rounds [cytology | microbiology/virology | immunology | hematology | histology]

Human Parvovirus B19 in the Bone Marrow With Negative Viral Serologic Results

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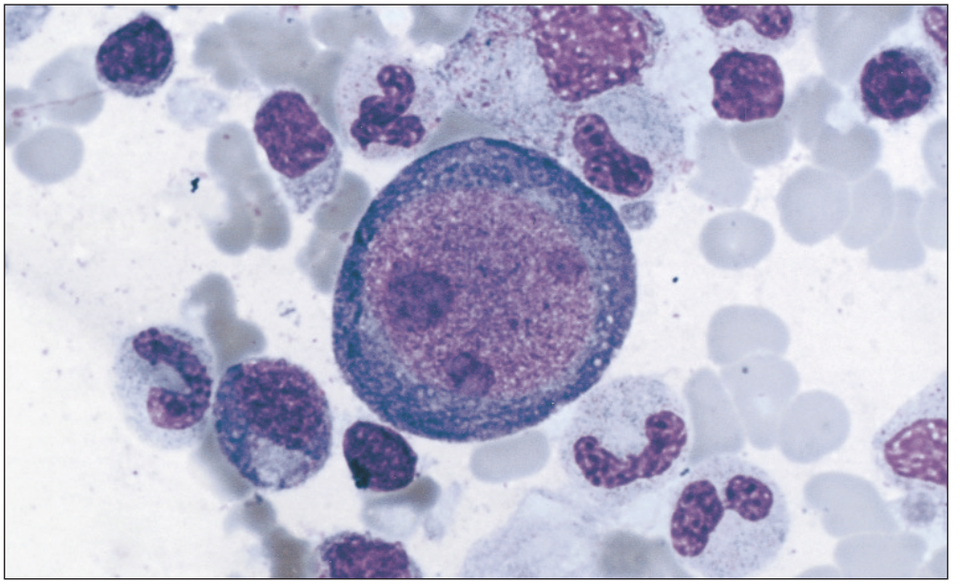
- Human parvovirus B19
- Tests to diagnose parvovirus B19 infection
- Persistent parvovirus B19 infection and suppressed serologic response
- Identification of inclusions in the erythroid precursors to diagnose active infection and confirmation with polymerase chain reaction for viral DNA



[I1] Giant proerythroblast with intensely blue, vacuolated cytoplasm; compact nuclear chromatin pattern; and multiple, distinct, large purple inclusions in the nucleus (Wright-Giemsa, oil immersion, ×1,000).

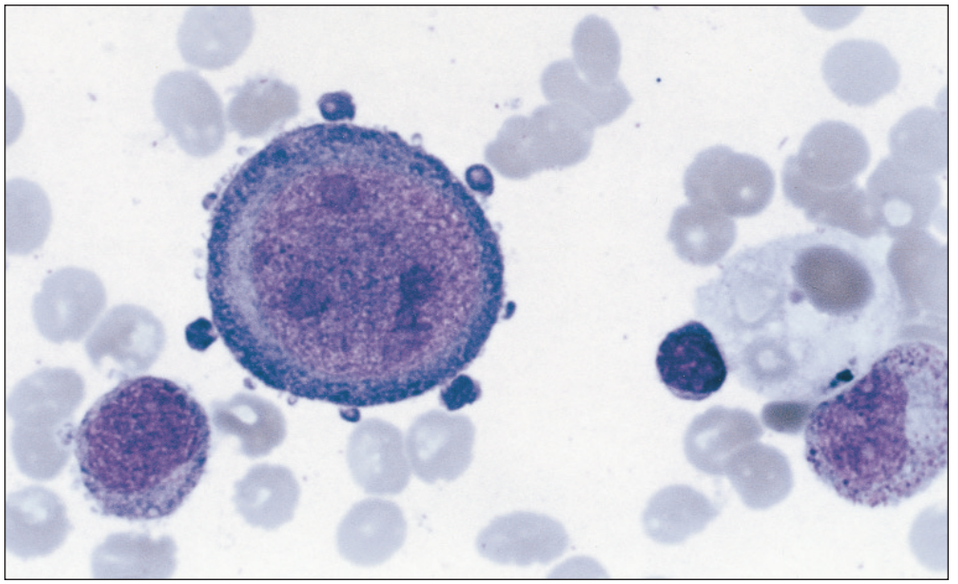
Case Presentation

A 28-year-old man with a history of alcohol and intravenous drug use presented with delirium tremens, fever, and progressive anemia. He had numerous tattoos. Physical examination revealed marked pallor. No organomegaly was identified.

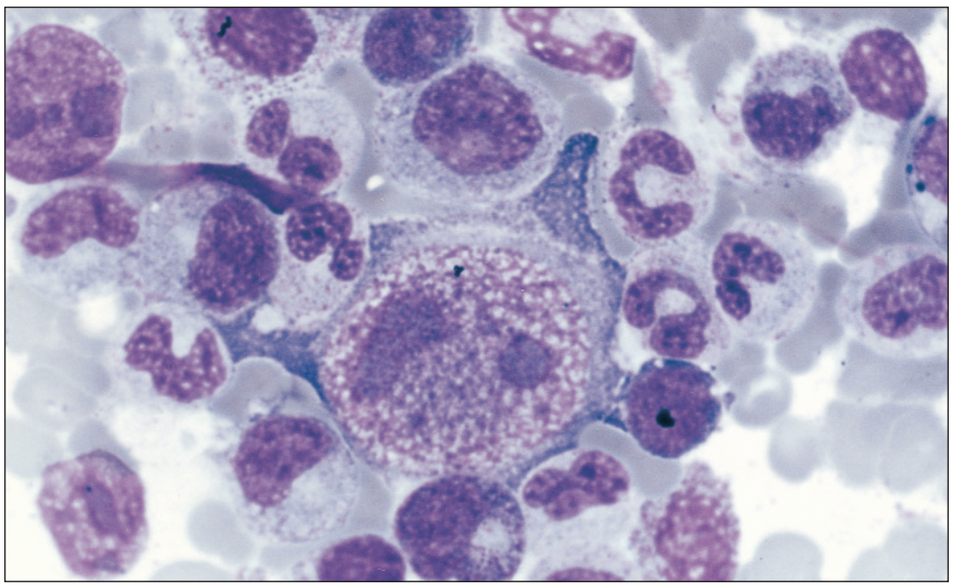
CBC count revealed hemoglobin, 10.3 g/dL (103 g/L); WBC, 800/µL (0.8 × 10^9/L); and platelet count, 14 × 10^3/µL (14 × 10^9/L). The WBC differential was as follows: 41% (0.41) neutrophils; 48% (0.48) lymphocytes; 6% (0.06) monocytes; 4% (0.04) eosinophils; and 1% (0.01) basophils. The reticulocyte count was less than 0.1% (0.001). Blood cultures were negative, and radiologic studies revealed no significant abnormality. No HIV-1 or HIV-2 antibodies were detected, and HIV-1 DNA by polymerase chain reaction (PCR) was negative. The CD4 count was 1,300/µL (1.3 × 10^9/L).

A bone marrow aspirate and biopsy was performed to evaluate the possible causes of pancytopenia. The marrow was hypocellular for the patient's age and showed a red cell aplasia (myeloid/erythroid ratio of 10.8:1). Numerous giant proerythroblasts were present in the aspirate, with a high nuclear/cytoplasmic ratio; a narrow rim of intensely blue, vacuolated cytoplasm; compact uncondensed nuclear chromatin pattern; and multiple, distinct, large purple inclusions in the nucleus [I1]. The large size of the proerythroblasts could be appreciated by comparison with adjacent neutrophils. Some of the early giant proerythroblasts showed multiple small cytoplasmic projections called "pseudopodia" or "dog ears" [I2]. Intermediate giant proerythroblasts having frayed cytoplasm with decreased basophilia and coarse nuclear chromatin with a large inclusion were also identified [I3]. This cell was larger than the early giant proerythroblast shown in [I1] and [I2].

In combination with pure red cell aplasia, these changes in erythroid precursors are highly suggestive of infection with human parvovirus B19. DNA analysis using PCR for human parvovirus B19 DNA was performed, and the results were positive. However, the IgG and IgM results for human parvovirus B19 were negative. The patient received intravenous immunoglobulins



[I2] Early giant proerythroblasts showing multiple small cytoplasmic projections called "pseudopodia" or "dog ears" (Wright-Giemsa, oil immersion, ×1,000).



[I3] Intermediate giant proerythroblasts with frayed cytoplasm with decreased basophilia and coarse nuclear chromatin with a large inclusion (Wright-Giemsa, oil immersion, ×1,000).

for parvovirus B19. He left against medical advice 3 days later.

Clinical Background

Human parvovirus B19 is a small, nonenveloped, single-stranded DNA virus, classified in the family Parvoviridae, the subfamily Parvovirinae, and genus *Erythrovirus*. Parvoviruses are icosahedral particles between 20 and 25 nm in diameter containing 60 copies of the structural proteins. Virion DNA contains 5,596 nucleotides and has identical inverted terminal repeat sequences at each end that permit self-priming. Nine mRNA species have been identified in B19-infected cells.[1]

Human parvovirus B19 is known to cause erythema infectiosum (fifth disease), polyarthritis, aplastic crisis in various hemolytic anemias, prolonged erythroid suppression in immunodeficient patients, and fetal death in many cases of vertical transmission.[2,3] It is cytotoxic to erythroid progenitor cells in vivo and in vitro. It is known to enter the red cell precursors through the blood group P antigen.[4]

The presence of pure red cell aplasia and full spectrum of characteristic giant proerythroblasts in the bone marrow are pathognomonic of parvovirus B19 infection.[5] The pathogenesis of the giant proerythroblasts and mechanism of erythroid hypoplasia in parvovirus B19 infection are not fully understood. Induction of apoptosis has been suggested due to the presence of cytoplasmic blebs; a direct cytopathogenic lytic effect of the virus on erythroid precursors has also been suggested.[5]

In immunocompetent patients, the infection typically resolves in 2 to 3 weeks with the production of IgG that neutralizes the virus infectivity for erythroid host cells.[6] Persistent or recurrent parvovirus B19 infection can be associated with suppressed serologic response. By the age of 30 years, 30% to 60% of the normal adult population is estimated to have IgG antibodies against parvovirus B19.[7] Immunocompetent hosts rarely remain ill due to this viral infection,[8] but in immunodeficient patients, the virus usually persists for prolonged periods[2] and can lead to chronic anemia.[9]

In persistently infected patients, an aberrant immune response can result in failure to produce IgG and IgM antibodies.[2] Identification of characteristic inclusions in the erythroid precursors is diagnostic of active infection. Recognition of these changes and confirmation with PCR for viral DNA will hasten appropriate therapy. This case represents the characteristic and unique morphologic features of erythroid precursors in parvovirus B19 infection with nonreactive serology in an immunocompetent host.

Role of the Laboratory

IgG and IgM antibodies specific for parvovirus B19 can be measured by indirect immunofluorescence antibody (IFA) technique or enzyme immunoassay (EIA). For IFA, the patient's serum is incubated with parvovirus B19 recombinant VP1 antigen in insect cells stabilized on a glass slide. In the IgM

test, specimens are pretreated to prevent interference with rheumatoid factors and to reduce IgG competition. If anti–parvovirus B19 antibodies are present in the specimen, a stable complex is formed with the antigen. Bound antibody then reacts with a fluorescein-labeled anti–human IgG or IgM antibody, and this 3-part complex is visualized with the aid of a fluorescence microscope.[10,11] The presence of IgM-class antibodies indicates recent infection. The presence of IgG antibodies is consistent with previous exposure. A negative test result for both IgG and IgM antibodies indicates the absence of antibodies to parvovirus.

Our patient had a negative test result for IgG and IgM antibodies, but the presence of red cell aplasia combined with typical changes in erythroid precursors prompted PCR testing for parvovirus B19 DNA. Positive results confirmed infection with the virus. The PCR was performed in a reference laboratory with the method described by Rogers et al.[12] Confirmation of parvovirus B19 infection can also be performed by immunohistochemistry.

Conclusion

Bone marrow changes, which include pure red cell aplasia and presence of giant proerythroblasts, are key to the diagnosis of human parvovirus infection. Immunocompetent hosts can also have negative IgG and IgM antibodies. In such cases, infection can be confirmed by PCR for viral DNA.

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interpretation [management/administration and training | generalist]

A Four-Part Approach to Competency Assessment

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- Competency programs must assess all core competencies and address employee preparation, theoretical testing, practical examination, and postevaluation follow-up.
- Competency programs must be consistently applied to both technical and nontechnical staff and should take an educational rather than a disciplinary approach.

Clinical Laboratory Improvement Amendments (CLIA) '88 mandate that clinical laboratories administer competency testing to all employees annually. Specifically, the amendments state, "The laboratory must have an ongoing mechanism to evaluate the effectiveness of its policies and procedures for assuring employee competence and, if applicable, consultant competence."[1]

New employees must be assessed prior to 6 months of service, and all employees must be assessed at least annually. Under CLIA '88, Health Care Financing Administration does not recommend how competency testing should be accomplished. However, the College of American Pathologists' laboratory general proposed checklist includes a checklist question and a suggested list of competency measures.[2] The Commission on Office Laboratory Accreditation, an independent association for laboratory accreditation, also has guidelines and suggestions for meeting the CLIA requirement.[3]

Competency is the ability to do a job correctly and safely and to recognize and solve minor problems without needing assistance.[4] The competency assessment for testing and support personnel should assess the following core competencies: technical skills, judgment and decision making, knowledge base, and communication.[5] The competency scheme should cover the critical steps in the preanalytic, analytic, and postanalytic processes; each individual who performs a step in the process, whether technical or clerical, should be assessed. Many laboratories do not consistently assess the competency of the nontechnical employees who are essential to providing quality results. Federal guidelines do not require the assessment of