte chemoattractant protein-1 (CCL2) in inflammatory disease and adaptive immunity: therapeutic opportunities and controversies. Microcirculation 2003, 10:247-257.
7.   Kennedy KJ, Strieter RM, Kunkel SL, Lukacs NW, KarpusWJ: Acute and relapsing experimental autoimmune encephalomyelitis are regulated by differential expression of the CC chemokines macrophage inflammatory protein-1alpha and monocyte chemotactic protein-1. Journal of neuroimmunology. 1998; 92:98-108.

8.   Thompson W, Karpus W, Van Eldik L. MCP-1-deficient mice show reduced neuroinflammatory responses and increased peripheral inflammatory responses to peripheral endotoxin insult. J Neuroinflammation. 2008; 15:35.

9.   Zisman DA, Kunkel SL, Strieter RM, Tsai WC, Bucknell K, Wilkowski J. MCP-1 protects mice in lethal endotoxemia. J Clin Invest 1997; 99: 2832-2836.

10. MatsukawaA, Hogaboam CM, Lukacs NW, Lincoln PM, Strieter RM. Endogenous monocyte chemoattractant protein-1 (MCP-1) protects mice in a model of acute septic peritonitis: cross-talk between MCP-1 and leukotriene B4. J Immunol 1999; 163:6148-6154.

11.   Aoki T, Kataoka H, Shimamura M, Nakagami H, Wakayama K: NF-κB Is a Key Mediator of Cerebral Aneurysm Formation Circulation. 2007 Dec 11; 116:2830-40.

12. Chalouhi N, Ali MS, Jabbour PM, Tjoumakaris SI, Gonzalez LF, Rosenwasser RH, et al. Biology of intracranial aneurysms: role of inflammation. Journal of Cerebral Blood Flow & Metabolism. 2012; 32:1659–1676.

13. Satish L. Deshmane, Sergey Kremlev, ShohrehAmini, Bassel E. Sawaya. Monocyte Chemoattractant Protein-1 (MCP-1): An Overview. J Interferon Cytokine Res. 2009; 29:313-26.

14. Aoki T, Kataoka H, Ishibashi R, Nozaki K: Impact of Monocyte Chemoattractant Protein-1 Deficiency on Cerebral Aneurysm Formation. Stroke. 2009; 40:942-51.

15. Terai M, Jibiki T, Harada A, Terashima Y, Yasukawa K, Tateno S, et al: Dramatic decrease of circulating levels of monocyte chemoattractant protein-1 in Kawasaki disease after gamma globulin treatment. J Leukoc Biol. 1999 ; 65:566-72.

16. Arakelyan A, Petrkova J, Hermanova Z, Boyajyan A, Lukl J, Petrek M. Serum Levels of the MCP-1 Chemokine in Patients With Ischemic Stroke and Myocardial Infarction. Mediators of Inflammation. 2005:3;175–179.

17. Komotar RJ, Starke RM, Connolly ES. Endovascular Therapy with MCP-1 Releasing Coils Promotes Inflammatory Intra-aneurysmal Tissue Healing. Neurosurgery. 2012; 71:N10-1.

18. Remmelts HH, Meijvis SC, Biesma DH, van Velzen-Blad H, Voorn GP, Grutters JC, et al. Dexamethasone downregulates the systemic cytokine response in patients with community-acquired pneumonia.Clin Vaccine Immunol. 2012; 19:1532-8.