The Quantitative and Functional Changes of Postoperative Peripheral Blood Immune Cell Subsets Relate to Prognosis of Patients with Subarachnoid Hemorrhage: A Preliminary Study

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OBJECTIVE: **It has been suggested that the preoperative (PRE) and postoperative (POST) immune system alteration triggered by aneurysmal subarachnoid hemorrhage (SAH) and surgical treatment itself may affect patients' prognosis and contribute to POST complications. The mechanisms may be attributed to immune suppression—triggered infection or immune overreaction—triggered aseptic inflammation. In this study, we investigated the dynamic changes in peripheral immune cell subsets as well as the alterations of inflammatory cytokines in patients with aneurysmal SAH who received craniotomy and clipping surgery. In addition, we studied the association of those changes with POST complications and clinical prognosis.**

METHODS: **We investigated 27 patients who received craniotomy and clipping surgery for aneurysmal SAH. The operations were all performed within 24 hours after the occurrence of aneurysm rupture. Detailed immune monitoring (peripheral blood leukocytes and lymphocyte subsets and inflammatory cytokines) was performed on PRE (on admission), day 1, day 3, and day 6 after operation.**

RESULTS: **Our data showed that the percentage of CD3+, CD8+, natural killer T (NKT), CD4+, and regulatory T (Treg) cells significantly decreased and the level of interleukin 4 (IL-4), interferon γ, and IL-2 significantly increased 1 day after surgery compared with the data in PRE. On the contrary, natural killer (NK), NK group 2 (NKG2D), and B cells increased and the level of IL-10 in plasma decreased. In study of the relationship between POST fever and the change in immune cell subgroups, the fever group had a lower percentage of CD3+, CD4+, NKT, Tregs, and B cells on day 1, day 3, and day 6 after surgery compared with the patients who did not have fever, whereas the CD8+, NK, and NKG2D subsets showed the opposite trend. Furthermore, we analyzed the association between immune profile changes and the prognosis of those patients. The patients were divided into those with an unfavorable prognosis ($n = 6$) and those with a favorable prognosis ($n = 21$) according to Glasgow Outcome Scale score and postoperation (POST) coma. Our results showed that except for B cells, patients with a favorable prognosis had a relatively higher percentage of CD3+, CD4+, CD8+, NK, NKT, NKG2D, and Treg cells compared with the unfavorable prognosis group from PRE to day 6 POST.**

CONCLUSIONS: **Our results indicated that patients with aneurysmal SAH undergoing craniotomy and clipping surgery had a profound transient deterioration in immune function. In addition, the changes in immune cell subgroups had a strong association with POST fever. The changes in immune cell subgroups were also directly associated with clinical prognosis of the patients. These association findings might be attributable to a better biomarker to predict patient diagnosis.**

Key words
- Clipping surgery
- Immune function
- Inflammatory cytokines
- Lymphocyte subsets
- Peripheral blood leukocytes
- SAH

Abbreviations and Acronyms
GOS: Glasgow Outcome Scale
IFN-γ: Interferon γ
IL-2: Interleukin 2
IVGTT: Intravenous glucose tolerance test
NK: Natural killer
NKG2D: NK group 2
PB: Peripheral blood
POST: Postoperation/postoperative
PRE: Preoperation/preoperative
SAH: Subarachnoid hemorrhage

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Citation: World Neurosurg. (2017) 108:206-215.
http://dx.doi.org/10.1016/j.wneu.2017.08.091

*Journal homepage: **www.WORLDNEUROSURGERY.org***

*Available online: **www.sciencedirect.com***

INTRODUCTION

Subarachnoid hemorrhage (SAH) is a complex and potentially devastating disorder, with only 25% of patients likely to live independently.[1,2] The clinical and socioeconomic burden of SAH is unacceptably high. SAH caused by intracranial aneurysm rupture, namely aneurysmal SAH (aSAH), are most common. Surgical treatment, both endovascular coiling and neurosurgical clipping, is the only therapy for aSAH, but high fever and other bad complications are still risk factors that contribute to patients' unfavorable postoperative (POST) outcome.[3,4]

POST infection, which is a leading cause of major disability and death, together with cerebral vasospasm play the rule of second strike. In neurosurgery intensive care units, infection is a common problem. The overall POST infection rate ranges from 6.5% to 11.2% in most hospitals. Among those patients staying longer than 48 hours in a neurosurgery intensive care unit, the infection rate is higher than 30%.[5,6]

Previous studies have shown that the occurrence of systemic infections after SAH may be a symptom of impaired immune competence.[7,8] Shortly after the onset of SAH, the blood components and irritated brain tissue expose antigens that are recognized by components of the innate immune system, which leads to its activation and acute inflammatory response.[9,10] The activated primary effector cells are phagocytes (polymorphonuclear neutrophils, macrophages, and monocytes)[11,12] and innate lymphocytes (natural killer [NK] and NKT).[13] These cells attach to the endothelium of proinflammatory vessels in the brain by binding with various adhesion factors. They produce cytokines[14,15] (interleukin 2 [IL-2], IL-6, interferon γ [IFN-γ], and IL-10) that induce and regulate inflammation, ingest and destroy microbes, and clear the damaged cells. Subsequently, innate immune responses generate molecules that present signals, in addition to antigens, to activate naive T and B lymphocytes.[10,16]

Immune activation after SAH has been proved to play an important role in host defense against infection. Besides neutrophils and monocytes, the activated lymphocytes also release proinflammatory cytokines, directly eliminating damaged cells, killing the infected cells, or neutralizing microbes.[17] There is increasing evidence that, after acute central nervous system injury, a temporary impairment of cellular immune function acts as an important risk factor in the occurrence of infections. For this reason, the occurrence of systemic infections with patients POST aSAH needs to be further explored. In this pilot study, we assessed the preoperative (PRE) and POST immune profile changes (peripheral immune subset count) of patients with aSAH undergoing clipping surgery. In addition, we studied the association of those changes with POST complication (fever), as well as the relationship between immune profile changes and clinical outcome of patients.

METHODS

Patients

Patients were enrolled from May 2015 to July 2015 in the Neurosurgery Department of the Second Xiangya Hospital of Central South University, Changsha, Hunan, China. We investigated 27 adult patients who received clipping surgery for an intracerebral aneurysm after SAH. The inclusion criteria were: 1) SAH confirmed by cranial computed tomography; 2) cerebral angiogram showing intracranial aneurysm (the location of aneurysms of these 27 patients was the anterior communicating artery, posterior communicating artery, and middle cerebral artery); and 3) the surgical operations were performed within 24 hours after the occurrence of the disease. Depending on the location of the aneurysm, we use the modified pterional approach. For each patient, we may change the approach slightly, but the skin incision and bone flap do not change more than 3 cm^2 or 4 cm^2, respectively. Clinical presentation was graded according to the World Federation of Neurological Surgeons.[18] Exclusion criteria were as follows: significant impairment of renal or liver function, severe myocardial dysfunction, coagulation abnormalities, preexisting infections, accompanying cancer diseases, or any other immune deficiency syndrome (e.g., AIDS, leukemia, lymphoma, or lymphocytopenia). Aneurysm location was assessed using 4-vessel angiography or computed tomography angiography or both on the day of admission.

All patients received the usual regimen of drugs until the morning of surgery, including atropine 500 μg and phenol-barbital 10 mg, intramuscularly, as PRE preparation, 45 minutes before they were sent to the operation room. All patients received antibiotic prophylaxis with use of the following protocol: body weight $<$ 80 kg: ceftriaxone 1 g intravenous glucose tolerance test (IVGTT), body weight $\geq$80 kg: ceftriaxone 2 g IVGTT. If allergic, vancomycin 15 mg/kg IVGTT over an hour was administered.

Induction and maintenance of anesthesia were similar for all patients, and consisted of weight-related doses of fentanyl (35 mg/kg), midazolam (0.25 mg/kg), and pancuronium bromide (0.15 mg/kg). Patients were moderately hyperventilated under capnometric control ($Paco_2$ [partial pressure of carbon dioxide, arterial], 32–36 mm Hg) with a gas mixture containing 65% air in oxygen. The antimicrobial prophylaxis was performed with 2 g of ceftriaxone after induction of anesthesia.

The study protocol was approved by the ethics committee of the Second Hospital of Central South University. Written informed consent from a next-of-kin was required for enrollment. Retrospective consent was obtained from patients, when possible.

Clinical Management

Heparinized peripheral blood (PB) samples from 27 patients were collected at 4 predetermined intervals: T1) immediately before induction of anesthesia; T2) day 1 POST; T3) day 3 POST; and T4) day 6 POST. The patients were divided into a fever group ($n = 9$) and a nonfever group ($n = 18$) according to whether patients had long-lasting fever ($>$ 3 days, highest body temperature reached 39°C) or not. Demographic and clinical characteristics are summarized in Table 1.

The patients were defined as having an unfavorable prognosis ($n = 6$) if they had a long POST coma (coma time of $>$2 weeks and a Glasgow Scale score of $<$9) or if they had to use a respirator to maintain adequate ventilation.

On the other hand, patients who had no serious complications were defined as having a favorable prognosis ($n = 21$). Demographic and clinical data for favorable prognosis and unfavorable prognosis in the study are listed in Table 2. Survival and