Platelet phagocytosis by leukocytes in a patient with cerebral hemorrhage and thrombocytopenia caused by gram-negative bacterial infection

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Abstract
Bacteria-induced thrombocytopenia is a common clinical disease that is often ignored by clinical and scientific research. Thus, exploring the mechanism and principle of bacteria-induced thrombocytopenia could facilitate the development of new diagnostic, preventative, and treatment modalities for thrombocytopenia. This case report describes a case of platelet phagocytosis by neutrophils and monocytes in a patient with cerebral hemorrhage and thrombocytopenia caused by gram-negative bacterial infection. After the infection was eradicated, platelet phagocytosis was alleviated, and his platelet count normalized. Cellular immunity may be an important cause of bacteria-induced thrombocytopenia in patients with cerebral hemorrhage.

Keywords
Cerebral haemorrhage, thrombocytopenia, leukocyte, phagocytosis, case report, neutrophil, bacterial infection

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Introduction

Thrombocytopenia caused by infection is common in clinical practice. Its mechanism may be related to the direct damage of megakaryocytes, the direct destruction or consumption of platelets by pathogens, and immune-mediated platelet destruction. However, the specific mechanism is unknown, and little clinical or scientific research has addressed this issue. We found that the phagocytosis of neutrophils and macrophages played an important role in a patient with intracerebral hemorrhage and thrombocytopenia caused by gram-negative bacterial infection, indicating that cellular immunity might be an important cause of thrombocytopenia caused by infection.

Case report

Patient details

A 15-year-old boy was hospitalized with head injury and coma after riding in an electric car on January 17, 2020. At the time of admission, his temperature was 36.8°C, his pulse was 108 beats/minute, his respiratory rate was 19 breaths/minute, and his blood pressure was 110/60 mmHg. His abdomen was soft without tenderness, and his liver and spleen were not swollen. Subarachnoid hemorrhage, left frontotemporal extradural hematoma, left frontotemporal subdural hematoma, and left frontotemporal bone fracture were detected by computed tomography (CT). On the same day, hematoma removal and cranial flap decompression were performed under emergency general anesthesia. Postoperative CT revealed that the intracerebral hemorrhage had been cleared, but his consciousness was not restored. We suspected the existence of a brain hernia that could affect cerebral function. His temperature was 39.5°C on January 17 and 18, 2020, gradually falling to 38.2°C on January 19, 2020 and 37.5°C on January 30, 2020. His temperature returned to normal on February 1, 2020, and he gradually recovered consciousness on February 5, 2020. The patient was healthy, and he had no family history of thrombocytopenia.

This work was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The reporting of this study conforms to the CARE guidelines. The patient provided written informed consent for the publication of this case report. Because all performed tests are routinely used in clinical diagnosis and treatment, the ethics committee of our hospital waived the requirement for ethics approval.

Laboratory examination

Routine blood testing was performed using an XN9000 automatic 5-part differential hematology analyzer (Sysmex, Kobe, Japan) and supporting reagents. Fibrinogen content was tested using a Ca7000 automatic coagulation analyzer (Sysmex) and supporting reagents. Blood biochemistry testing was performed using a Dxc800 automatic biochemical analyzer (Beckman-Coulter, Brea, CA, USA) and supporting reagents. The isolation, culture, and identification of sputum bacteria were performed in accordance with the national guide for clinical laboratory procedures (3rd edition) using the VITEK2 COMPACT automatic microbiology analyzer (BioMérieux, Marcy-l’Étoile, France).

Results of laboratory examination

The platelet count was normal at admission, but it decreased after infection before gradually returning to normal after the infection was cured (Tables 1–2). There was no obvious abnormality regarding bleeding and coagulation. After hematoma
removal, the patient continued to have a fever. The patient had no history of infection outside the hospital, and his fever started after admission, strongly suggesting nosocomial infection. *Haemophilus influenzae* was detected in sputum culture, indicating the presence of pulmonary infection. On January 19, 2020, peripheral blood smear analysis revealed high levels of platelet phagocytosis by neutrophils and monocytes, toxic granules and vacuoles in the cytoplasm of the neutrophils, and a small amount of aggregation and mild satellite phenomena in platelets. After the infection was cured, platelet phagocytosis was no longer observed (Figures 1–2). After treatment, the patient recovered, after which he was discharged from the hospital (Figure 3).

### Discussion

Thrombocytopenia caused by infection is a common clinical phenomenon, but it has not been widely investigated. In addition, the specific mechanism is unclear. We found that gram-negative bacteria played an important role in the development of thrombocytopenia in a patient with subarachnoid hemorrhage. The patient was admitted to our hospital with cerebral hemorrhage after a fall. On admission, his body temperature and platelet count were normal, but his leukocyte and neutrophil counts were elevated. After hematoma removal, the patient continued to have a fever. *H. influenzae* was detected in sputum culture, indicating the presence of pulmonary infection. On January 19, 2020, his leukocyte count (mainly neutrophils) remained elevated, and his platelet count was decreased. Other findings included high numbers of neutrophil- and monocyte-phagocytized platelets, satellite phenomena, and aggregation of a small number of platelets, which were observed 20, 2020, and cefoperazone sodium was injected on February 2, 2020.

### Treatment

On January 17, 2020, intracranial hematoma removal surgery was performed under general anesthesia. One unit of platelets was transfused on January 19, 2020. Meropenem and phenobarbital sodium were injected intramuscularly on January 25, 2020, and cefoperazone sodium was administered from January 20, 2020.

### Table 1. Changes in the patient's blood routine test data.

| Date    | Platelets (10^9/L) | Hemoglobin (g/dL) | Erythrocyte (10^12/L) | Leucocytes (10^9/L) | Lymphocyte ratio (%) | Neutrophil ratio (%) |
|---------|--------------------|-------------------|-----------------------|---------------------|----------------------|----------------------|
| 1-17-2020 | 126                | 157               | 5.27                  | 22.22               | 2.1                  | 93.8                 |
| 1-19-2020 | 89                 | 95                | 3.4                   | 15.9                | 4.5                  | 91.1                 |
| 2-5-2020  | 119                | 94                | 3.35                  | 6.26                | 29.1                 | 58.8                 |

### Table 2. Changes in the patient's biochemistry test data.

| Date    | Blood glucose (mmol/L) | Triglyceride (mmol/L) | Lactate dehydrogenase (U/L) |
|---------|------------------------|-----------------------|-----------------------------|
| 1-17-2020 | 12.66                  | 0.33                  | 262                         |
| 1-19-2020 | 5.5                    | 0.36                  | 312                         |
| 2-50-2020 | /                      | 1.11                  | 177                         |
after the peripheral blood smear was stained with Wright's staining.

Intracerebral hemorrhage can cause endothelial cell damage and activate platelets. After activation, platelets adhere to the vascular endothelium and react with neutrophils and monocytes. The main physiological functions of platelets include hemostasis, coagulation, inflammation, and immune reactions. Thrombin-sensitive protein (TSP) can mediate the interaction between activated platelets and monocytes because both leukocytes and activated platelets have receptors for TSP on their surfaces. Relevant studies have illustrated that platelet membrane protein-140 can also mediate the interaction between activated platelets and neutrophils and cause thrombocytopenia by participating in the binding of activated platelets and leukocytes. In addition, vascular adhesion protein-1 can mediate the interaction between platelets and neutrophils. Static platelets cannot react with neutrophils, but thrombin-activated platelets react with neutrophils in a calcium-dependent manner.

In this case, the patient had a stress reaction caused by an accident, elevated blood glucose levels, and marked leukocytosis. Acute stress and infection mainly trigger innate immune responses. Cells involved in innate immunity do not target any specific pathogen; rather, they widely attack a variety of different pathogens quickly (minutes to hours) when challenged. Neutrophils and macrophages play major
Figure 2. Sodium citrate anticoagulant. (a) Platelets are phagocytized by neutrophils and monocytes (Wright–Giemsa stain, ×1000). (b) Platelets are phagocytized by neutrophils (Wright–Giemsa stain, ×1000). (c) Platelets are phagocytized by neutrophils (Wright–Giemsa stain, ×1000). (d) Platelet aggregation (Wright–Giemsa stain, ×1000).

Figure 3. Platelet phagocytosis was alleviated after thrombocytopenia was resolved. (a–b) Patient’s blood smear after recovery (Wright–Giemsa stain, ×1000).
roles in innate immunity, and activation of these cells can lead to inflammation. Neutrophils and macrophages concentrate at the injured or infected site, releasing toxic substances such as oxygen-free radicals to damage the invading pathogens and simultaneously phagocytize pathogens and damaged tissues. Furthermore, it has been reported that the thrombocytopenia associated with increased lactate dehydrogenase (LDH) levels is often caused by infection. In this case, the observations of increased LDH levels and gram-negative bacterial infection were consistent with changes in platelet counts (Table 2). In summary, it was likely that cerebral hemorrhage and gram-negative bacterial infection led to these overreactions that sensitized the platelets, resulting in their phagocytosis by leukocytes.

To confirm thrombocytopenia in this case, the possibility of reduced platelet counts caused by the use of the anticoagulant EDTA-K2 had to be discounted. Therefore, in this study, sodium citrate and heparin were used as anticoagulants to detect platelets. The results showed that platelet counts were still depressed, and leukocyte-phagocytized platelets were detected.

The pathogenic mechanism of human granulocytic anaplasmosis (HGA) is mainly that the function of neutrophils is damaged following infection by pathogens, resulting in abnormal adhesion, migration, neutrophil degranulation, and phagocytosis, which in turn cause a series of immunopathological changes. In this case, the patient had fever and thrombocytopenia, and the possibility of Anaplasma phagocytophilum infection existed. However, considering that the cause of the disease was clear in this case, HGA could be excluded.

The etiology of hemophagocytic lymphohistiocytosis (HLH) is diverse, and infection is the most common cause. Because leukocyte-phagocytized platelets and consistent fever were detected in this case, the possibility of HLH had to be considered. This patient had normal triglyceride levels, his spleen could not be touched under the rib margin, his hemoglobin level exceeded 90 g/L, and his prognosis was good. Therefore, a HLH diagnosis was excluded.

Viral infection can cause platelet aggregation and destruction, resulting in thrombocytopenia. Acute thrombocytopenia is more common in children. In this case, cerebral hemorrhage and gram-negative bacterial infection were clearly present, and thus, virus-induced thrombocytopenia was not considered.

Infection following cerebral hemorrhage has rarely been reported as a cause of thrombocytopenia associated with the phagocytosis of platelets by leukocytes in peripheral blood. We cannot clarify whether the stress of intracerebral hemorrhage or infection alone leads to platelet phagocytosis. It is unclear why thrombocytopenia caused by infection is common in such patients even though platelet phagocytosis is rare. However, few studies have examined thrombocytopenia caused by craniocerebral hemorrhage alone. It remains unknown whether platelet phagocytosis is caused by craniocerebral trauma and intracerebral hemorrhage alone. We hope this will be clarified in the future.

Declaration of conflicting interests
No conflict of interest exists in the submission of this manuscript, and the manuscript was approved for publication by all authors. Signed consent to publish from the patient has been obtained. We have de-identified all patient details.

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Supplemental material

Supplemental material for this article is available online.

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