Clinical Utility of DNA Based Testing for Parvovirus B19 Virus

Critical Review
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Introduction

Of all the parvoviruses, B19 stands out as essentially the only one of clinical importance to humans. Within the family Parvoviridae, the densoviruses infect members of the order Insecta but not vertebrates, and the dependoviruses infect a number of animal species but are not known to be associated with human disease (10). Parvovirus B19 is one of the autonomous parvoviruses, which are widespread in nature and frequently cause disease in their hosts (other examples are feline panleukopenia virus and canine parvovirus).

Parvovirus B19 is an unenveloped single stranded DNA virus (5.5 kb). There are two capsid proteins of approximately 83 and 60 kDa. B19 is resistant to lipid solvents and is relatively sensitive to acid and alkali and to heat denaturation. Spread is by the respiratory route. Approximately one week after exposure, an intense viremia develops, which is associated with flu-like symptoms. The viremia lasts several days and is followed by an IgM response, which is detected 10-12 days after exposure. An IgG response develops approximately 2 weeks after exposure. The IgM may persist for several months, and the IgG probably persists for life (3). Life long immunity most likely occurs after infection, even though IgG levels may drop.

Clinical Illness

B19 is often associated with an erythematous maculopapular rash which in its most distinct form is termed erythema infectiousum (EI). Classically, the rash starts with an intense erythema of the cheeks, which then progresses to the trunk and the limbs where it may have a reticular appearance. The rash is believed to be caused by immune complex formation and develops after the viremic stage has passed. Typically, it lasts only a few days. Because it was the fifth of six erythematous-rash illnesses of childhood in an old classification system, it is still sometimes known as "fifth disease." The term "slapped-cheek disease" has also been applied due to the characteristic rash.

In a study of 11-15 year old boys in a residential school during a B19 outbreak (5), 72% of those who were seronegative before the outbreak seroconverted. Of these, approximately two thirds were asymptomatic, one quarter had an associated influenza-like illness, and only 10% had a typical erythematous rash. Often the rash is atypical and erythema of the cheeks is not always prominent, there is no reticular appearance on the limbs, and there may be involvement of the palms and soles (10).

Approximately 80% of infected adult females and 10% of infected children report joint symptoms with B19 infection (10). These usually present as a symmetrical arthralgia or arthritis in the small joints of the hands, although the wrists, knees and ankles may also be involved. The arthropathy usually resolves within two weeks, but rarely may persist for months or years.

Pure Red Cell Aplasia

By far the most serious sequalae of B19 infection are due to B19 induced selective inhibition of erythroid colony formation in the bone marrow. Erythroid colony formation by the late erythroid progenitor cell (CFU-E) and the more primitive erythroid progenitor cell, the burst-forming unit (BFU-E), is strongly inhibited by the virus.

B19 is directly cytotoxic to the host cell. The viral cytopathic effect in the marrow manifests as giant pronormoblasts of about 25-32 μ. The cells are scattered throughout the aspirate smear, and their number roughly correlates to the viral content (3). The giant pronormoblasts themselves, however, may not contain detectable B19 viral capsid proteins.

Typically, no mature erythroid precursors are present in the bone marrow of healthy individuals 10 days after inoculation. The expected disappearance of reticulocytes from the peripheral blood and a small fall in the hemoglobin level follow during week two after inoculation (10). The cessation of erythropoiesis lasts 5 to 7 days. This stage corresponds to the viremic stage, with return of red cell production usually coinciding with detection of antibody response. Commercial immunoglubulin preparations contain antibodies to B19, and infusion is associated with decline in viral concentration within hours, followed by reticulocytosis (4).

In otherwise healthy individuals, the short-lived red cell aplasia usually goes unnoticed. When complicating a chronic hemolytic anemia where the patients are relying on a constant supply of red blood cells due to continual destruction, however, this may result in aplastic crisis. Evidence suggests that B19 infection has been the principal cause of aplastic crisis in sickle cell anemia patients for the last 20 years (10). Any chronic hemolytic state, however, such as hereditary spherocytosis, pyruvate kinase deficiency, and transfusion-independent beta-thalassemia may be affected.

Infection During Pregnancy

Parvovirus B19 is not associated with birth defects (10), but infection during pregnancy has been associated with spontaneous abortion in about 10% of cases (3, 10). Also, infection during the second and third trimesters have been associated with fetal hydrops. This is most likely due to erythrocyte aplasia in the effected fetus.

Pathologically, these hydropic infants show leukoerythroblastosis, iron deposition in the liver, and typical giant pronormoblasts. The risk for fatal fetal outcome is highest with infection in the first two trimesters.Most pregnancies complicated by B19 infection continue to full term delivery of healthy infants (10).

Chronic Infection

In patients with underlying immunodeficiency states, persistent B19 infection may occur. It is thought that persistently infected patients fail to mount a neutralizing antibody response to the virus and thus remain chronically infected. They therefore usually lack the immune-complex-mediated symptoms of EI and arthropathy. Persistent parvovirus infection and pure red cell aplasia have been documented in congenital immunodeficiency, children with lymphoblastic leukemia, patients with acquired immunodeficiency syndrome, and recipients of solid organ transplants (3).

In chronically infected patients, the anemia may be intermittent , with periods of relapse (associated with viremeia) and remission (associated with spontaneous disappearance of virus from the circulation) (3). Clinically, the degree of anemia may be severe.

The bone marrow in the well documented cases of chronic infection contains cytopathic signs of infection including giant pronormoblasts and pure red cell aplasia (3, 4, 8,9). Viral concentrations in the serum of cloned parvovirus DNA are in the range of 10^8-10^9 / μl (4,9). This is compared to levels of 10^9-10^11/μl in acute infections (4,9). Administration of intravenous immune globulin is associated with a drastic reduction of viremia (4, 8)

Serology and DNA Based Diagnosis

A variety of antibody assays including EIA, antibody capture assays, and immunoblot may be used to detect antibodies to parvovirus B19. A rise in IgM titer is usually seen within 3-4 days after the onset of symptoms, but may not appear until 7-10 days after onset of symptoms. IgM usually remains for 3-4 months after acute infection. IgG antibodies are usually detectable about two weeks after exposure.

DNA based assays have been used in the diagnosis of in-utero or neonatal infections. PCR can be performed on amniotic fluid for identification of fetuses at high risk.

Review of DNA Based Testing for HIV Infected Patients

Since chronic infections in immunodeficiency states typically lack an immune response, DNA based tested have also been proposed for these circumstances. Unfortunately the studies have been conflicting and contradictory as to their relevance and utility. With the main questions arising from whether detectable virus in immunocompromised patients actually correlates with symptomatic disease (i.e. anemia), or whether a certain number of these patients may have detectable virus without symptoms.

Some studies show that detection of B19 DNA is associated with anemia. Gyllensten et al (6) measured serum for B19 DNA by polymerase chain reaction in 69 AIDS patients with anemia of undiagnosed cause and 37 AIDS patients without anemia. Among the anemic patients, 5/69 had B19 detectable by PCR and among the non-anemic patients 0/37 had B19 detectable by PCR. In the patients with anemia, the presence of parvovirus correlated with the degree of anemia. From this, it was concluded by the authors that PCR detection of the virus was associated with anemia in AIDS patients. The bone marrow was not examined.

In another study, Naides et al (12) examined serial sera from 14 HIV patients with PCR. Nine of fourteen (9/14) had detectable viremia at some point by PCR. Four of the nine had serially positive samples. Since all four with serially positive samples had severe anemia, the author concluded that B19 infection should be considered in anemic patients with AIDS. Notable in this study is the lack of control groups. This study was also notable for the detection of anti-parvovirus IgG in 5/9 patients with viremia. From this, the authors concluded that reactivation may be a mechanism for chronic infection in AIDS patients.

Something not considered in this paper was that low-grade viremia detectable by PCR could be without clinical significance. If AIDS patients without anemia were to show detectable B19 DNA, these assumptions would be in question. Bone marrow biopsies in these patients would also be useful to see if characteristic signs of infection were visible (pure red cell aplasia with giant pronormoblasts).

Musiani et al (11) looked at some of these issues in a study of 7 hemophiliacs with concomitant HIV-1 infection. Serum was tested for medium-high viremia by DNA dot blot hybridization and low grade viremia by polymerase chain reaction. The studies were over 3-4 years in each patient. Three of the seven were negative for B19 over the entire study, while four were persistently positive by PCR. Some of these patients were intermittently detectable by dot blot hybridization, indicative of a rise in viral titer. Only during these times were acute manifestations found. No detectable disease was noted in patients with low-grade viremia detectable only by PCR.

Kerr et al (7) examined bone marrow biopsies from 61 HIV patients and 23 non-HIV patients with a nested PCR assay for B19. Thirteen of the 61 HIV patients showed detectable B19 DNA. None of the non-HIV patients showed detectable B19 DNA. Only two of the 13 with PCR positivity had significant anemia (defined by hemoglobin less than 9 g/dl). Also marrow morphology in patients with PCR positivity was not suggestive of parvovirus. From this, the authors concluded that B19 detectable by PCR in AIDS patients is without clinical significance.

Abkowitz et al (1) found 4 of 77 (5%) of randomly selected HIV positive patients to be positive for B19 DNA by PCR. Five of 54 (9%) control patients were also positive (not statistically different). None of these patients had anemia. When assayed by dot blot hybridization , a less sensitive assay, 1 HIV (0.5%) and 2 non-HIV (2%) were positive. Again, none of these patients had anemia. When patients with HIV and anemia (Hct < 24) were tested 5/30 patients had detectable parvovirus by DNA dot blot. Two of four who had bone barrow biopsies showed characteristic signs of B19 infection. These findings suggest that detection of higher grade viremia may be of value in HIV patients.

On the other hand, other studies have not been able to detect parvovirus as easily. Van-Elsacker-Niele et al (13) studied single serum samples from 317 consecutive HIV infected patients with PCR to B19. Anemia was present in 176/317 patients. None of the patients had detectable parvovirus. The authors concluded that chronic B19 infection should not be considered a frequent cause of anemia in HIV infected individuals.

Chernak et al (2) tested 105 patients in a general population of HIV patients and 22 HIV patients with anemia for B19 with a PCR assay. one of the 105 patients from the general population and one of the 22 from the anemia group were positive. From this the authors concluded that infection with parvovirus B19 is uncommon in some groups of HIV patients.

Conclusion

In summary, the literature concerning DNA based testing for parvovirus B19 DNA in HIV patients is contradictory and confusing. While some studies conclude that PCR based testing is associated with symptomatic disease, other studies suggest that PCR is too sensitive and detects insignificant viremia. Yet other studies conclude that PCR positivity is rare in these patients, even with anemia. The studies which claim that PCR is too sensitive suggest that higher titer viremia detectable with non-amplified dot blot hybridization assays may be associated with clinically significant disease.

Clearly no consensus has been reached. Different PCR techniques may have different sensitivities and specificities and are difficult to compare. Many of the studies lack adequate control groups. Many of the studies lack comparison to a gold standard and just look at anemia, which is common in HIV patients and may be multifactorial. Correlation with bone marrow biopsy findings has been done in a few studies, but is inconsistent. Until these issues have been addressed, significant treatment decisions, such as IVIg infusion, should not be based on these test results. The tests should not be offered by the clinical laboratory for general patient care purposes.

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