# Dynamic changes in serum monocyte chemoattractant protein-1, and regulated upon activation, normal T cell expressed and secreted levels in patients with minor intracerebral hemorrhage

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## ABSTRACT

**الأهداف:** التحقق من وقت إنتاج البروتين الكيميائي الجاذب للخلايا وحيدة النواة، وإفراز خلايا تي (رانتيز) وتنظيمها بعد التنشيط وذلك بعد حدوث نزيف المخ.

**الطريقة:** أُجريت هذه الدراسة في قسم الأعصاب، المستشفى الخامس التابع لجامعة سِن يات سين، زوهاي، الصين. شملت الدراسة 15 مريضاً مصاباً بنزيف طفيف في المخ وذلك خلال الفترة من يوليو إلى ديسمبر 2008م. لقد قمنا بقياس مستويات البروتين الكيميائي الجاذب للخلايا وحيدة النواة، ورانتيز باستخدام اختبار إليزا في الأيام 3–1، و7، و14، و30 وذلك بعد حدوث نزيف المخ، وبعد ذلك قمنا بعمل مقارنة بين مجموعة المرضى ومجموعة الشاهد المكونة من 10 أشخاص.

**النتائج:** أشارت نتائج الدراسة إلى زيادة مستويات البروتين الكيميائي الجاذب للخلايا وحيدة النواة مباشرة خلال الأيام 3–1 من بدء نزيف المخ ($p$=0.000)، وبعد ذلك أخذت هذه المستويات بالانخفاض الطفيف على اليوم 7 ($p$=0.001)، واليوم 14 ($p$=0.000). ولكن بعد اليوم 14 أخذت هذه المستويات بالارتفاع حتى وصلت إلى أعلى معدلات الارتفاع على اليوم 30 ($p$=0.000). ولم يكن هنالك أي علاقة إحصائية واضحة بين مستويات البروتين الكيميائي الجاذب للخلايا وحيدة النواة على طول الفترات الزمنية. وكانت مستويات الرانتيز في كل الفترات الزمنية في مجموعة المرضى غير مختلفة عن مجموعة الشاهد. كما لم يكن هناك علاقة بين مستويات البروتين الكيميائي الجاذب للخلايا وحيدة النواة، والرانتيز من جهة ومستويات بروتين سي التفاعلي، أو أحجام الأورام الدموية الدماغية، أو الضعف العصبي المزمن، أو النتائج المترتبة بعد مرور 3 أشهر من جهة أخرى.

**خاتمة:** أظهرت هذه الدراسة بأن الارتفاع المستمر في مستويات البروتين الكيميائي الجاذب للخلايا وحيدة النواة قد يساهم في تقليل الضرر المزمن الناتج عن حدوث نزيف المخ الطفيف.

**Objective:** To investigate the time expression profile of serum monocyte chemoattractant protein (MCP)-1, and regulated upon activation, normal T cell expressed and secreted (RANTES) after minor intracerebral hemorrhage (ICH).

**Methods:** This study was carried out in the Department of Neurology in the Fifth Affiliated Hospital of Sun Yat-Sen University, Zhuhai, China. Fifteen consecutive hospitalized patients with minor ICH were enrolled from July 2008 to December 2008. The patients' serum levels of MCP-1 and RANTES were measured by enzyme-linked immunosorbent assay (ELISA) on days 1-3, 7, 14, and 30 after the onset of ICH, and compared with that of 10 controls.

**Results:** The serum MCP-1 levels increased immediately on days 1-3 ($p$=0.000) after onset, and then decreased slightly on day 7 ($p$=0.001), and day 14 ($p$=0.000); after day 14, the levels continued to increase and reached their highest levels on day 30 ($p$=0.000). No statistical differences in MCP-1 levels were found between any time points. The RANTES levels at any time point did not differ significantly from the controls. The levels of MCP-1 and RANTES were not correlated with the serum C-reactive protein (CRP) levels, brain hematoma volumes, acute neurologic impairment, or 3-month outcome.

**Conclusion:** The persistent elevation of serum MCP-1 levels after minor ICH may contribute to the acute damage and the repair following an ICH.


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Stroke is one of the 3 most frequent causes of death including cardiovascular diseases and lower respiratory infections worldwide, and 10-20% of strokes involve intracerebral hemorrhage (ICH).[1,2] Satisfactory therapies for ICH remain lacking because its pathological mechanism is unclear. In recent years, many studies have shown that inflammation was one of the important mechanisms in the acute phase reaction and secondary brain injury after ICH.[3-6] Chemokines are necessary chemotactic cytokines in inflammatory reactions. Based on the arrangement of cysteine residues, they are divided into 4 subfamilies: CXC, CC, C, and $CX_3C$.[7] The binding of the chemokines to their respective receptors leads to shape rearrangement and the directed migration of inflammatory cells.[7] There have only been a few studies on the chemokines involved in ICH. Studies indicate that monocyte chemoattractant protein (MCP)-1/CCL2, macrophage inflammatory protein (MIP)-2/Cxcl2, growth-related oncogene (GRO)γ/CXCL3, CCR1, IL-8, MIP-1α/Ccl3, immediate-early serum-responsive JE gene/Ccl2, and GRO/CXCL2 are involved in the early pathophysiology of ICH.[8-11] However, the precise time profile of chemokines expression after ICH onset is unclear. The MCP-1 and regulated upon activation, normal T cell expressed and secreted (RANTES)/CCL5 are primary members of the biggest chemokine subfamily – the CC subfamily, and they induce the migration of capital inflammatory cells - monocytes. This study aimed to determine the changes in the serum levels of MCP-1 and RANTES during ICH over 30 days, exploring the association among the changes of the 2 chemokines with C-reactive protein (CRP) variations, acute neurologic impairment, and 3-month outcome in patients with ICH.

## Methods.

*Patients.* This study enrolled consecutive patients with ICH from July 2008 to December 2008. All of the patients who were hospitalized in the Fifth Affiliated Hospital of Sun Yat-sen University, Zuhai, China and fulfilled the following criteria: (1) age ≥ 18 years, (2) first-ever stroke, (3) supratentorial ICH within 1-3 days of stroke onset, and (4) hematoma volume on brain CT ≤ 30 ml on days 1-3 after stroke onset were included. The diagnosis of ICH was made according to the World Health Organization (WHO) criteria, and was certified by brain CT.[12] Patients were excluded if they had any of the following conditions: mixed stroke; hemorrhagic cerebral infarct; comorbidities including malignant/autoimmune disease; severe infection; systemic inflammatory response syndrome (SIRS) with onset within one week of ICH; severe dysfunction of the heart, lung, liver, or kidney; use of immunomodulators; or neurologic impairment or disability caused by trauma or other diseases. The study was approved by the local research ethics committee, and was carried out according to the principles of the Helsinki declaration. Written informed consent was obtained from all participants.

*Controls.* Volunteers for health examination at the hospital were enrolled. The volunteers fulfilled the following criteria: (1) age ≥ 18 years, (2) normal neurological examination, and (3) without a history of neurological disease. Patients were excluded if they had any of the following: infectious diseases in the previous 2 weeks; severe dysfunction of the heart, lung, liver, or kidney; malignant/autoimmune disease; or use of immunomodulators.

*Serum samples collection.* Two milliliters of ulnar venous fasting blood samples were collected from each patient on the mornings of days 1-3, 7, 14, and 30 after stroke onset. Similarly, the blood samples were also taken from the control group on the morning of the health examination. The samples were allowed to clot at room temperature for 30 minutes, and the serum was immediately frozen and stored at -80°C after being centrifuged at 3,000 rpm for 5 minutes.

*Determination of MCP-1, RANTES, and CRP.* The MCP-1 and RANTES levels were measured with human enzyme-linked immunosorbent assay (ELISA) kits (Wuhan Boster Biological Technology, Ltd, Wuhan, China. No. EK0441, EK0494) according to the manufacturer's instructions and based on the quantitative sandwich enzyme immunoassay technique.[13] The sensitivity of the assay was 15.6 pg/mL. The CRP levels were measured routinely with immunoturbidimetry in the clinical laboratory of the hospital.

*Evaluation of hematoma volume, neurologic impairment, and outcome.* The hematoma volume on brain CT was calculated with the Tada formula on days 1-3 and 7 after stroke onset.[14] Neurologic impairment was determined using the National Institutes of Health Stroke Scale (NIHSS) at days 1-3 and 7 after stroke onset.[15] Three-month outcomes were estimated with the modified Rankin scale (mRS) and the Barthel index (BI).[15] The calculation of hematoma volume and score of NIHSS, mRS, and BI were performed independently by 2 attending neurologists.

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***Statistical analysis.*** We used the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) version 13 software for all analyses. Data of continuous variables were expressed as median ± interquartile distance. Categorical variables presented using frequency counts were compared between the patient and control groups by a chi-square test. Statistical differences in age and the serum levels of MCP-1, RANTES, and CRP between the patients with the controls were determined by the Mann-Whitney test. A Kruskal-Wallis test was used to compare the differences in MCP-1, RANTES, and CRP levels at each time point. Correlations among the levels of MCP-1 and RANTES with the CRP levels and other laboratory measurements were determined using Spearman's correlation analysis. Statistical significance was set as $p<0.05$.

**Results.** Baseline characteristics of patients and controls. Only 15 patients (11 males and 4 females) with minor ICH were enrolled because of the low frequency of ICH in South coastal China. The patients' median age was 68±21 years, with a range of 40-85 years. There were 11 patients with a striatum hemorrhage, 3 with a lobar hemorrhage, and one with an internal capsule hemorrhage. Concomitant diseases included 10 patients with primary hypertension, 2 with II type diabetes, and one with coronary heart disease. All of the patients survived to 3 months after the onset. Ten volunteers (7 males and 3 females) for health examination were enrolled, with a median age of 69±20 years (range: 42-83 years). Five had primary hypertension, and one had II type diabetes. There were no differences in the demographic characteristics, concomitant diseases, smoking and drinking between the groups. The number of patients receiving angiotensin-converting enzyme inhibitors (ACEIs) and statins in the patient group were significantly higher than those in the control group (**Table 1**).

***Variation of serum MCP-1, RANTES, and CRP levels.*** **Table 2** shows the variation in the serum levels of MCP-1, RANTES, and CRP. Serum MCP-1 levels in the stroke patients were significantly higher than those in the controls at 4 time points. The MCP-1 levels increased immediately at days 1-3 ($p$=0.000) after onset, and then decreased slightly at days 7 ($p$=0.001), and 14 ($p$=0.000), but were still higher than in the controls. After day 14, the levels continued to increase and reached their highest levels at day 30 ($p$=0.000). The serum RANTES levels decreased slightly on days 1-3 days after onset, but increased slightly at day 7. They then continued to increase until day 14, and reached their highest levels at day 30. However, the RANTES

**Table 1 -** Baseline characteristics of hospitalized ICH patients, and matched controls.

| Characteristics | Patient group (n=15) | Control group ( n=10) | *P*-value |
|---|---|---|---|
| | n (%) | | |
| Age, year (interquartile distance) | 68 (21.0) | 69 (20) | 0.253 |
| Male | 11 (73.3) | 7 (70) | 0.162 |
| Hypertension | 10 (66.7) | 5 (50) | 0.341 |
| Diabetes mellitus | 2 (13.3) | 1 (10) | 0.780 |
| CHD | 1 (6.7) | 0 0 | ND |
| Smoking | 9 (60.0) | 6 (60) | 0.059 |
| Drinking | 11 (73.3) | 8 (80) | 0.073 |
| ***Medication*** | | | |
| CCB | 10 (66.7) | 5 (50) | 0.353 |
| ACEI | 8 (53.3) | 1 (10) | <0.0001 |
| ARB | 3 (20.0) | 1 (10) | 0.265 |
| β-blocker | 2 (13.3) | 0 0 | ND |
| Nitrates | 1 (6.7) | 0 0 | ND |
| Sulfonylureas | 2 (13.3) | 1 (10) | 0.077 |
| Statins | 11 (73.3) | 4 (40) | 0.006 |

ICH - intracerebral hemorrhage, CHD - coronary heart disease, CCB - calcium-channel blocker, ACEI - angiotensin-converting enzyme inhibitor, ARB - angiotensin II receptor blocker, ND - not determined

**Table 2 -** Serum MCP-1, RANTES, and CRP levels at different time points in patients with ICH.

| Groups | n | MCP-1 (pg/mL) (95% CI) | RANTES (pg/mL) (95% CI) | CRP (mg/L) (95% CI) |
|---|---|---|---|---|
| ***Patients*** | | | | |
| Day 1-3 | 15 | 111.62±114.63[a*] (70.51-140.08) | 27.31±24.29[#] (17.24-35.60) | 7.59±19.73[c†] (4.16-20.71) |
| Day 7 | 15 | 87.91±64.42[b*] (61.03-125.45) | 32.67±31.93[#] (14.24-46.17) | 6.42±7.45[d†] (4.00-8.91) |
| Day 14 | 15 | 86.32±126.48[a*] (55.10-104.51) | 38.03±44.21[#] (14.46-58.17) | 9.29±18.20[d†] (3.50-21.70) |
| Day 30 | 15 | 129.80±101.19[a*] (71.70-144.43) | 45.02±43.78[#] (9.89-53.67) | 3.42±5.70[c†] (2.64-6.07) |
| Controls | 10 | 33.76±23.82 (29.01-49.96) | 30.50±26.68 (15.39-41.89) | 0.50±1.88 (0.2-2.0) |

MCP-1 - monocyte chemoattractant protein-1, RANTES - regulated upon activation, normal T cell expressed and secreted, CRP - C-reactive protein, ICH - intracerebral hemorrhage, [a]versus controls, $p$=0.000, [b]versus controls, $p$=0.001, [c]versus controls, $p$=0.000, [d]versus controls, $p$=0.001, [c]verus controls, $p$=0.004, *versus different time points, $p$=0.478, #versus different time points, $p$=0.735, †versus different time points, $p$=0.094

**Table 3 -** Correlation between the MCP-1, RANTES, with CRP levels, and correlation between the MCP-1, RANTES, and CRP levels with brain hematoma volume in patients with ICH.

| Time | CRP | | | | Hematoma volume (day 1-3) | | | | | | Hematoma volume (day 7) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MCP-1 | | RANTES | | MCP-1 | | RANTES | | CRP | | MCP-1 | | RANTES | | CRP | |
| | r | *p* | r | *p* | r | *p* | r | *p* | r | *p* | r | *p* | r | *p* | r | *p* |
| Day 1-3 | 0.36 | 0.89 | 0.04 | 0.99 | 0.27 | 0.32 | 0.14 | 0.63 | 0.49 | 0.63 | 0.23 | 0.42 | 0.19 | 0.49 | 0.28 | 0.29 |
| Day 7 | 0.30 | 0.27 | 0.21 | 0.45 | 0.15 | 0.59 | 0.01 | 0.99 | 0.11 | 0.69 | 0.16 | 0.58 | 0.01 | 0.97 | 0.23 | 0.41 |
| Day 14 | 0.23 | 0.93 | 0.25 | 0.36 | 0.12 | 0.68 | 0.12 | 0.68 | 0.17 | 0.55 | 0.11 | 0.69 | 0.08 | 0.79 | 0.11 | 0.70 |
| Day 30 | 0.11 | 0.67 | 0.45 | 0.08 | 0.01 | 0.98 | 0.01 | 0.99 | 0.01 | 0.99 | 0.03 | 0.92 | 0.71 | 0.80 | 0.03 | 0.93 |

CRP - C-reactive protein, MCP-1 - monocyte chemoattractant protein-1, RANTES - regulated upon activation, normal T cell expressed and secreted, ICH - intracerebral hemorrhage

**Table 4 -** Correlation between the MCP-1, RANTES, with CRP levels with NIHSS scores in patients with ICH.

| Time | NIHSS scores (day 1-3) | | | | | | NIHSS scores (day 7) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MCP-1 | | RANTES | | CRP | | MCP-1 | | RANTES | | CRP | |
| | r | *p* | r | *p* | r | *p* | r | *p* | r | *p* | r | *p* |
| Day 1-3 | 0.11 | 0.71 | 0.19 | 0.49 | 0.06 | 0.84 | 0.18 | 0.53 | 0.14 | 0.61 | 0.16 | 0.56 |
| Day 7 | 0.35 | 0.21 | 0.38 | 0.16 | 0.08 | 0.77 | 0.16 | 0.58 | 0.41 | 0.13 | 0.04 | 0.89 |
| Day 14 | 0.27 | 0.33 | 0.26 | 0.34 | 0.05 | 0.85 | 0.14 | 0.63 | 0.24 | 0.39 | 0.08 | 0.78 |
| Day 30 | 0.28 | 0.31 | 0.21 | 0.42 | 0.06 | 0.83 | 0.02 | 0.95 | 0.11 | 0.69 | 0.09 | 0.75 |

ICH - intracerebral hemorrhage, CRP - C-reactive protein, MCP-1 - monocyte chemoattractant protein-1, RANTES - regulated upon activation, normal T cell expressed and secreted, NIHSS - National Institutes of Health Stroke Scale

**Table 5 -** Correlation between the MCP-1, RANTES, with CRP levels with BI and mRS scores in patients with ICH.

| Time | BI (3 months) | | | | | | mRS (3 months) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MCP-1 | | RANTES | | CRP | | MCP-1 | | RANTES | | CRP | |
| | r | *p* | r | *p* | r | *p* | r | *p* | r | *p* | r | *p* |
| Day 1-3 | 0.04 | 0.86 | 0.26 | 0.35 | 0.13 | 0.63 | 0.12 | 0.97 | 0.31 | 0.27 | 0.14 | 0.63 |
| Day 7 | 0.31 | 0.25 | 0.49 | 0.64 | 0.22 | 0.43 | 0.29 | 0.30 | 0.44 | 0.10 | 0.33 | 0.23 |
| Day 14 | 0.11 | 0.71 | 0.32 | 0.24 | 0.00 | 0.99 | 0.21 | 0.45 | 0.30 | 0.29 | 0.15 | 0.59 |
| Day 30 | 0.06 | 0.83 | 0.21 | 0.46 | 0.13 | 0.96 | 0.32 | 0.91 | 0.26 | 0.35 | 0.03 | 0.91 |

ICH - intracerebral hemorrhage, CRP - C-reactive protein, MCP-1 - monocyte chemoattractant protein-1, RANTES - regulated upon activation, normal T cell expressed and secreted, BI - Barthel index, mRS - modified Rankin scale

levels in the patients did not differ significantly from those in the controls at any time points. Serum CRP levels increased significantly at day 1-3 ($p$=0.000) after onset, and then decreased slightly at day 7 ($p$=0.001). After day 7, they continued to increase and reached their highest levels at day 14 ($p$=0.001), but then decreased to their lowest levels at day 30, although they were still higher than in the controls ($p$=0.004). There were no statistical differences in the serum levels of MCP-1, RANTES, and CRP between any time points.

***Correlation of the MCP-1 and RANTES levels with the CRP levels.*** There was no correlation between the MCP-1 and RANTES levels with the CRP levels at the corresponding time points (**Table 3**).

***Correlation of the MCP-1, RANTES, and CRP levels with the brain hematoma volume.*** The brain hematoma volumes at days 1-3 after onset were 7.06±12.30 (95% confidence interval [CI]: 1.77-10.02) mL (minimum 0.25 ml, maximum 27.56 mL). The brain hematoma volumes at day 7 after onset were 5.05±16.88 (95% CI: 2.83-12.66) ml (minimum 0.37 mL, maximum 89.80 mL). The brain hematoma volumes in 10 patients were less than 10 ml at 2 time points, 2.60±6.34 (95% CI: 0.79-7.07) mL at days 1-3, and 2.94±4.55 (95% CI: 0.89-5.05) mL at day 7. The brain hematoma volumes in 5 patients were larger than 10 ml at 2 time points, 13.23±9.64 (95% CI: 13.18-14.92) mL at days 1-3, and 19.22±49.61(95% CI: 17.87-39.94) mL at day 7. The MCP-1, RANTES, and CRP levels at any time point were not correlated with the brain hematoma volumes at the 2 time points (**Table 3**).

***Correlation of the serum MCP-1, RANTES, and CRP levels with neurological impairment and outcome.*** The NIHSS scores were 3±9 (95% CI: 1.00-5.00) and 2±6 (95% CI: 1.00-5.00) at days 1-3, and 7 after the onset of ICH. The mRS scores, and BI scores were 1±1 (95% CI: 1.00-2.00), and 100±10 (95% CI: 90.00-100.00) at 3 months after ICH onset. The MCP-1, RANTES, and CRP levels were not correlated with the NIHSS, mRS, and BI scores at any time point (**Tables 4 & 5**).

**Discussion.** The MCP-1 is also called CCL2, and its corresponding receptor is CCR2.[7] The MCP-1 is a classic mediator of inflammation that specifically induces the migration of mononuclear macrophages, which may be generated from brain glial cells, neurons, the endothelium, and macrophages.[7] A previous study observed that serum MCP-1 levels increased immediately within 48 hours after ICH onset.[8] Our study found that not only did serum MCP-1 levels in patients with minor ICH increase significantly in the early phase after onset, but they also increased in a persistent process. Maybe this result supports the role of MCP-1 participation in the pathophysiology of ICH.

The increase in serum MCP-1 can originate from several sources. The first of these are brain glia and neurons. The upregulation of MCP-1 mRNA in the brain tissue of ICH rats has been observed, which might increase expression and release of MCP-1.[11] The MCP-1 can pass through the broken blood-brain barrier into the bloodstream, which leads to an increase in serum MCP-1 levels.[8] White blood cells can also be a source of serum MCP-1.[3] In addition, MCP-1 can be derived from vascular endothelial cells. First, atherosclerosis, diabetes mellitus, and hypertension all induce the endothelium to express increased levels of MCP-1.[16-19] Secondly, the activation of the endothelium is enhanced in the acute phase of stroke, which promotes the expression of MCP-1.[20] Activation of peripheral immune organs can lead to increased serum MCP-1 after experimental stroke, which indicates that stroke induces rapid activation of the peripheral immune system.[21] The results of our study showed that the upregulation of MCP-1 was by no means a transitory process. The MCP-1 levels initially increased but were stable at days 7, 14, and 30 after the onset. This process might indicate the time profile of inflammation activity after ICH, where central and peripheral inflammation activity are the most active in the early phase after the onset, and then continues to the recovery phase. A recent study[22] found that lack of CCL2 or CCR2 decreased the hematoma volume early after mice ICH, but delayed its recovery. Therefore, we speculated that the inflammatory activities might promote the regeneration and repair of nerves in the few days to weeks after ICH onset. However, it is unclear why the serum MCP-1 levels were also elevated at day 30 after ICH. Considering the pathological process of ICH, this time point should represent the recovery phase in which the hematoma is almost entirely absorbed, and the primary pathology shows gliosis and neovascularization. Thus, we speculated that the proliferation of abundant gliocytes and endothelial cells could upregulate MCP-1. Some experimental studies have reported that astrocytes and microglia in the ischemic brain region upregulated the expression of MCP-1 after brain ischemia, which induced neuroblast and neural progenitor cell migration to the ischemic region to participate in nerve repair.[23,24] It is worth investigating whether there was a similar mechanism in repairing brain damage in the recovery phase of ICH.

The RANTES, also known as CCL5, binds through the CCR1, CCR3, and CCR5 receptors to produce a chemotaxis effect.[7] The RANTES are produced by T lymphocytes, platelets, endothelial cells, smooth muscle cells, and glial cells;[7] it promotes the directed migration of leukocytes and monocytes into damaged or inflamed sites.[7] There have been no previous reports on the expression of RANTES in ICH prior to this study. The RANTES levels did not change statistically in this study. The following processes could produce this result: (1) the RANTES response to stroke was inferior to that of MCP-1; (2) the cell sources of the 2 chemokines were different - the expression of the MCP-1 increased rapidly because the microglia of the brain were initially activated when the stroke first occurred, but the RANTES might originate principally from platelets;[25] (3) the RANTES

bound efficiently to the vascular endothelium because its affinity with the glycosaminoglycans of the vascular endothelial cell surface exceeded the affinity of MCP-1, which resulted in an insufficient increase in serum RANTES levels;[26] or (4) there might have been inter-individual determination variations among the patients. Nonetheless, the RANTES levels still inclined to increase gradually, which suggested that RANTES probably also contributed to the inflammatory reaction of ICH. However, this needs to be demonstrated in future research.

The CRP is an accepted acute phase protein and an inflammatory marker of acute cardio-cerebrovascular diseases.[3,27] Serum CRP was elevated in this study, and the levels at every time point after the onset were also stable. Although the influence of determination variations could not be excluded. This pattern of CRP changes suggested continuous inflammation after ICH onset as well. It should be noted that some studies have indicated that CRP can induce endothelial cells and monocytes to express MCP-1.[28,29] Therefore, although there was no statistical correlation between the increases of CRP levels and that of MCP-1 levels, the increases of serum MCP-1 levels still might be influenced by CRP in our study. Although it was unclear if CRP could induce the expression of RANTES, the possibility exists.

Some studies have found that serum MCP-1, RANTES, and CRP levels in the acute phase of stroke could predict the patient's neurologic deterioration and outcome.[30-32] However, our study did not find that serum MCP-1, RANTES, and CRP levels were correlated with hematoma volume or NIHSS, BI, and mRS scores. This finding might be due to the small sample size and the small hematoma volume in most patients who recover rapidly and have better outcomes. Moreover, if the patients have used statins and ACEIs, the inflammatory activity could be inhibited.[33,34] In addition, bleeding sites and determination variability could obstruct the results. Similarly, the above-mentioned factors might interfere with determinations of serum MCP-1, RANTES, and CRP levels and statistical significance. To verify the results, future studies should use an increased sample size, including different patient ages, bleeding volumes and sites, interventions, comorbidities, and so forth.

A recent study on ICH in rats found that the antiepileptic drug valproic acid downregulated the mRNA of MCP-1 and other inflammatory factors, reduced brain inflammatory activities, and peri-hematomal cell death, and also improved neurofunctional recovery.[35] Thus, interfering with chemokine activity might be a method to treat ICH.

In conclusion, the serum levels of MCP-1 were elevated, but that of RANTES did not change in the acute and recovery phase of minor ICH. The changes of MCP-1 levels may contribute to damage in the acute phase of ICH, and the repair of the CNS in the recovery phase of ICH. However, whether it can predict the severity and outcome of ICH still needs further confirmation.

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