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Case Study

Cytopenias and clonal expansion of gamma/delta T-cells in a patient with anaplasmosis: a potential diagnostic pitfall.

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Human granulocytic anaplasmosis is a rare, tick-borne infectious disease caused by *Anaplasma phagocytophilum*. Herein, we report a rare case of human granulocytic anaplasmosis associated with cytopenias and clonal expansion of gamma/delta T-cells in the bone marrow. A 77-year old man presented multiple times to the emergency department complaining of muscle weakness. Complete blood count detected cytopenias and peripheral blood smear showed pseudo Pelger-Huet neutrophils. These findings prompted bone marrow evaluation with ancillary studies including flow cytometry, karyotyping and T-cell rearrangement studies. Careful examination of peripheral blood smear revealed very rare neutrophils with intracytoplasmic inclusions, suggestive of ehrlichiosis/anaplasmosis. Bone marrow evaluation showed dyserythropoiesis, dysmegakaryopoiesis and prominence of hemophagocytic histiocytes. Furthermore, an increased number of T-cells was seen in the bone marrow and flow cytometry showed excess of gamma/delta T-cells, while T-cell rearrangement studies detected a T-cell clone. Serologic evaluation confirmed the diagnosis of anaplasmosis. This case nicely illustrates hematologic sequelae of infection with *Anaplasma* and potential diagnostic pitfalls, such as myelodysplastic syndrome and T-cell lymphoproliferative disorder. To our knowledge, this is the first reported case of clonal expansion of gamma/delta T-cells associated with anaplasmosis. Pathologists should be careful and vigilant when screening peripheral blood smears, as they are often the first to raise the suspicion of anaplasmosis. 〔*J Clin Exp Hematop 56(3):160-164, 2017*〕

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INTRODUCTION

Human granulocytic anaplasmosis (HGA), previously known as human granulocytic ehrlichiosis, is an emerging, but frequently underreported infectious disease that is caused by a tick-borne, obligate intracellular bacterium, *Anaplasma phagocytophilium*.[1] Anaplasmosis is most frequently reported from the upper Midwest and northeastern United States (US), although cases have been reported in Europe and Asia.[1, 2] In the average pathology practice, cases of anaplasmosis are very rarely encountered. However, pathologists are often instrumental for raising the suspicion of this infectious disease. Therefore, careful and vigilant screening of peripheral blood smears is required.

Herein, we present a rare case of a patient with anaplasmosis associated with cytopenias and clonal expansion of gamma/delta T-cells in the bone marrow.

CASE REPORT

A 77-year-old male from rural southeastern Manitoba, Canada (close to the border with the US) presented multiple times to the emergency department over a two-month period, complaining of muscle weakness, particularly leg weakness with history of multiple falls. He was febrile during several encounters in the emergency department with temperature ranging from 37.3 to 38.9 degrees Celsius. There was no rash. The patient's physical examination was unremarkable and he denied any recent travels. The complete blood count was performed and showed marked thrombocytopenia (platelets - 26x10E9/L; normal range, 140-440x10E9/L), low normal hemoglobin (141 g/L; normal range, 140-180 g/L), and mild leukopenia (white blood cells - 3.91x10E9/L; normal range, 4.5-11x 10E9/L) with mild lymphocytopenia (absolute

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lymphocyte count - 1.13x10E9/L; normal range, 1.3-3.2x10E9/L) and mild monocytopenia (absolute monocyte count - 0.12x10E9/L; normal range, 0.3-0.8x10E9/L). Absolute reticulocyte count was 10x10E9/L (normal range, 20-100x10E9/L). Other significant laboratory findings included elevated creatine kinase (2532 U/L; normal range, 52-175 U/L), elevated plasma myoglobin (232 ug/L; normal range, <50 ug/L), and elevated lactate dehydrogenase (993 U/L; normal range, 120-230 U/L). Due to persistent cytopenias, peripheral blood smears were sent for review and bone marrow aspiration and biopsy were performed.

Peripheral blood and bone marrow findings

Peripheral blood and bone marrow aspirate smears were stained with Wright-Giemsa. Significant findings on the blood smear included decreased number of platelets with some large platelets and occasional reactive-appearing lymphocytes. Furthermore, the neutrophils showed numerous hypolobated forms, including pseudo Pelger-Huet neutrophils. Very rare neutrophils showed intracytoplasmic inclusions (morulae-like structures), suggestive of ehrlichiosis/anaplasmosis (Figure 1A).

Bone marrow was mildly hypercellular for age showing the cellularity of 40-50%. Aspirate smears revealed mild dyserythropoiesis with megaloblastoid changes and binucleation of red blood cell precursors. Dyspoiesis was also seen in megakaryocytes that showed some large hyperlobated forms and separation of nuclear lobes (Figure 1B). Less that 10% of cells in erythroid lineage and less than 10% of megakaryocytes showed dyspoietic changes. Other significant finding included increased number of hemophagocytic histiocytes (Figure 1C), but without evidence of microorganisms. Bone marrow core biopsy showed increased number of small mature lymphocytes. Immunohistochemical studies were performed on the bone marrow core and showed significantly increased CD3-positive T- lymphocytes, scattered and in small clusters (Figure 1D).

Flow cytometry

Five-color flow cytometry analysis was performed and detected 11% lymphocytes, of which 88% were T-cells. A predominant T-cell population detected (78% of lymphocytes) was positive for CD3, CD2, CD5, CD7 (dim), and TCR gamma/delta, and was negative for CD4, CD8, and TCR alpha/beta, consistent with expansion of gamma/delta T-cells (Figure 2A).

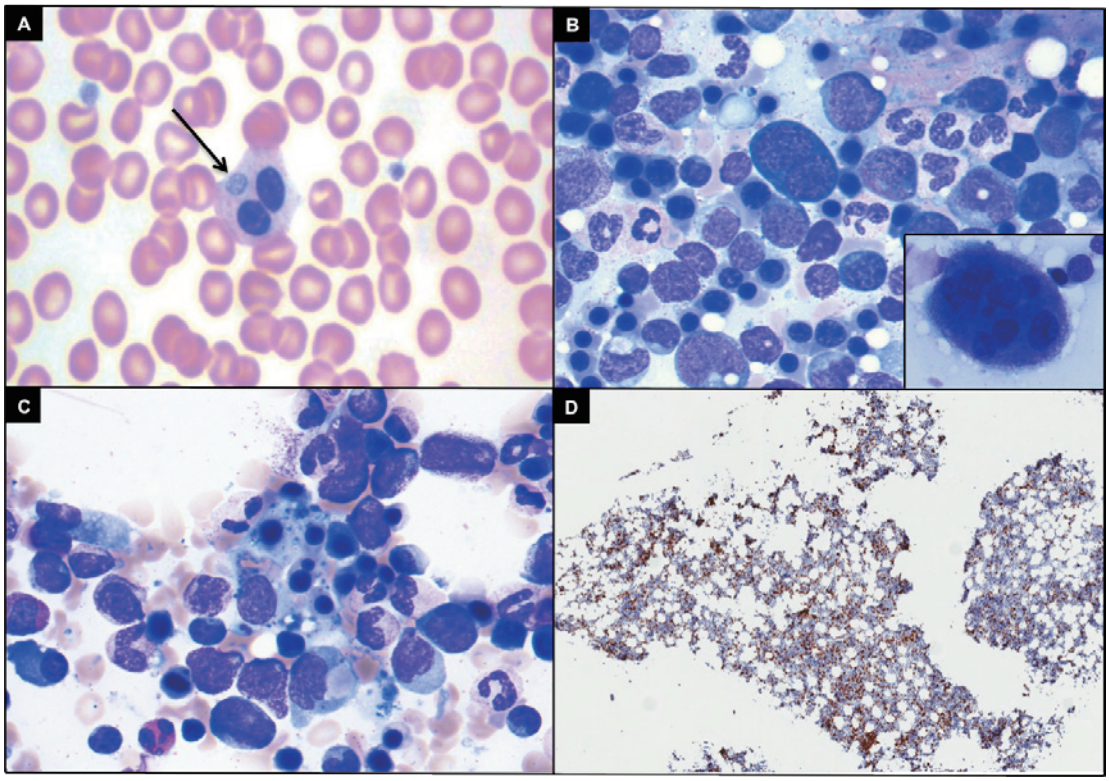

Figure 1. A – Pseudo Pelger-Huet neutrophil with intracytoplasmic morula - arrow (Wright-Giemsa x1000); B – Bone marrow aspirate showing megaloblastoid and binucleated red blood cell precursors; Inset – large megakaryocyte with separation of nuclear lobes (Wright-Giemsa x1000); C – Bone marrow aspirate showing hemophagocytic histiocytes (Wright-Giemsa x 1000); D – Bone marrow core showing increased number of CD3 positive T-cells (Immunohistochemistry x40)

Chromosome analysis and T-cell rearrangement studies

The bone marrow cells were harvested after 24-hour culture, and the karyotypes were analyzed following the standard protocols. Karyotypes were recorded according to the International System for Human Cytogenetic Nomenclature.[3] Conventional cytogenetic analysis showed a normal male karyotype of 46, XY[20].

T-cell receptor β and γ rearrangements were studied by polymerase chain reaction (PCR) and capillary electrophoresis of fluorescently labeled PCR products, using standardized kit (In VivoScribe, Technologies, San Diego, CA) and showed a clonal population (Figure 2B).

Clinical follow-up

Due to the presence of possible morulae in the patient's neutrophils, serological testing for *Anaplasma* was performed and showed a positive result. When asked explicitly about possible exposure, the patient did recall being bit by a tick a couple of weeks prior to the onset of the muscle weakness. The patient was treated with doxycycline (100 mg bid) and improved dramatically shortly after initiation of treatment. One month later, follow-up blood work showed normal platelet count (257x10E9/L), as well as normalized values of creatine kinase, myoglobin and lactate dehydrogenase. Follow-up bone marrow and T-cell clonality studies were not performed in this patient.

DISCUSSION

Anaplasmataceae is a diverse family of obligate intracellular bacteria that includes the genera *Ehrlichia* and *Anaplasma*.[1, 2] The genus *Anaplasma* consists of the human granulocytic agent, *Anaplasma phagocytophilum* which causes HGA.[1] This tick borne infectious disease is transmitted through the tick vector *Ixodes scapularis* in the eastern and midwestern US, *I. pacificus* in the western US, and *I. ricinus* in Europe.[2] Although rare in Canada, HGA has been described in many parts of the US and Europe.[1]

Most patients with HGA present with an acute illness (including high fever, malaise, myalgia, headache, and chills), approximately 12 days after the tick bite.[4] Our patient presented to the emergency department with muscle weakness, but no fever. Examination of the peripheral blood showed thrombocytopenia, lymphocytopenia and pseudo Pelger-Huet neutrophils. The presence of unexplained cytopenias in an elderly male, and the morphology of neutrophils, prompted a bone marrow examination to evaluate the patient for myelodysplastic syndrome (MDS). Upon closer

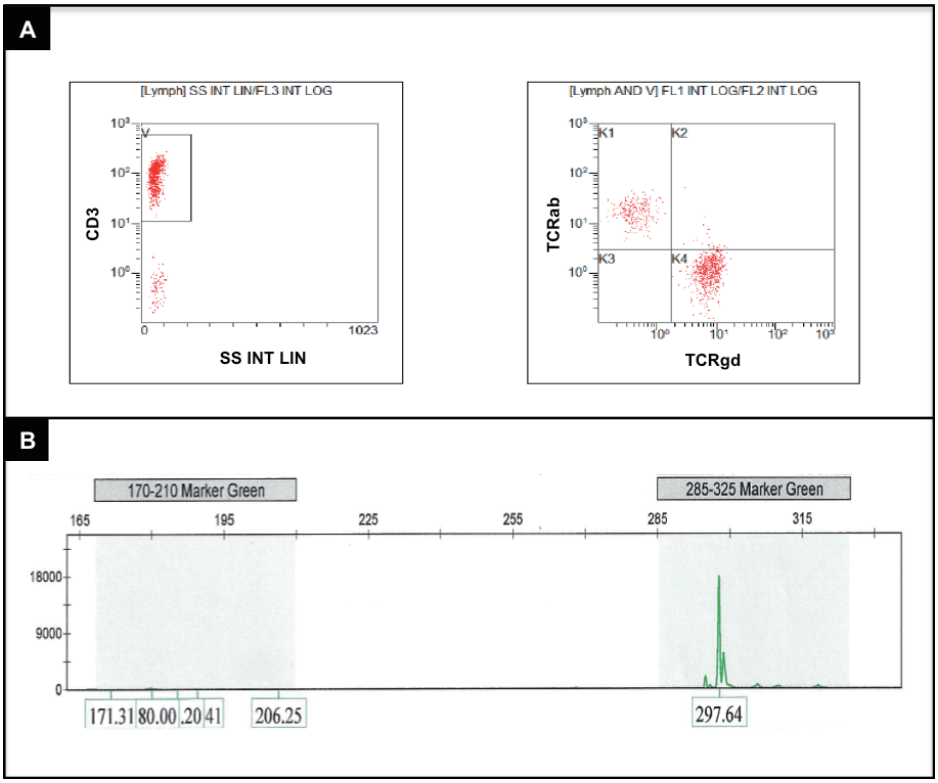

Figure 2. A – Flow cytometry of the bone marrow. Expansion of CD3-positive gamma/delta T-cells. B - T-cell receptor gene rearrangement study using BIOMED-2 primers shows a large clonal peak in the Beta C panel

examination of the peripheral blood, a very rare pseudo Pelger-Huet neutrophils had intracytoplasmic inclusions which raised the possibility of ehrlichiosis/anaplasmosis. Cytopenias, and especially thrombocytopenia is a common manifestation of all tick-borne diseases, but its pathogenesis is not completely understood. Multiple mechanisms are likely to contribute to thrombocytopenia in these patients and include decreased platelet production (e.g. decreased number of megakaryocytes, ineffective formation of platelets), hypersplenism, immune platelet destruction, non-immune platelet consumption (e.g. disseminated intravascular coagulation), and direct infection of platelets, as seen in *Ehrlichia* infection.[1,5] Our patient did not have splenomegaly on clinical examination and further testing to assess for the immune-mediated process was not done. Furthermore, during the course of infection, nearly all patients with HGA develop lymphopenia; however, after initiation of antibiotic therapy, the lymphopenia recovers, and a lymphocytosis usually ensues.[4] Bone marrow examination in our patient showed mild dyserythropoiesis and dysmegakaryopoiesis. Although pseudo Pelger-Huet neutrophils and dyspoietic changes in the bone marrow, in a patient with pancytopenia, should definitely raise the possibility of MDS, it is important to exclude reactive causes, including infection, which can also cause the similar changes.

One of the most striking findings in the bone marrow of our patient was the presence of hemophagocytic histiocytes. Hemophagocytic lymphohistiocytosis (HLH) is an aggressive, life-threatening syndrome of excessive inflammation and tissue destruction due to abnormal immune activation and an exaggerated inflammatory response (due to a lack of normal downregulation of activated macrophages and lymphocytes).[6, 7] The diagnosis of systemic HLH syndrome is based on clinical and laboratory criteria. The current diagnostic criteria for the diagnosis of systemic HLH syndrome require meeting any 5 of 8 criteria that include fever, splenomegaly, bicytopenia, hypertriglyceridemia or hypofibrinogenemia, hemophagocytosis, low/absent NK-cell activity, hyperferritinemia, and high soluble interleukin-2-receptor levels.[8] Our patient did not meet 5 criteria for the HLH diagnosis. Hemophagocytic lymphohistiocytosis can occur as a familial or sporadic disorder, and it can be triggered by disruption of normal immune homeostasis (e.g. infections, autoimmune disorders, lymphomas). The initiating trigger for an acute episode, in patients with a genetic predisposition and in sporadic cases, is often the immune activation by an infection.[7] The majority of infection induced HLH is caused by viral infections, most commonly of the Herpesviridae family (Epstein-Barr virus, cytomegalovirus, etc.), as well as other types of viruses (e.g. Human Immunodeficiency Virus, influenza).[7] Only a few cases of *Ehrlichia* and *A. phagocytophilum*-induced HLH have been reported.[6, 9]

To our knowledge, our case is the first reported case of clonal expansion of gamma/delta T-cells associated with anaplasmosis. Less than 5% of all normal T-cells express the gamma/delta T-cell receptor and are usually restricted to the splenic red pulp, intestinal epithelium, and other epithelial sites. Gamma/delta T-cells can be associated with certain infections, such as intracellular infections, including viral (e.g. Epstein-Barr virus), parasitic (e.g. malaria, leishmaniasis), and bacterial (e.g. listeriosis).[10] Caldwell, *et al*.[10] previously reported clonal expansion of gamma/delta T-cells in human ehrlichiosis, but reasons for its occurrence are presently unclear. It is paramount not to confuse the possibility of an intracellular infection with lymphoma. Clonal expansion of gamma/delta T-cells does not automatically equate to a T-cell lymphoproliferative disorder, and possibility of an infectious etiology must be considered.

In summary, the morphological findings in our case were concerning for MDS and HLH, and clonal expansion of gamma/delta T-cells raised the differential diagnosis of a T-cell lymphoproliferative disorder. However, the summation of the clinical history, presence of morulae in neutrophils, and serological testing all supported the diagnosis of HGA. Diagnosis of myelodysplastic syndrome or T-cell lymphoproliferative disorder should not be made until reactive/secondary causes are ruled out. Correlation with clinical and cytogenetic findings is critical in these cases. This rare case of anaplasmosis nicely illustrates hematologic sequelae of this infection and potential diagnostic pitfalls.

CONFLICT OF INTEREST

The authors disclose no potential conflicts of interest.

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