ABSTRACT  Primary parvovirus B19 infection is an infrequent, but serious and treatable, cause of chronic anemia in immunocompromised hosts. Many compromised hosts have preexisting antibody to B19 and are not at risk. However, upon primary infection, some patients may be able to mount a sufficient immune response to terminate active parvovirus B19 infection of erythroid precursors. The most common consequence of B19 infection in the compromised host is pure red-cell aplasia, resulting in chronic or recurrent anemia with reticulocytopenia. Anemia persists until neutralizing antibody is either produced by the host or passively administered. Parvovirus B19 should be suspected in compromised hosts with unexplained or severe anemia and reticulocytopenia, or when bone-marrow examination shows either giant pronormoblasts or absence of red-cell precursors. Diagnosis is established by detection of B19 DNA in serum in the absence of IgG antibody to B19. In some cases, IgG antibody is detected but is not neutralizing. Anti-B19 IgM may or may not be present. Therapy includes any or all of the following: red-cell transfusion, adjustment in medications to restore or improve the patient’s immune system, and administration of intravenous immunoglobulin (IVIG). Following treatment, patients should be closely monitored, especially if immunosuppression is unchanged or increased. Should hematocrit trend downward and parvovirus DNA trend upward, the therapeutic options above should be revisited. In a few instances, monthly maintenance IVIG may be indicated. Caregivers should be aware that B19 variants, though rarely encountered, can be missed or under-quantitated by some real-time polymerase-chain reaction methods.

DESCRIPTION OF THE AGENT AND PATHOPHYSIOLOGY OF INFECTION

In 1975, Yvonne Cossart first identified parvovirus particles by electron microscopy while evaluating tests for hepatitis B (1). This new virus, detected in serum number 19 in plate B, has since been known as parvovirus B19. The most common diseases subsequently associated with parvovirus B19 include fifth disease or erythema infectiosum (EI) of childhood, polyarthralgias/polyarthritis, fetal hydrops, transient aplastic crisis in individuals with chronic hemolytic anemias, and chronic red cell-aplasia in immunocompromised hosts.

Parvovirus B19 was the only member of the large Parvoviridae family to be unequivocally associated with human disease until the discovery of human bocavirus in 2005 (2). Human bocavirus (HBoV) is associated with respiratory infections in very young children and has been linked to gastroenteritis (3, 4). HBoV most often occurs as a coinfection, is detected in asymptomatic patients, and can persist at low levels for months; thus its role in disease has been questioned (5). HBoV has also been reported in severely immunocompromised hosts with respiratory or gastrointestinal disease, but rarely as the sole pathogen (6–13). Detection of very high HBoV titers in the respiratory tract, blood, and/or stool suggests a possible role in disease (7, 9–11, 14). However, in clinical practice, quantitative HBoV testing is not routinely available. While several commercially available respiratory-virus molecular-amplification panels can detect HBoV, results are qualitative (15). There is no treatment.

PARV4, first reported in an intravenous-drug user with hepatitis B virus, has since been found mostly in blood or tissues of persons with hepatitis C or human immunodeficiency virus (HIV) (16, 17). Bufavirus, a
highly divergent parvovirus, was identified in feces of children with diarrhea in Burkina Faso and was subsequently found in feces of gastroenteritis patients in northern Europe (18, 19). Since the impact of these viruses in the compromised host is not well understood (20), this chapter will focus on parvovirus B19.

Parvoviridae are named for their small size, parvum being Latin for small. The family, which includes both animal and insect pathogens, is divided into two subfamilies based on infection of vertebrate or invertebrate cells. The vertebrate Parvovirinae subfamily consists of eight genera and five are known to include human pathogens: Protoparvovirus (bufavirus), Bocaparvovirus (human bocavirus), Dependoparvovirus (adeno-associated virus), Erythroparvovirus (parvovirus B19), and Tetraparvovirus (PARV4). The new phylogeny is based on the protein sequence of the replication-initiator protein NS1 (21).

Parvovirus B19, of the genus Erythroparvovirus, consists of a highly stable nonenveloped icosahedral virion, 22–24 nm in diameter, with a single-stranded 5.6 kilobase DNA genome. The stability of parvovirus allows infectious virus to persist despite standard heat treatments of blood products, and to be transmitted in products like albumin, immunoglobulin, and pooled factor VIII and factor IX concentrates. The genome encodes a major nonstructural (NS) 1 protein, and two structural proteins, viral protein (VP) 1 and VP2. NS1 initiates and mediates most aspects of viral DNA replication, and serves as the motor for progeny-strand encapsidation. VP2 is the predominant protein, comprising 95% of the virus capsid. VP1 is the same as VP2 except for an additional 226 amino acids at its amino terminal (22). VP1 makes up only 5% of the capsid, has its unique region external to the viral capsid itself, and is thought to be the main target of neutralizing antibodies (22). Sequence analysis reveals that NS1 is highly conserved, while VP1 and VP2 show greater variation (23). Despite variations in VP1 and VP2, the antigens are commonly and successfully used in serologic tests (24–26).

Several novel variants of parvovirus B19 have been discovered (27–29), leading to a proposal that the Erythroparvovirus genus should include the following: genotype 1 (parvovirus B19), genotype 2 (strains A6 and K71), and genotype 3 (strain V9) (30). While the prevalence and significance of these variants remains to be determined, sporadic infections in both healthy and compromised hosts have been reported in Europe (31, 32) and the United Kingdom (33), especially in older patients.

Acquisition of parvovirus B19 infection is thought to occur via the respiratory route. After virus enters the respiratory tract, it is presumed to replicate in nasopharyngeal lymphatic tissue (34). Approximately one week after inhalation of infected droplets, viremia is observed, associated with nonspecific symptoms of fever, malaise, and myalgia. Viremia leads to infection of erythroid-precursor cells, predominantly in bone marrow and in fetal liver, by binding of the virus to P antigen or globoside (Gb4) on the cell surface (35). Conformational changes in virus VP1 then allows binding to a second as-yet unidentified surface molecule, which appears to mediate virus entry (36). Erythroid-progenitor cells produce infectious virus and are destroyed, leading to a drop in circulating reticulocytes, which lasts 2 to 5 days (34), followed by a drop in hematocrit. A transient decline in white cell and platelet counts can also occur. Although infectious virus is not produced in leukocyte and platelet precursors, these cells can be infected via P antigen followed by expression of the cytotoxic NS1 protein. Virus titers in the blood reach very high levels of up to 10^{13} particles per milliliter (22, 37, 38). With the appearance of IgM antibodies in the second week, virus titer in the peripheral blood begins to fall. Within 3 to 5 days of IgM, IgG is detectable in the blood and the symptoms associated with EI appear, namely erythematous rash and arthralgia. These symptoms are coincident with the immune response, have been observed in chronically infected patients following administration of immunoglobulin, and are considered to be immune mediated.

During acute infection, parvovirus B19 has been detected in nasopharynx, blood, bone marrow, liver, skin, cerebrospinal fluid (CSF), and synovium, and is known to infect erythroblasts, megakaryoblasts, granulocytes, macrophages, follicular dendritic cells, B and T lymphocytes, and endothelial cells.

Parvovirus B19 has been shown to persist in solid tissues of infected individuals for life (39). Parvovirus B19 DNA has been detected in bone-marrow samples in the absence of disease and can be detected at low levels in peripheral blood of normal hosts for months and even years after primary infection and resolution of symptoms (40–44). Virus has also been found to persist in synovium, myocardium, and skin (45–47). Of note, older persons carry genotype 2, which was apparently supplanted by the currently circulating genotype 1 around 1973. The cells harboring the virus and the mechanism of persistence, however, remain uncertain. It is possible that B19 may integrate into the human genome, as occurs with other parvoviruses, namely the adeno-associated viruses.
EPIDEMIOLOGY AND CLINICAL DISEASE

Immune-Competent Patients

Infection with parvovirus begins in childhood and continues throughout life. By young adulthood, up to 40% to 60% have been infected, and in old age, ~90% are antibody-positive. Transmission occurs year-round, but may peak late winter to early summer. Every 3 to 4 years, epidemics of increased activity occur. Although acute infections can manifest as EI, infection is asymptomatic in up to 50% of children. A prodromal nonspecific illness consisting of fever, chills, headache, malaise, and myalgias, coinciding with B19 viremia, can occur, followed by a typical “slapped cheek” facial rash and a lacy, reticulate erythematous rash on the trunk and extremities coincident with the immune response. The typical EI rash often waxes and wanes over a period of days.

In adults, the typical EI rash is much less common (48); rather arthralgias predominate, particularly in women. Symmetric painful, swollen joints, especially wrists, knees, and hands, can last for weeks to months, and be confused with Lyme disease or rheumatoid arthritis. Indeed, some features of B19 infection resemble autoimmune diseases and autoantibodies, such as antinuclear antibody and rheumatoid factor, are commonly found (22). Prolonged fatigue has been associated with elevated levels of interferon-γ and tumor-necrosis factor-α (49–52), and some cases meet the criteria for chronic fatigue syndrome.

In healthy individuals with a normal red-cell lifespan of 120 days, the drop in hematocrit associated with parvovirus B19 infection of erythroid-precursor cells is modest and not clinically significant. However, in anemic individuals, especially those with a high reticulocyte count to compensate for chronic hemolysis, such as sickle-cell anemia or hereditary spherocytosis, parvovirus B19 infection leads to a dramatic fall in hematocrit and an acute “transient aplastic crisis” requiring red-cell transfusion. These individuals have a red-cell lifespan as short as 15 to 20 days, and present with severe anemia early in the course of infection, often before IgM antibodies are detectable. Once IgG antibodies develop, the virus is neutralized, reticulocytosis reappears, and the hematocrit returns to baseline. Transient declines in whiteblood cell or platelet counts may also be seen, but are rarely clinically significant.

When infection occurs during pregnancy, maternal viremia can lead to transplacental transmission of virus. Infections early in pregnancy can lead to spontaneous abortions. Fetal infection leads to interruption of erythropoiesis and fetal anemia; in a minority of cases, parvovirus-associated myocarditis and severe anemia can lead to heart failure, fetal hydrops, and/or fetal demise. Other rashes can occur, in a generalized or localized distribution. These include petechial rashes, Henoch-Schönlein purpura, papular-purpuric gloves and socks syndrome, Gianotti-Crosti syndrome, desquamation, erythema multiforme, and erythema nodosum (53–56).

Thrombocytopenia can be mediated by bone-marrow suppression, NS1 toxicity to megakaryocytes, and by anti-platelet antibodies. Virus-associated hemophagocytic syndrome (VAHS) has also been associated with B19 (57, 58).

Parvovirus B19 has also been linked to myocarditis, a variety of neurologic syndromes, uveitis, hepatitis, renal syndromes, vasculitis, and chronic fatigue syndrome (49, 59–62). The mechanisms responsible are not clear, including which cell types are infected, and whether disease is mediated by immune complexes, NS1 toxicity, cytokine up-regulation, or autoantibodies. Of note, detecting B19 DNA in tissue extracts by polymerase-chain reaction (PCR) may reflect viremia and not actual tissue replication. Human leukocyte antigen (HLA) and cytokine gene polymorphisms have been linked to chronic arthritis and chronic fatigue syndrome following acute B19 infection, and it is possible that inherited variations in antigen presentation and cytokine response are responsible for symptoms (52).

Immunocompromised Patients

Disease spectrum

Many compromised hosts have preexisting antibody to B19 and may reactivate B19 with low-level, asymptomatic, transient DNAemia (63). Even in the face of primary infection, some compromised hosts may be able to mount a sufficient immune response. However, in others, neutralizing antibodies are not produced (64). Consequently, active parvovirus B19 infection of erythroid precursors is not terminated and viremia persists. The most common consequence of persistent active infection is pure red-cell aplasia (PRCA) resulting in chronic or recurrent anemia with reticulocytopenia (65, 66). Bone-marrow involvement can also manifest as neutropenia, agranulocytosis, pancytopenia, thrombocytopenia, and hemophagocytosis (67–72). Nonspecific symptoms of fever, myalgias, and malaise can occur (67, 73). Immune-mediated symptoms, such as the rash and arthralgias of EI, are not observed unless antibody develops. However, atypical rashes, some due to endothelial-cell infection, can be seen (68, 74). Myocarditis, glomerulopathy, and central nervous system vasculitis have also been reported (74–79).
Host factors and subgroups

Persistent parvovirus B19 infection can occur in congenital, acquired, and iatrogenic immunodeficient states, including acute and chronic leukemias (68, 80, 81), patients on chemotherapy for cancer (82–86), hematopoietic stem-cell and solid-organ transplant recipients (70, 87), immunosuppressive therapy for autoimmune disease (67, 88), congenital immunodeficiencies such as Nezelof syndrome (89, 90), and acquired immune deficiency syndrome (AIDS) (91). The common denominator is inability to produce sufficient neutralizing antibodies to the virus.

Although B19 is responsible for a minority of PRCA cases (92), B19-associated PRCA tends to be more severe and is more likely to follow primary B19 infection. B19 should be considered in patients with oncologic or hematologic malignancies undergoing either induction or maintenance chemotherapy with unexplained or severe anemia and reticulocytopenia (93). Rituximab therapy, which inhibits B cells and leads to a reduction in immunoglobulins, has been linked to persistent B19 infection in lymphoma patients (71). Persistent B19 infection can be the first manifestation of congenital immunodeficiencies, such as X-linked hyper IgM syndrome (94) and Nezelof’s syndrome (89).

Numerous cases of PRCA due to B19 have been reported in solid-organ transplants, especially renal transplants, and occur from weeks to years after transplant (70). Regimens that include the potent antirejection drugs mycophenolate mofetil (MMF) and tacrolimus may carry a greater risk of PRCA than cyclosporine (87, 95), although cyclosporine has also been implicated. Persistent B19 in renal transplants has led to collapsing glomerulonephritis and allograft loss (75, 76). In a lung-transplant patient with cystic fibrosis, total lymphoid irradiation for bronchiolitis obliterans was considered a contributing factor to persistent B19 infection (96).

Parvovirus B19 has been linked to engraftment failure after peripheral-blood stem-cell transplant (97), as well as PRCA (98). However, problems are infrequent in hematopoietic stem-cell transplant recipients due to the common practice of administering periodic IVIG (99).

PRCA due to B19 has been described in AIDS patients with low CD4-cell counts, predominantly in homosexual men. In contrast, AIDS patients with hemophilia generally have not had problems with persistent B19 (100), probably because they have a very high prevalence of B19-neutralizing antibodies from repeated exposure to B19-contaminated blood products and clotting factors.

In compromised hosts, B19 infection can be acquired via the respiratory route, from endogenous reactivation, or from blood products. Exposure to other compromised hosts who shed very high titers for prolonged periods has been implicated in outbreaks in cancer and transplant patients (101, 102). In one large study, patients with hematologic malignancies who received B19-positive transfusions did not develop symptomatic infections (103). However, severe transfusion-transmitted B19 infection has been reported in a naïve, severely immunocompromised renal-transplant recipient (104).

Morbidity and mortality

End-organ involvement

Destruction of erythroid precursors in the bone marrow is the hallmark of B19. As in normal hosts, pancytopenia, neutropenia, or thrombocytopenia can occasionally be seen and rare cases of hemophagocytosis, myocarditis, hepatitis, or central nervous system disease have been reported.

Prognostic factors

If immunosuppressive agents can be stopped, reduced, or changed, the host may be able to mount an effective antibody response and control or terminate active infection. In AIDS cases, highly active antiretroviral therapy (HAART) can result in increased CD4-cell counts, restoration of the immune system, and resolution of the anemia (105, 106). Likewise, once cancer chemotherapy has been completed, the immune system hopefully rebounds and is able to control the infection. Some immunocompromised patients may have a delayed antibody response, yet recover with some transfusion support, even with no other change in regimen (107). However, if the underlying immune deficiency cannot be corrected or worsens, spontaneous resolution is less likely.

Therapeutic considerations

Spontaneous resolution can occur if the immune system can reconstitute. Red-cell transfusions may be required in the interim. If immune reconstitution is not expected or will be delayed, passive administration of B19 antibodies will lead to virus neutralization, resumption of reticulocytosis, and a rise in hematocrit. However, relapses may occur as the passive antibody wanes if the host has not yet been able to produce neutralizing antibody. In the majority of reported cases, only one course of IVIG has been needed for long-term remission (87). Of note, immunocompromised hosts shed very high titers of B19, which can be a significant source of transmission to others. Since treatment with IVIG
reduces virus shedding a million-fold, IVIG should reduce transmission as well (108). However, IVIG also has side effects, including allergic reactions, transfusion-transmitted diseases, and a decline in renal function. It is also expensive and can be in limited supply. Unfortunately, a recent trial of a candidate B19 vaccine was terminated due to the development of rashes in several recipients (109).

GOALS OF LABORATORY TESTING

Screening and Prevention
No vaccine or antiviral is available. It is not practical to prevent community acquisition of parvovirus infection, since transmission is via respiratory droplets, usually from persons without recognized symptoms. In addition, B19 can be transmitted in plasma, bone marrow, stem cells, erythrocyte and platelet concentrates, clotting factors, immunoglobulin, serum albumin, and other products from human blood. The incidence of positive blood products is highest at times of peak B19 community transmission (110, 111). In one study, 1% of blood products administered to patients with hematologic malignancies tested positive for B19 DNA, including 17.6% of allogeneic peripheral-blood-progenitor cells and 2% of pooled products (2%) (103). However, median B19 DNA titers were low (700 genome equivalents/ml) and no symptomatic infections occurred. The Food and Drug Administration (FDA) and the European Pharmacopoeia have determined that, since titers of >10^7 IU/ml have been associated with clinical disease, 10^4 IU/ml is the maximum level of B19 DNA acceptable in plasma pools and their products (37). Manufacturers now perform B19 PCR on plasma mini-pools in order to remove plasma with high viral loads. Of note, B19 PCR assays may either miss or under-quantitate B19 variants. However, newer assays and international standards have been developed to address this issue (112–114). Fortunately the prevalence of genotypes 2 and 3 in blood products is likely low (115–117). Nevertheless, better methods for pathogen inactivation, filtration, and detection in blood products are needed.

Diagnosis and Prognostication
Since PRCA can have other causes, and the manifestations of EI that allow clinical diagnosis of B19 are not present, laboratory diagnosis of parvovirus B19 is essential in immunocompromised hosts. Available methods are shown in Table 1. In the compromised host, B19 DNA PCR of blood, either quantitative or real-time PCR with cycle threshold (Ct) values, is of greatest value in establishing a diagnosis, differentiating low-level persistence from clinically relevant infection, following response to treatment, and predicting relapse (37, 88, 114).

Treatment and Monitoring
The goal of therapy for PRCA is resolution of anemia. Therefore, reticulocytosis and hematocrit are monitored, and red cells are transfused as needed. In individuals treated with IVIG, PRCA may recur once passive antibody wanes, especially if immunosuppression stays the same or intensifies (108). If the hematocrit begins to fall, B19 viral load should be determined to confirm the etiology and a second course of IVIG administered if clinically indicated by the degree of anemia. Quantitative B19 DNA can also be monitored prospectively as an early indicator of relapse (37, 88). It should be noted, however, that B19 DNA can be detected by PCR in serum for months to years at lower levels in the absence of disease, even following IVIG therapy (22, 41–43, 69, 118). Therefore, a positive PCR alone should not lead to therapy, and elimination of B19 DNA from the blood should not be a goal of treatment. Rather, significant increases in viral load, in concert with a drop in reticulocytosis, should be indications for concern.

LABORATORY TESTING OPTIONS, APPLICATIONS, AND INTERPRETATION

Laboratory testing options and applications for diagnosis of parvovirus B19 are summarized in Table 1.

Serology
Since symptoms are often immune-mediated, detection of IgM and IgG antibodies is the mainstay of parvovirus B19 diagnosis in the normal host. Nevertheless, serology can be transiently falsely negative due to antibody-virus complexes in highly viremic samples (119). In general, IgM to B19 appears 7 to 10 days after infection, is followed within a few days by IgG, and remains positive for 2 to 4 months. In contrast, immunocompromised hosts may not develop antibodies, or IgM can develop but remain positive for months or years as an indicator of persistent infection, without development of IgG (73). A variety of methods can be used to detect parvovirus B19 antibodies (Table 2), and an international standard for B19 IgG assays has been developed and tested in collaborative studies (120). However, the only FDA-approved assay is in enzyme immunoassay (EIA) format and uses baculovirus-expressed conformational VP2 antigen (Biotrin International, Dublin, Ireland). This format and antigen source have given the best results...
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<thead>
<tr>
<th>Method</th>
<th>Use</th>
<th>Advantages</th>
<th>Limitations</th>
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<tr>
<td>IgM antibody by EIA</td>
<td>Diagnosis of acute or recent infection</td>
<td>Widely available</td>
<td>Antibody may not develop in immunocompromised hosts</td>
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<td>Mainstay of diagnosis in healthy hosts</td>
<td>Less expensive than molecular tests</td>
<td>The source and type of viral antigen impacts test sensitivity</td>
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<td>Detects antibodies to all known genotypes</td>
<td>IgM assays prone to false-positive results</td>
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<td>Class-capture EIA provides best results</td>
<td>Indirect EIA less sensitive and specific than class capture for IgM</td>
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<td>Presence of IgM generally correlates with immunity to reinfection</td>
<td>In compromised hosts, IgG may not develop</td>
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<td>Detects antibodies to all known genotypes</td>
<td>IgG may be poorly neutralizing</td>
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<td>EIA does not assess neutralizing ability</td>
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<td>Neutralization and immunoblot tests are confined to research laboratories</td>
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<td>The source and type of viral antigen impacts test sensitivity</td>
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<td>IgG can reflect passive transfer of antibody from blood products and not host immune response</td>
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<td>Protocols in different laboratories give varying results</td>
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<td>Quantification may not be standardized among labs</td>
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<td>May not detect or accurately quantify all genotypes</td>
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<td>Real-time probes more likely to miss mutations due to short length</td>
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<td>PCR can remain positive at low levels months to years after treatment or resolution of disease, even in normal hosts</td>
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<td>Cross contamination leads to false positives</td>
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<td>IgG Antibody by EIA</td>
<td>Diagnosis of current or past infection</td>
<td>Development of IgG is usually associated with neutralization of virus, fall in virus titer, and resolution of acute infection</td>
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<td>Can detect seroconversion</td>
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<td>Presence of IgG generally correlates with immunity to reinfection</td>
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<td>DNA amplification (e.g., PCR)</td>
<td>Detection and quantification of virus in serum, plasma, bone marrow or biopsy tissues</td>
<td>Main diagnostic test for use in hosts who may not produce antibody</td>
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<td>Real-time PCR allows wider availability and quantification</td>
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<td>Quantitative real-time commercial tests available</td>
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<td>Viral DNA can be quantitated and monitored for response to therapy and for risk of recurrence</td>
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<td>International B19 quantitation standard and genotype panels are available</td>
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<td>Available in reference laboratories and larger centers</td>
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<td>Cytopathology in bone marrow</td>
<td>Detection of giant pronormoblasts, viral inclusions, and red-cell aplasia</td>
<td>Can be first clue to parvovirus B19 infection</td>
<td>Giant pronormoblasts or inclusions may not be present, or may be missed</td>
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<td>Standard technique available in most hospitals</td>
<td>Atypical findings reported in compromised hosts</td>
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<td>Pure red-cell aplasia is not specific to parvovirus B19</td>
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<td>Antigen</td>
<td>Detection of viral antigen in bone marrow, tissues</td>
<td>Immunohistochemistry localizes viral antigen to specific cells</td>
<td>Not as sensitive as in situ hybridization</td>
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<td>Not widely available</td>
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<td>Not standardized</td>
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<tr>
<td>DNA hybridization</td>
<td>Detection of DNA in serum, bone marrow, tissues</td>
<td>Positive result in serum has strong predictive value for disease or recent infection</td>
<td>Less sensitive than DNA-amplification methods</td>
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<td></td>
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<td>In situ hybridization can localize infected cells</td>
<td>Not widely available</td>
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<td>Detects all 3 genotypes</td>
<td>Not standardized</td>
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<tr>
<td>Virus isolation</td>
<td>Isolation of infectious virus</td>
<td>Can assay infectious virus, develop neutralization tests, and study mechanisms of replication</td>
<td>Requires specialized cultures (human erythroid-progenitor cells and specialized cell lines)</td>
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<td>Available only in research laboratories</td>
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(25, 26, 121, 122). Bacterial-based expression systems, such as *Escherichia coli*, produce B19 linear epitopes suitable for immunoblots, but not EIA (121, 122). Although B19 shows genetic variability that can affect DNA PCR, immunologic properties are not affected, and routine serology detects all three B19 genotypes (24). However, EIA does not assess neutralizing ability. Consequently, a positive IgG by EIA does not exclude active B19 disease in the compromised host. Of note, IgG can also reflect passive transmission of antibody from blood products or IVIG therapy.

VP1-specific immunoblot assays and neutralization assays of virus in cell culture are generally confined to the research setting (64, 89). Antibodies to NS1 have been linked to persistent infection in some studies, but not in others (110).

**Bone-Marrow Examination**

Bone-marrow findings can provide the first clue to an unrecognized B19 infection (93). The hallmark is erythroid hypoplasia, as erythrocyte precursors are destroyed by B19 infection. Giant pronormoblasts, early erythroid cells in which B19 is actively replicating, are considered pathognomonic for B19, but they may be absent or missed. Typical giant pronormoblasts contain large eosinophilic nuclear-inclusion bodies, cytoplasmic vacuolization, and occasional “dog-ear” projections (123). Atypical bone-marrow findings have also been reported in compromised hosts, including near-normal erythroid precursors and inclusions throughout the erythroid spectrum (124).

### Nucleic Acid

**In situ** DNA hybridization is used for tissue localization of B19 DNA in formalin-fixed tissues, including bone-marrow and tissue biopsies (125). Direct hybridization of blood samples, usually in the dot-blot format, has a detection limit of $\sim 10^3$ genome copies/ml, can be readily quantified, and with use of a full-length probe, detects all known variants (37, 126). However, it has been replaced in clinical laboratories by more sensitive genome-amplification methods, such as PCR that can detect as little as 1 to 10 genome copies/ml. Real-time technology has replaced nested PCR, allowed wider clinical use, and made quantification simpler (121, 122, 127–129). Even with qualitative real-time assays, viral loads can be estimated and trends in Ct values can be monitored. By convention, serum is the most commonly used sample, but plasma is also acceptable and whole blood may have the highest viral loads during acute infection (130).

As shown in Table 3, assays have targeted conserved regions of either NS1 (117), VP1, or VP1/VP2 genes (129, 131, 132). Laboratory-developed real-time assays have been reported by a number of laboratories (88, 129, 131–133), and a number of commercial B19 quantitative assays are available in Europe. A few are also available in the U.S. as analyte-specific reagents (ASR). In early reports, commercial assays equaled or exceeded the sensitivity of nested PCR for genotype 1, but the Roche assay (no longer available) failed to detect genotypes 2 and 3, and the Artus assay under-quantitated genotype 3 by 3 log$_{10}$ (88, 115–117). In addition, some

### TABLE 2 Parvovirus B19 serologic assays

<table>
<thead>
<tr>
<th>Manufacturer (City, Country)</th>
<th>Method$^a$</th>
<th>Antigen and Source</th>
<th>FDA approved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotrin International (Dublin Ireland)</td>
<td>Indirect IgG EIA and class-capture IgM EIA</td>
<td>Baculovirus-expressed VP2$^a$</td>
<td>Yes</td>
</tr>
<tr>
<td>Diasorin (Saluggia, Italy)</td>
<td>Indirect IgG and class-capture IgM CLIA -Liaison platform</td>
<td>Baculovirus-expressed VP2$^a$</td>
<td>No</td>
</tr>
<tr>
<td>Denka Seiken (Tokyo, Japan)</td>
<td>Indirect IgG and IgM EIA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medac Diagnostika (Wedel, Germany)</td>
<td>Indirect IgG EIA and class-capture IgM EIA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Euroimmun (Lübeck, Germany)</td>
<td>Indirect IgG EIA and class-capture IgM EIA</td>
<td>Yeast-expressed VP2</td>
<td>No</td>
</tr>
<tr>
<td>IBL (Hamburg, Germany)</td>
<td>Indirect IgG and IgM EIA</td>
<td>E.coli-expressed VP1</td>
<td>No</td>
</tr>
<tr>
<td>Focus Parvovirus DxSelect (Cyprus, CA)</td>
<td>Indirect IgG and IgM EIA</td>
<td>Recombinant VP1</td>
<td>No</td>
</tr>
<tr>
<td>Mikrogen (Martinsried, Germany)</td>
<td>Indirect IgG and IgM EIA and strip immunoassay</td>
<td>E.coli-expressed VP1, baculovirus-expressed VP2</td>
<td>No</td>
</tr>
<tr>
<td>Biotrin International (Dublin Ireland)</td>
<td>Indirect IgG and IgM immunofluorescence (IFA)$^b$</td>
<td>Baculovirus-expressed VP1</td>
<td>No</td>
</tr>
</tbody>
</table>

$^a$VP2 comprises >95% of capsid antigen and appears to be conserved among genotypes.

$^b$Pretreatment with adsorbent reagent needed to prevent interference from rheumatoid factors.

$^c$EIA, enzyme immunoassay; CLIA, chemiluminescence immunoassay.
strains of genotype 1 were missed (116). This is a particular concern in persistent infections since genome variability is reportedly more common (134). Due to the short probe lengths used in real-time assays, ability to detect variants may be reduced unless multiple primers and probes are incorporated (121). Recently, a multiplex genotype-specific assay (112) and an assay shown to quantify all three genotypes with sensitivities of \( \leq 10 \) copies/reaction (114) have been published. An international World Health Organization (WHO) standard for B19 nucleic-acid-amplification techniques has been established (135), which is particularly important in screening blood products. In addition, an international B19 panel that includes all 3 genotypes has been developed to assist in assay validation (113). An internal control to detect inhibition is also recommended, and is provided with the commercial assays. In one study, inhibition occurred in 2 of 165 serum samples, but 3 of 5 bone-marrow samples extracted on a MagNA Pure instrument (116).

### Culture

Isolation of B19 requires specialized cultures, such as bone-marrow erythroid-progenitor cells or fetal liver. While useful for assaying virus infectivity, developing neutralization tests, and studying viral replication, these methods are confined to research laboratories (37, 38).

### Antigen

Detection of B19 antigens in bone marrow or tissue samples by immunostaining using monoclonal antibodies may be available in some settings; however, it is not as sensitive as in situ hybridization (125). Organ-system and Sample-Type-Specific Interpretation of Data

Prior to development of neutralizing antibodies, viral loads of \( 10^{11} \) to \( 10^{13} \) B19 genome copies/ml of serum are commonly seen even in persons without symptoms. Levels fall as antibodies develop, but B19 DNA can be detected by PCR at low levels in serum and bone marrow for months to years after symptoms have resolved (22, 41–43). As PCR assays become more sensitive, more low-level positives of questionable significance will be detected. Quantitation of viral load may help with interpretation of clinical significance in the individual patient. Rising titers in compromised hosts merit close monitoring of reticulocyte count and hematocrit, assessment of immunosuppressive or anti-retroviral regimens, and the need for IVIG.

The frequency and clinical relevance of missing or under-quantitating genotypes 2 and 3 is unclear and needs further study in various geographic locations and patient groups (121). It should be recognized that detection of B19 by PCR in tissue extracts may reflect viremia, or infection of endothelial cells lining blood vessels, and not true tissue replication. In some cases of encephalitis and meningitis, B19 DNA has been detected by PCR in CSF (59, 78, 132), but the site of replication has not been determined.

### Therapy

**Therapeutic Options**

No antiviral therapy is available. The first goal should be stabilization by transfusion if needed, followed by an assessment of options for facilitating the host’s produc-
tion of neutralizing antibody. This may include holding cancer chemotherapy if possible, changing or reducing immunosuppressive drugs, or optimizing HAART. IVIG, which contains neutralizing B19 antibodies, can be administered, especially when anemia is severe and improvement in the immune system in the short-term is not anticipated. The optimal dose and duration of IVIG has not been determined and published reports have varied widely (68, 70, 87, 108). The regimen of 0.4 g/kg/day for five to ten days has been suggested (22). Symptomatic disease is often associated with viral titers of $10^6$–$10^{12}$ IU/ml and a decline up to 6 log10 can be observed following IVIG administration (37). For those patients that relapse, a second course may be needed. In rare circumstances, monthly therapy of 0.4 g/kg/day for one day per month has been used. Preparations are now available that are not sucrose-based, and should be used when possible in renal transplants and other patients with impaired renal function. Again, the goal is to restore reticulocytosis and hematocrit, not generate a negative PCR result.

Erythropoietin has been used in a few cases; however, some investigators report worsening disease, presumably due to an increase in target cells for B19 replication (136, 137).

Impact of Diagnostic and Screening Assays on Therapy
Therapy is guided by the hematocrit and the reticulocyte count. Detection of high titers of B19 DNA by PCR confirms the etiologic role of B19 in the patient’s anemia. A low or negative B19 viral load in the serum and absence of supporting pathology in the bone marrow should call into question the diagnosis of B19-induced PRCA.

Use of Different Diagnostic Modalities to Monitor Therapeutic Response
The reticulocyte count and hematocrit are the most relevant indicators of the clinical impact of B19 infection. Reticulocyte counts rise dramatically once neutralizing antibody is produced, or within a week of IVIG therapy. If IVIG is not administered, but rather a change in immunosuppressive or antiviral drugs is initiated, quantitative PCR should be helpful in assessing the effectiveness of the strategy. Viral load should be documented prior to therapy and once the patient is stable. Should the hematocrit start to fall again, a repeat viral load to confirm the role of B19 is warranted. The patient should be closely monitored to confirm the downward trend in hematocrit and reticulocyte count, which should correspond to rising B19 levels, and therapeutic options reviewed. While monitoring B19 viral load as an early predictor of relapse is now feasible, the viral-load threshold for intervention is not defined and there is no data yet to support the benefit or cost effectiveness of this approach (88).

When IVIG is administered, serum creatinine should be monitored and IVIG decreased or withheld as needed. Documenting IgG seroconversion in patients who have not been treated with IVIG, or after IVIG has waned, is recommended.

SUMMARY
Parvovirus B19 is an infrequent, but serious and treatable, cause of chronic anemia in immunocompromised hosts. Patients with congenital, acquired, and iatrogenic immunodeficiencies are susceptible, and the common denominator is inability to produce sufficient neutralizing antibodies to control the virus after primary infection. The anemia persists until neutralizing antibody is either produced by the host or passively administered. B19 should be suspected in compromised hosts with unexplained or severe anemia and reticulocytopenia, or when bone-marrow examination shows giant pronormoblasts or absence of red-cell precursors. Diagnosis is established by detection of B19 DNA in serum in the absence of IgG antibody to B19. In some cases, IgG antibody is detected by EIA, but is not neutralizing. Anti-B19 IgM may or may not be present. Therapy includes any or all of the following: red-cell transfusion, adjustment in medications to restore or improve the patient’s immune system, and administration of IVIG. Following treatment, patients should be closely monitored, especially if immunosuppression is unchanged or increased. Should hematocrit trend downward and parvovirus DNA trend upward, the therapeutic options above should be revisited. In a few instances, monthly maintenance IVIG may be indicated. Caregivers should be aware that B19 variants, though rarely encountered, can be missed or under-quantitated by some real-time PCR methods.

ACKNOWLEDGMENTS
Conflicts of interest: We declare no conflicts of interest.

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