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Short Communication

A case of human granulocytic anaplasmosis diagnosed by a peripheral blood smear test in South Korea

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Running head: Intragranulocytic morulae in human granulocytic anaplasmosis

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Summary

We report the case of a 76-year-old woman whose infection was rapidly diagnosed as human granulocytic anaplasmosis (HGA) through a peripheral blood smear that showed characteristic intragranulocytic morulae. The smear was prepared on the day of hospitalization, which was 1–2 weeks before results were available from serology or the polymerase chain reaction (PCR). On the basis of the blood smear test, we started timely and appropriate antimicrobial treatment. The peripheral blood smear is known to have the lowest sensitivity when compared with serological or PCR tests for the diagnosis of HGA, but we suggest that the sensitivity of peripheral blood smear tests could be increased by employing experienced staff. The patient was confirmed as having HGA by PCR 7 days after the positive peripheral blood smear test, and 14 days later by serology. Morulae in neutrophils are an important diagnostic indicator of HGA, especially in febrile patients with a history of tick-bites or outdoor activities in rural areas.
Early symptoms and signs of tick-bone rickettsial diseases are nonspecific and overlap, and so it is challenging to diagnose such cases at a time when antibacterial therapy is most effective (1). Leukopenia and thrombocytopenia, which are characteristic of these diseases, can be observed in many other infections. By contrast, intracellular morulae in blood smears can be crucial diagnostic findings for these diseases. *Anaplasma phagocytophilum* is observed as morulae in neutrophils of the host, whereas morulae are observed in monocytes of hosts infected with *Ehrlichia chaffeensis* (2). Human granulocytic anaplasmosis (HGA) can be a self-limiting illness, but severe cases may result in a septic shock-like syndrome, coagulopathies, acute respiratory distress syndrome, acute abdominal syndrome, or acute renal failure (3). In South Korea, human HGA cases have been consistently observed since being first reported in 2013 (4, 5).

A 76-year-old woman who lived in Jeongseon, Gangwon province, South Korea, was admitted following a 5 day history of fever, chill, and mild headache. The patient did not have any skin rash or suspect tick bite sites. She had no other medical conditions or history of overseas travel, but she had visited a nearby farm 7 days before the onset of illness. Physical examination revealed a fever of 38.8°C, blood pressure 120/80 mm Hg, pulse rate 90 beats/minute, and respiratory rate 16/minute. Results from the initial complete blood count (CBC) were as follows: a hemoglobin concentration of 11.6 g/dL, white blood cell count 2.36 × 10^3/µL (segmented neutrophil 72.8%, lymphocyte 16.3%, monocyte 10.5%), and platelet count 43 × 10^3/µL. Other laboratory findings showed slightly elevated liver enzyme values with aspartate aminotransferase at 80 U/L, alanine aminotransferase at 53 U/L, and alkaline phosphatase at 128 U/L. The concentration of C-reactive protein was notably elevated at 15.35 mg/dL, and procalcitonin was slightly increased at 1.33 ng/dL. Coagulopathy laboratory findings were as follows: activated partial thromboplastin time prolongation at 47.3 seconds,
elevated d-dimer at 4.1 μg/mL, and elevated fibrinogen degradation product at 16.2 μg/mL. These findings suggested an infection with coagulopathy, but we could not specify the patient’s infection focus. Ceftriaxone was administered empirically.

Based on the patient’s history of visits to rural areas and the identification of leukopenia with thrombocytopenia, tick-borne diseases were included in differential diagnosis. EDTA anticoagulated peripheral blood samples were smeared on glass slides. A completely dried smear slide was fixed with methanol for 5 min and allowed to air dry completely. The slide is covered with the Wright’s staining solution for 5 min. The staining procedure is repeated once and the slide is dried for 3 min. After being rinsed with distilled water for 7 min, the slide was washed gently under running tap water and dried. Five infected neutrophils containing intragranulocytic morulae per 100 white blood cells were observed in the blood smear performed on admission day (Fig. 1), thus antibiotic was changed to doxycycline the next day. After 12 hours of doxycycline administration, the patient’s temperature returned to normal. On the second day of doxycycline treatment, CBC results also returned to normal following a temporary lymphocytosis.

The patient’s Anaplasma antibody titer was 1:32 for IgM and <1:80 for IgG measured by the indirect immunofluorescent antibody assay (IFA) commercial kit (Fuller Laboratories, Fullerton, CA) on admission day. The Anaplasma antibody titers determined 2 weeks after the symptom onset were 1:2,048 for IgM and 1:1,280 for IgG. Polymerase chain reaction (PCR) tests detecting *A. phagocytophilum* and *Coxiella burnetii* were performed on genomic DNA from the patient’s blood sample and reverse-transcription PCR to detect Severe Fever and Thrombocytopenia Syndrome Virus (SFTSV) RNA was done on patient’s serum (6, 7, 8). The PCR test for *A. phagocytophilum* was positive (Fig. 2), while the others were negative. PCR
results were available 7 days after the peripheral blood smear that showed morulae in granulocytes.

When determining the cause of the fever of unknown origin, early identification of the causative pathogen is crucial to guide antibiotic therapy. Bone marrow examination is commonly performed in such patients to diagnose the cause (9). Yi et al. (10) conducted a retrospective study on patients who presented with fever and underwent bone marrow examination before the first HGA case was reported in 2013 (4, 10). Excluding patients with clear hematological or microbiological diagnosis, they reviewed 70 patients with blood samples stored at -70°C, and 7.1% (5/70) of them were diagnosed as HGA cases by PCR (10). Twenty percent (1/5) of these cases were fatal, and none had received doxycycline treatment, suggesting that early diagnosis and initiation of appropriate antibiotics are important.

To confirm HGA, PCR, serological, and blood smear tests are usually used (2). While culture is the reference standard, propagation takes more than 10 days and needs biosafety level 3 facilities. Serological tests are important diagnostic methods for HGA, but the sensitivity is not satisfactory because of false negative results in the acute phase and false positive results from cross reactive antigens (2). According to a previous report (11), the specificity of PCR, serology, and blood smear for HGA was close to 1, but sensitivity was lowest for blood smears at 0.21. The sensitivity of serology was 0.32 when HGA was diagnosed with seroconversion or a 4-fold increase in antibody titer in the convalescence phase, while it was 0.58 if HGA was diagnosed when a single antibody titer $\geq 1:256$ was used as a diagnostic criterion. The sensitivity of PCR was 0.79 (11). However, only a few laboratories are capable of performing these tests. By contrast, peripheral blood smear tests can be easily performed in any laboratory, and HGA could be diagnosed by detecting morulae in neutrophils (12). Because the genus
Anaplasma is transmitted to leukocytes from ticks, intracellular morulae present as membrane and luminal features caused by proliferative bacteria (3, 13). The sensitivity of blood smear tests in the diagnosis of HGA is dependent on the experience of the examiner, so the sensitivity of the test is better in experienced centers. Active communication between the laboratory medical department and clinicians is also important for early diagnosis of HGA using the blood smear test. The sensitivity of the blood smear test for detecting intragranulocytic morulae could be improved if the buffy coat of the blood is used for staining, as described for the diagnosis of leishmaniasis (14) or if a thick smear is used (15). Whether buffy coat staining or thick smears improve the sensitivity for diagnosing HGA will be examined in a future study.

Pathogens are constantly emerging, and tests to detect them pose a constant challenge. Although standard diagnosis of HGA is based on serological or PCR tests, this case shows that a conventional peripheral blood smear test can be a useful and fast method for detecting intragranulocytic morulae.

Acknowledgments

Not applicable.

Competing interests

The authors declare no conflict of interest.

References

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**Figure legends**

Figure 1. Intragranulocytic morulae observed in the peripheral blood smear of the patient performed on the day of hospital admission (Wright stain, ×1000).

Figure 2. Phylogenetic tree based on the sequences of (A) 16SrRNA (837bp) and (B) groEL (332 bp) from GenBank and this case (▶). Trees were made by using the neighbor-joining method. Locations (country/province or city), hosts, and GenBank accession numbers are shown. Scale bars indicate (A) 0.005 and (B) 0.02 base substitutions per site. PCR conditions are described in reference 6.
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