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LETTER TO THE EDITOR

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Importance of the hematology laboratory in infectious disease diagnosis by morphology: Four educational case studies

Dear Editors,

With expanding global mobility and advances in medical knowledge with increased vulnerable populations, the rapid diagnosis of infectious disease has never been more critical. To illustrate the important role the hematology laboratory can provide, four educational cases are described with emphasis on morphologic diagnosis. Morphological interpretation provides a rapid and unique perspective which supplements other diagnostic investigations. The cases are organized by diagnosis based on peripheral blood film (case 1), bone marrow aspirate (case 2), bone marrow aspirate and biopsy (case 3), and bone marrow biopsy (case 4).

1 | CASE 1. *PLASMODIUM FALCIPARUM* INFECTION IN A PATIENT SUSPECTED FOR HAVING EBOLA INFECTION

An HIV-positive man in his 60s presented with fever, malaise, vomiting, and confusion upon return from Sierra Leone where an ongoing Ebola outbreak was occurring and infection with Ebola virus was suspected. A peripheral blood film was made in a biosafety cabinet with appropriate personal protective equipment, fixed in 100% methanol for 5 minutes, followed by complete immersion of the slide in 10% buffered formalin for 15 minutes for viral inactivation, and then stained with Wright-Giemsa stain. The blood film showed ring forms compatible with *Plasmodium falciparum* (Figure 1A), with a parasitemia level of 20%. Testing for Ebola virus was canceled once the diagnosis of severe malaria was made. The patient was treated for severe *Plasmodium falciparum* with intravenous artesunate and recovered. It is recommended that all handling of laboratory specimens be performed in a biosafety cabinet, Level 3 (Figure 1B), which includes small instruments for basic hematology, coagulation, and biochemistry testing placed within the biosafety cabinet.[1]

1.1 | Educational message

Healthcare professionals should be suspicious of possible Ebola virus infection in persons with compatible signs and symptoms, including but not limited to fever, body aches, weakness, vomiting, diarrhea, bleeding, and an epidemiologic risk factor within 21 days before the onset of symptoms.[2] Healthcare providers must be cognisant that many patients suspected of having Ebola often have other infections, such as malaria or influenza, and it is important to quickly investigate for malaria in patients with possible Ebola infection, either with peripheral blood film or using rapid diagnostic testing (RDT).[3]

2 | CASE 2. BIOLOGICS FOR INFECTION-RELATED HEMOPHAGOCYTOSIS

A previously healthy 19-year-old male was admitted to hospital with fulminant hepatitis in the context of acute infectious mononucleosis with positive Monospot and EBV-IgM antibody. Initial laboratory work-up showed increased liver enzymes and ferritin levels, elevated INR and PTT, high triglycerides, low fibrinogen, and pancytopenia. The bone marrow aspirate showed granulocytic and megakaryocytic hyperplasia and hemophagocytosis (Figure 2A,B) compatible with reactive changes due to underlying infection-induced hemophagocytic lymphohistiocytosis (HLH). Testing for soluble IL-2 receptor was not performed, as there were already sufficient criteria for HLH diagnosis and due to long turnaround time for this send out test. He was started on intravenous Acyclovir, an antiviral drug, and aggressive supportive management, with minimal improvement. On the 4th day of his admission, a single dose of 400 mg intravenous infliximab, a TNF inhibitor, was administered. An immediate good clinical response was seen with defervescence and resolution of all clinical and laboratory manifestations of liver failure, along with improvement of other laboratory values including platelet count recovery to normal and ferritin level reduction (Figure 2C,D). His follow-up visits over the following 8 years confirm that he recovered fully and is in good health. Testing for primary HLH mutations was negative for STX11, RAB27A, PRF1, STXBP2, and BIRC4 mutations. Test for X-linked mutations associated with fulminant infectious mononucleosis with hemophagocytosis (SH2D1A and XIAP mutations) was not performed.[4]

Hemophagocytic lymphohistiocytosis is characterized by hyperinflammatory cytokine storm and multiorgan involvement. HLH

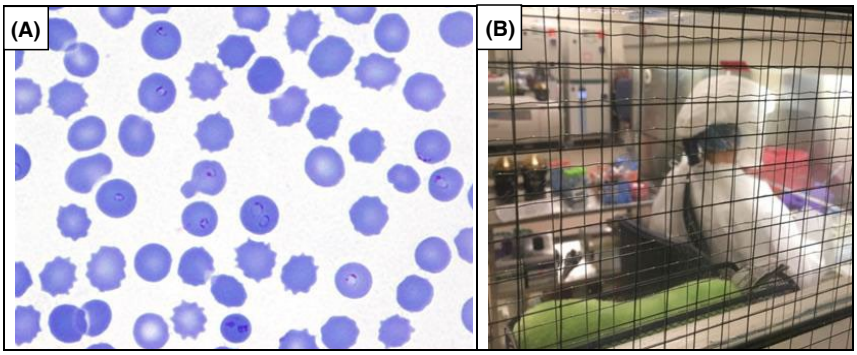

FIGURE 1 Peripheral blood and microbiology laboratory from Case 1. A. Methanol/Formalin fixed peripheral blood film with morphology typical for *Plasmodium falciparum*, with high parasitemia level composed only of delicate ring forms, including one red cell containing two ring forms and absence of maturing trophozoites or schizonts. (Wright-Giemsa, 60x). B. Biosafety cabinet in microbiology laboratory ready for laboratory investigation of possible Ebola virus

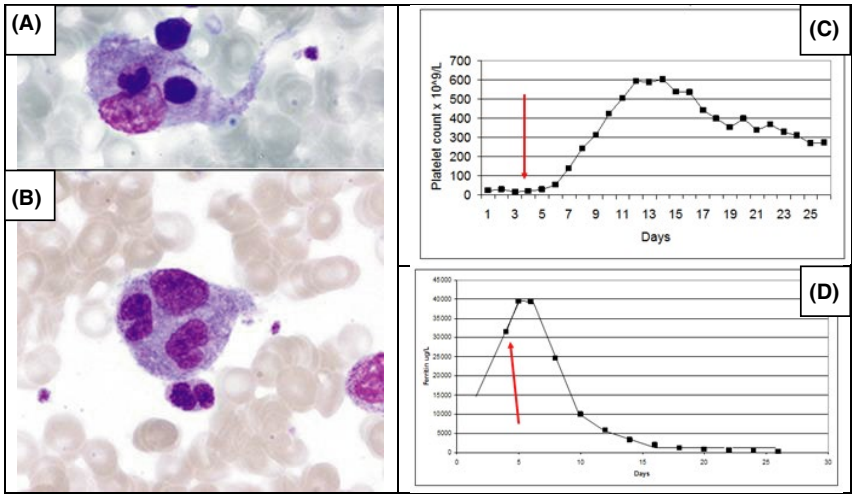

FIGURE 2 Bone marrow aspirate morphology and response of serological markers to infliximab from Case 2. A and B. Bone marrow aspirate done on the 2nd day of admission showing hemophagocytosis (Wright-Giemsa, 100x). C. Trend of platelet counts before and after infliximab-red arrow indicates infliximab use. D. Trend of Ferritin levels before and after infliximab-red arrow indicates infliximab use

may be due to an inherited mutation or may be acquired. Diagnosis is made by demonstrating an HLH-associated gene defect and/or meeting the specific clinical and laboratory criteria set by HLH-2004 Diagnostic Guidelines.[5] Treatment options are based on the type of HLH and include immunoglobulins, steroids, cyclosporine A, and etoposide according to HLH-94 and HLH-2004 treatment protocols. Anticytokine treatment options have been published over the years but there has been no consensus agreement on this type of treatment.[6] Secondary hemophagocytic syndrome has been associated with the use of anti-TNF-α in the treatment of inflammatory bowel disease.[7]

2.1 | Educational message

Bone marrow aspirate is an accessible way to provide tissue to look for hemophagocytosis in patients with pancytopenia and infection. Hemophagocytic syndrome and multiorgan failure are rare but well recognized complication of infectious mononucleosis and may respond to biologics.

3 | CASE 3. ANEMIA AFTER CHEMOTHERAPY: UNEXPECTED PARVOVIRUS INFECTION IN AN IMMUNOCOMPROMISED PATIENT

A female patient in her 50s with precursor B-acute lymphoblastic leukemia (B-ALL) underwent induction chemotherapy. The platelets and neutrophils recovered to normal, and the hemoglobin recovered to near normal, but the patient then unexpectedly developed a significant normocytic anemia with hemoglobin of 81 g/L (N 115-155), RBC 2.72 × 10^{12}/L (N 3.50-5.00), hematocrit 0.227 L/L (N 0.380-0.500), MCV 83.5 fL (N 80-100) with marked reticulocytopenia, absolute reticulocyte count of 2.8 × 10^{9}/L (N 22-92). The bone marrow aspirate demonstrated persistent B-ALL, with 40% blasts, and paucity of red cell precursors was also noted. Very rare giant proerythroblasts were identified in the bone marrow aspirate as well as two mononuclear cells with refractile nuclear inclusions in the bone marrow biopsy (Figure 3A-C). Immunohistochemical stain for parvovirus highlighted the nuclear inclusions (Figure 3D). A diagnosis of persistent B-ALL and

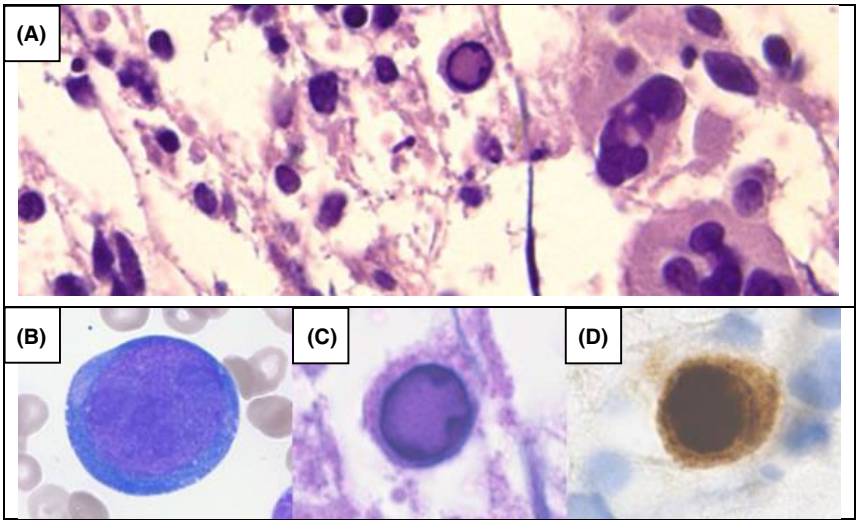

FIGURE 3 Bone marrow morphology from Case 3. Proerythroblasts with parvovirus nuclear inclusions in: A. bone marrow biopsy (H&E, 40x), B. bone marrow aspirate (Wright Giemsa, 100x), C. bone marrow biopsy (H&E, 100x), and D. bone marrow biopsy (Parvovirus immunohistochemistry, 100x)

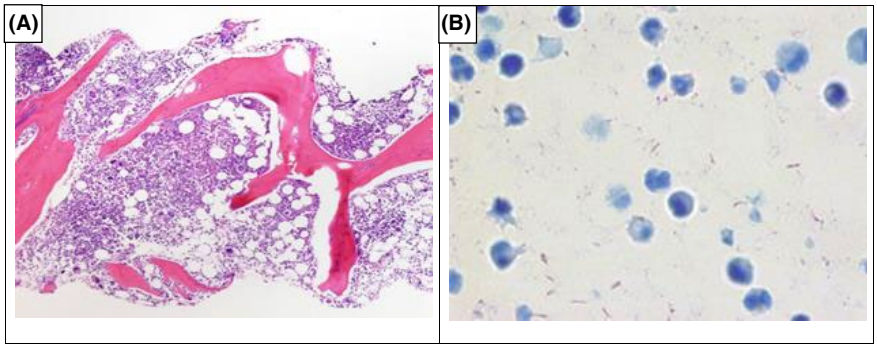

FIGURE 4 Bone marrow morphology from Case 4. A. Bone marrow biopsy showing trilineage hematopoiesis, normocellular for age. No granuloma and no necrosis identified. (H&E, 4x). B. Ziehl-Neelsen stain of bone marrow biopsy demonstrating acid fast bacilli, approximately 4um in length, and with some appearing beaded. (100x)

concurrent parvovirus infection was made. Parvovirus DNA and IgG antibodies were detected in the blood, but serology for IgM antibodies was negative. The patient had a desquamating rash which in retrospect was attributed to parvovirus infection. The parvovirus infection was treated with IVIG 0.4 g/kg (30 grams) once a week for 4 weeks to prevent relapse in this immunocompromised patient.[8] Recovery of the hemoglobin level (122 g/L) and red cell indices (RBC 4.24 × 10^{12}/L, hematocrit 0.348 L/L) and a mildly increased reticulocyte count (145 × 10^{9}/L) were demonstrated 6 weeks after starting IVIG.

Human parvovirus B-19 is a small, single-stranded, nonenveloped DNA virus with tropism for erythroid progenitor cells in the marrow. Parvovirus infection has various manifestations, and in patients with underlying hematological disorders can cause transient aplastic crisis with severe anemia. Parvovirus infection can also have various other inflammatory sequelae and extra-hematological manifestations.[9] A high index of suspicion is required when examining a bone marrow with anemia and marrow erythroid hypoplasia/aplasia as serology can be negative.[10] Marrow infection is characterized by giant proerythroblasts, 25-45 μm in diameter with increased cytoplasm and 1-3 viral nuclear inclusions. The bone marrow biopsy can demonstrate scattered infected erythroid precursors with glassy refractile nuclear inclusions, "lantern cells" which can be highlighted by nuclear immunohistochemical stain against parvovirus.

3.1 | Educational message

Immunocompromised patients with anemia, reticulocytopenia, and erythroblastopenia should trigger a careful search for morphologic features of parvovirus infection, including immunohistochemistry for parvovirus antigen.

4 | CASE 4. BONE MARROW EXAMINATION FOR FEVER OF UNKNOWN ORIGIN (FUO): MILIARY TUBERCULOSIS DEMONSTRATED BY ZIEHL-NEELSEN STAIN

A 19-year-old Inuk female from Nunavut presented in the second trimester of pregnancy with fever, weight loss, and jaundice. CBC showed anemia, hemoglobin 78 g/L (N 115-155), elevated WBC with neutrophilia 24.1 × 10^{9}/L (N 2.0-7.5), and thrombocytosis, platelet count 857 × 10^{9}/L (N 125-400). The lungs were unremarkable by chest radiograph. Cultures for tuberculosis (blood, urine, bronchoalveolar lavage, marrow aspirate) were negative. Bone marrow biopsy showed normocellular trilineage hematopoiesis, and no granulomas and no necrosis (Figure 4A). Ziehl-Neelsen stain demonstrated scattered acid fast bacilli in the marrow (Figure 4B). The patient showed dramatic clinical improvement after the initiation of quadruple therapy for tuberculosis (rifampicin, isoniazid,

pyrazinamide, ethambutol). The patient had premature rupture of membrane at 31 weeks gestation and a healthy baby was delivered by urgent caesarian section. Culture of the placenta for tuberculosis was negative.

Tuberculosis (TB) incidence among Inuit in Canada is approximately 300 times higher than the Canadian born non-Indigenous population (170 vs 0.5 cases per 100 000).[11] Miliary TB accounts for approximately 1% of all TB.[12] Maternal and fetal outcomes are commonly poor if TB develops during pregnancy.[13] This case illustrates the need to take into account the known local TB epidemiology regarding high risk groups when investigating cases. This case also highlights that prevention of TB is paramount, with national and global strategies to end the tuberculosis epidemic.

One of the indications for bone marrow examination is evaluation for fever of unknown origin (FUO).[14] In the work-up of FUO, it is important to obtain bone marrow aspirate for microbiology culture, including aerobic and anaerobic bacteria, fungus, and mycobacteria by placing 1-2 mLs in each of anaerobic media bottle, aerobic media bottle, and fungus/mycobacterium culture bottle (BACTEC MYCO/F Lytic (Becton Dickinson Diagnostic Instrument Systems). Dissemination of *Mycobacterium tuberculosis* throughout the body (miliary tuberculosis) may occur in immunocompromised patients, either with primary infection or from reactivation from a latent focus from prior infection. Although granuloma formation and necrosis are typical for tuberculosis infection, they are not always present[15]; thus, special stains for acid fast bacilli such as Ziehl-Neelsen are indicated on marrow biopsies performed for work-up of FUO. Miliary tuberculosis is one of the rare causes of morning temperature spike (along with typhoid fever and periarteritis nodosa). It should be noted that spurious diagnosis of tuberculosis can arise due to atypical or nontuberculous mycobacteria contaminating water sources used in pathology processes.[16]

4.1 | Educational message

Bone marrow biopsy in immunocompromised patients may lack well-formed granulomas, and special stains for acid fast bacilli should be considered in the appropriate clinical context.

5 | CONCLUSION

These four educational cases illustrate the importance of the hematology laboratory in the diagnosis of infectious disease. Morphologic features of infection should be sought in samples from patients with increased infection risk, such as geographical location and/or immunosuppression, and followed up with special stains, microbial cultures, molecular testing, serological testing, or other special techniques where indicated.

KEYWORDS

bone marrow, infection, morphology

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CONFLICT OF INTERESTS

Nothing to disclose.

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