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Parvovirus B19 in Anemic Liver Transplant Recipients

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Five hundred thirty-three liver transplant recipients were seen for follow-up care over a 6-month period. Of these, 23 (4.3%) had a hemoglobin level of ≤9 g/dl, with 19 being eligible for inclusion in this study. The median hemoglobin level was 8.7 g/dl with a low of 7.1 g/dl. Two patients had iron-deficiency anemia. All of the patients were on therapeutic drugs which can suppress erythropoiesis or shorten the lifespan of mature erythrocytes. Six patients (31.6%) were viremic for human parvovirus B19 but none was B19 immunoglobulin M seropositive. Two patients were immunoglobulin M seropositive for cytomegalovirus. The patients with circulating B19 DNA were not easily distinguished from those without the virus by their laboratory results. The absence of reticulocyte counts for these patients contributed to this inability to differentiate B19 infection from other causes of anemia, particularly drug myelotoxicity. The high likelihood of making a specific diagnosis with the increasing availability of PCR should spur the search for this virus in the liver transplant population.

The anemic state in patients with chronic liver disease can be associated with a number of factors including decreased erythropoietin production due to concomitant renal insufficiency, drug- or virus-induced marrow suppression, iron loss from bleeding, other nutritional deficiencies, hypersplenism, and acquired hemolytic anemia associated with congestive splenomegaly (18). In advanced liver disease associated with marked liver fibrosis and portal hypertension, the differential diagnosis of anemia must also include upper gastrointestinal bleeding.

As in the case of patients with chronic liver disease, liver transplant recipients are subject to several different medical conditions, each of which can produce an anemia. For example, they are invariably on immunosuppressive regimens such as tacrolimus (FK506), a recognized cause of myelosuppression (20). An additional myelosuppressive agent, trimethoprim-sulfamethoxazole, which interferes with folic acid metabolism, is administered routinely to these patients for prophylaxis against common microbial pathogens and various opportunistic infections.

In 1975, the list of putative causes of anemia in individuals with liver disease grew with the discovery of human parvovirus B19 (B19) (2, 8, 14). This viral agent causes anemia by infecting and lysing globoside-containing erythrocyte precursors (5). Initial reports link B19 infection with the aplastic crisis that occurs in patients with underlying hereditary anemias (17, 21). Other studies demonstrate a relationship between the agent and arthritis (19), hydrops fetalis (6, 9), and anemia in pediatric liver transplant recipients (16) and in individuals transplanted for fulminant liver failure (7).

To date, however, there have been no large-scale studies of B19 infections in adults who have undergone liver transplantation for chronic liver diseases. Furthermore, the available literature insufficiently characterizes the anemia and/or clinical status of patients reported with B19 infection, making it difficult to generate medically useful paradigms. The goal of this investigation was to determine the prevalence of B19 infection in a cohort of anemic liver transplant recipients. Concurrent causal factors for anemia were surveyed to permit a fuller characterization of anemia in this unique group of patients.

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MATERIALS AND METHODS

Informed consent was obtained from all patients as part of the liver transplantation evaluation. Human experimental guidelines of the U.S. Department of Health and Human Services and the University of Pittsburgh Medical Center were followed. As part of the routine follow-up care of liver transplant recipients, the investigators (E.F., and D.H.V.T.) collected and stored sera at −70°C from persons seen at Falk Clinic, an ambulatory care clinic of the University of Pittsburgh Medical Center, in Pittsburgh, Pa. A laboratory data review was conducted on all patients seen between March and August 1992. The medical records and sera from those persons with a hemoglobin level of ≤9 g/dl were pulled for review and testing, respectively. These elements including date of and reason for liver transplant; serum sample collection dates; potential bone marrow-suppressive drugs; transfusion history within 1 month prior to and up to 5 months after the index sample collection date; the administration of exogenous growth factors such as erythropoietin, granulocyte colony-stimulating factor, and granulocyte-macrophage colony-stimulating factor as well as that of intravenous immunoglobulin; and values for hemoglobin, platelet count, leukocyte count, blood urea nitrogen, and serum creatinine were collected for analysis. The stored sera were tested for the presence of B19-specific immunoglobulin M (IgM) and IgG (11) and B19 DNA (9). Cytomegalovirus (CMV)-specific IgM and IgG were determined by SIA (Sigma Diagnostics, St. Louis, Mo.) and FIAX CMV (International Diagnostic Technology, Inc., San Jose, Calif.), respectively, and antibodies to Epstein-Barr virus (EBV) were assayed by indirect immunofluorescence (Gull Laboratories, Inc., Salt Lake City, Utah). The CMV and EBV serologic testing was performed at the University of Pittsburgh Medical Center, according to the manufacturers' instructions.

Human parvovirus B19 assays. Human parvovirus B19-specific IgG and IgM levels were determined by an antibody-capture enzyme-linked immunosorbent assay which has been described previously (11). Samples were tested at a standard dilution of 1:100 with results recorded as positive, negative, or equivocal, based on the A_{405} reading with cutoff values for a positive response having been determined previously for this assay. Samples giving equivocal results in this assay were retested.

PCR-based amplification of parvovirus B19 DNA was performed on aliquots of frozen sera by the methodology of Jordan (9). Amplified samples were analyzed in duplicate, and specific parvovirus B19 DNA was detected by a microtiter plate-based enzyme immunoassay (10). Reactions were read at A_{490}. Results were considered negative for absorbance values of <0.4. Equivocal results were reanalyzed in triplicate, and the final interpretation was based on all five readings: the final result was negative if three or more of the five readings were <0.4, or positive if three or more of the five readings were ≥0.4.

TABLE 1. Anemia in adult liver transplant recipients[a]

Patient code	Age (yr) and sex	No. of samples (March–May/ June–August)	No. of mo post-transplant(s)	Native liver pathology	Hemoglobin (g/dl)	B19 IgG	B19 PCR	CMV IgG/IgM	EBV IgG/IgM	Other medical data
A	56F	1/0	4 and 5	PNC-B, HCC	8.2	+	−	+/NT	NT/NT	Iron deficient
B	24F	0/1	86	PNC-E	8.8	+	−	+/NT	+/NT	
C	55F	0/1	13	PBC	8.7	+	−	+/+	+/NT	Iron deficient
D	45F	1/0	8	PNC-B, HCC	8.7	+	−	+/−	+/−	
E	43F	0/1	13	PNC-E	8.8	+	−	+/NT	+/NT	Hemolytic anemia
F	67F	0/1	5	PNC-Crypt	8.8	−	−	+/NT	+/NT	Azathioprine therapy
G	68F	1/0	4 and 16	PNC-AI	9.0	+	−	+/−	+/−	
H	52F	2/0	3, 3*	PBC, C	8.5, 8.9*	+	−, −	−/−	+/−	
I	62F	0/2	2, 3*	PNC-C	8.9, 7.9*	−	−, +	+/NT	+/NT	Colonic polyp
J	62F	1/0	2	PBC	7.1	+	+	+/−	+/−	
K	70F	3/0	35, 36, 36*	PNC-C, HCC	8.6, 8.8, 8.5*	−	−, −, +	+/NT	+/NT	
L	45F	2/0	56, 56*	PNC-C	8.5, 8.9*	−	−, +	−/NT	NT/NT	Upper GI bleeding
M	53M	1/1	9, 11*	HCC	9.0, 9.0*	−	−, −	+/NT	+/NT	
N	44M	0/1	1	PSC, HCC	9.0	−	−	+/−	+/−	
O	40M	1/0	14	PNC-E	8.6	−	−	−/−	+/−	
P	46M	1/0	10	PNC-E	8.1	−	−	+/NT	+/NT	
Q	36M	2/0	2, 3*	PNC-E	8.5, 9.0*	−	−, +	+/+	+/NT	
R	52M	1/0	13	PNC-E	8.9	+	−	−/−	+/−	
S	66M	2/0	8, 9*	PNC-C, PNC-B	8.6, 8.1*	+	+, −	+/NT	+/NT	Renal transplant

[a] Abbreviations: F, female; M, male; B, hepatitis B; HCC, hepatocellular carcinoma; E, ethanol; PBC, primary biliary cirrhosis; crypt, cryptogenic; AI, autoimmune; C, hepatitis C; PSC, primary sclerosing cholangitis; NT, not tested; GI, gastrointestinal. Symbols: *, multiple visits; +, positive; −, negative.

RESULTS

Five hundred thirty-three liver transplant recipients were seen for follow-up care at Falk Clinic in the 6 months between March and August 1992, encompassing the study period. Of these, 23 (4.3%) had a hemoglobin level of ≤9 gm/dl. Four patients were not studied further because of concurrent sepsis or graft rejection. The remaining 19 (82%) had 27 serum samples available for testing. The patients were characterized by the following: (i) hemoglobin level of ≤9 g/dl, (ii) being ≥30 days posttransplant, (iii) having no clinical signs of sepsis (no fever, leukocyte count less than 8,000/ml), (iv) having normal lactate dehydrogenase level, (v) having no evidence of graft rejection, (vi) having no underlying hematological or nonhepatic neoplastic disorder, and (vii) having no evidence of active blood loss.

This group consisted of seven men (mean age, 48 years; age range, 36 to 66 years) and 12 women (mean age, 53.3 years; age range, 24 to 70 years). The main indications for liver transplantation in these patients (Table 1) were postnecrotic cirrhosis (PNC) due to hepatitis C (four patients) or B (two patients), PNC due to ethanol consumption (six patients), primary sclerosing cholangitis (one patient), primary biliary cirrhosis (three patients), hepatocellular carcinoma (one patient), and cryptogenic (one patient) or autoimmune (one patient) PNC. Six patients had two diagnoses. Two patients had more than one liver transplant (patients A and G), while one had a renal transplant and the liver transplant (patient S). The mean time from the most recent liver transplant to the study date was 15.4 months (median, 9 months; range, 1 to 86 months). The diagnosis of hepatitis B or hepatitis C was documented by positive enzyme immunoassay serology for the virus and liver biopsy consistent with viral hepatitis.

Eighteen samples (from 12 patients) and nine samples (from eight patients) were collected in the months of March through May and June through August, respectively. Patient M had a sample in each time period. Sixteen of the 19 patients (84%) had blood urea nitrogen levels of ≥20 mg/dl (range, 20 to 96 mg/dl; reference range, ≤20 mg/dl) and blood urea nitrogen/creatinine ratios of up to 24 (patient I). The hemoglobin levels of the 19 patients ranged from 7.1 to 9 g/dl with a median hemoglobin level of 8.7 g/dl. In fact, all but two of the patients had a hemoglobin level of ≥8.1 g/dl. Twelve patients had platelet counts under 150,000 cells per mm^3; the lowest was 73,000 cells per mm^3. Reticulocyte counts were available on two participants with counts of 3.6 (E) and 0.5% (F).

Therapeutic regimens. Patient follow-up revealed specific causes for the anemia in a minority of cases. These included iron deficiency (patients A and C), hemolytic anemia (treated by splenectomy, patient E), upper gastrointestinal bleeding (patient L), colonic polyp (patient I), and azathioprine therapy (patient F). Patients A and C had transferrin saturation of 5 and 9% (normal, 20 to 55%), respectively. The serum iron, total iron binding capacity, and ferritin for the remainder of the patients were normal or increased. B_{12} and folate were available for six of the patients (A, E, L, O, P, and S); they were within the normal range. The majority of patients were empirically treated with iron supplements, erythropoietin, and transfusions of packed erythrocytes (PRBC). One patient received intravenous immunoglobulin (patient M) 8 months prior to the period of observation for suspected B19 infection.

Five of the 19 patients did not have a transfusion in the month prior to the index hemoglobin, nor in the ensuing 5 months (patients A, B, K, Q, and S). In contrast, nine patients received 2 to 51 U (median of 4) of PRBC in the month preceding the clinic visit for reasons including gastric ulcer bleeding and resection (patient L) or bone marrow suppression secondary to azathioprine therapy (patient F). An additional four persons received 1, 2 (two persons), or 5 U of PRBC coincident with the index hemoglobin determination.

All 19 patients received tacrolimus (FK506) and prednisone for immunosuppression. Six patients were on erythropoietin at the time of the clinic visit, but none received intravenous immunoglobulin over the period that the index samples were drawn. Eight patients were on sulfa drugs, specifically trimethoprim-sulfamethoxazole. Several other patients were on additional drugs that are linked directly or indirectly to the development of anemia. These drugs include florinef, dexa-

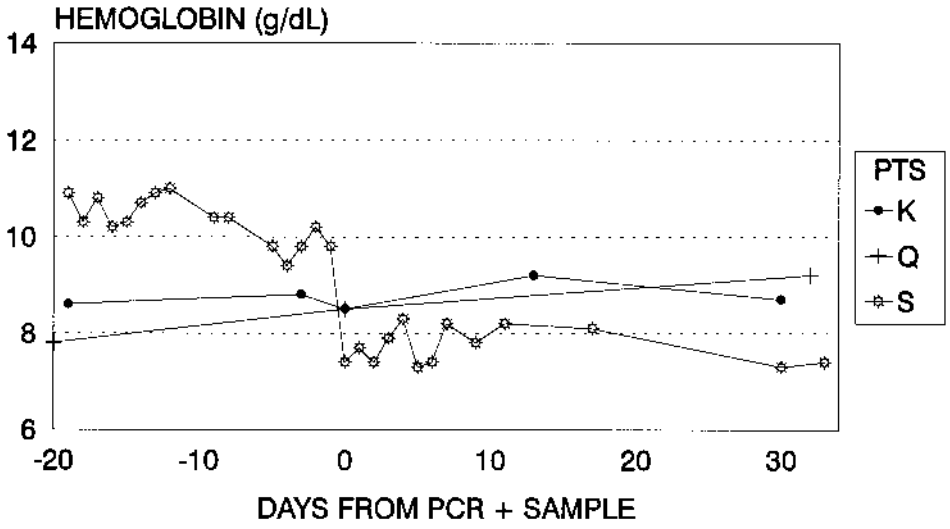

FIG. 1. Hemoglobin levels of three parvovirus B19 DNA-positive liver transplant recipients (PTS) who were not transfused over 1 month before and 5 months after the PCR-positive sample.

methasone, furosemide, carboplatin, dapsone, azathioprine, ceftizoxime, doxorubicin, and/or alfa interferon.

Viral assays. Data on CMV serologic testing were available for all 19 patients (Table 1). Of these, two were seropositive for both CMV IgM and IgG (C and Q) and three (H, O, and R) were seronegative for both indices. Historical or recent EBV serology was available for all but one patient (L). All 18 patients were known to be EBV IgG seropositive. Seven patients with anti-EBV testing concurrent with an index hemoglobin determination were found to be EBV IgM seronegative.

Data on the presence of B19-specific antibodies and B19 DNA were available for all 19 patients (27 samples). Ten of the 19 patients (53%) were B19 IgG seropositive, but none of the 19 patients was seropositive for B19 IgM. Six of the IgG-seropositive patients were seen during the peak B19 incidence period, between the months of March and May. Results of the PCR-based assay for B19 DNA revealed that 32% of the patients were viremic (I, J, K, L, Q, and S) with patients J and S being B19 IgG seropositive. Patients K, L, Q, and S were seen between March and May, 36, 56, 3, and 9 months after liver transplantation, respectively. Patients I and J were seen 2 months after transplantation. Interestingly, the B19 DNA-positive samples from patients I and J were collected within 1 day of each other. Excluding patient J, all B19 DNA-positive samples were in patients who had multiple samples. All four patients with a primary diagnosis of PNC due to hepatitis C were B19 DNA positive. Hepatitis viruses, especially hepatitis C virus, are known causes of anemia (21). All six B19 DNA-positive patients were ABO blood group O. The time plots of the hemoglobin levels of the B19 DNA-positive patients are shown in Fig. 1 and 2.

DISCUSSION

The importance of human parvovirus B19 infection as an etiologic agent of anemia in adult liver transplant recipients is not characterized fully. It is clear, however, that the problem is aggravated in hospitalized persons because of the greater risk

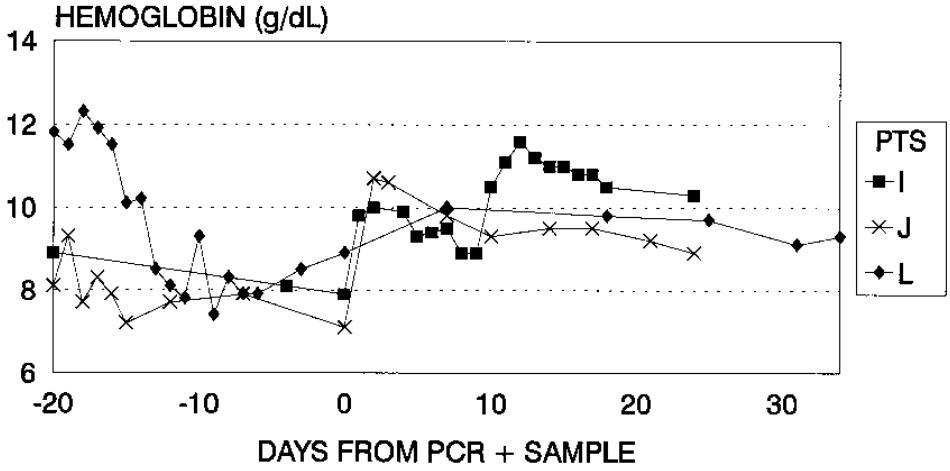

FIG. 2. Hemoglobin levels over time of three parvovirus B19 DNA-positive liver transplant recipients (PTS) who received PRBC transfusions within 20 days before and 34 days after the PCR-positive sample. Patient I had 2, 1, and 2 U of PRBC transfused on days 1, 7, and 10, respectively; patient J had 2 U of PRBC transfused on each of days −32 and 2; and patient L had 33 U of PRBC transfused between days −19 and −14 and none thereafter.

of nosocomial infection by person-to-person spread of respiratory secretions as well as the use of blood products, which are not currently screened for B19, in treating anemia and several coagulopathies.

The risk of transmission of human parvovirus B19 by the administration of blood products has been estimated to be 1 in 3,300 blood units (13). Donated blood units collected over a 5-month period from the Pittsburgh metropolitan area volunteer donor population were screened for B19 by PCR. The study detected B19 DNA in 13 of 10,000 U (0.13%) (9a), a figure more than fourfold higher than that published by McOmish et al. (13). The main problem arises from the fact that B19 is not inactivated by solvent-detergent treatment as happens with enveloped viruses (3). Therefore, patients who require factor concentrate support are at particular risk for infection or reinfection with B19 (3, 4, 15). In fact, 47% of the study population was B19 seronegative and therefore still susceptible to the infection.

Parvovirus B19 infection is seen most frequently between the months of March and May each year. Thirty-two percent of the liver transplant recipients showed B19 viremia, 67% of which was seen in the peak incidence period. None of these patients was IgM seropositive. However, this finding is not surprising given the immunosuppressive regimens that the patients were on and the fact that IgM levels peak as early as 2 to 3 weeks postinfection and then decline rapidly.

In this study, there were a number of noteworthy findings relating to the diagnosis and management of the anemia commonly seen in the liver transplant setting. First, it is quite striking that five of the seven patients with multiple samples were B19 DNA positive. It is reasonable to predict that the chance of detecting B19 in this population is directly proportional to how often one looks for the infection. For an accurate diagnosis of this disease, serious consideration should be given to the use of PCR-based testing on multiple occasions at the time of the initial evaluation of the anemia. With this in mind, there is evidence to suggest that in this patient population, a diagnosis of B19 infection was not strongly entertained, as reticulocyte counts were generally not obtained, and intravenous immunoglobulin was not administered. In part, this may be due to the fact that the anemia was longstanding in many of the patients studied. B19 replication occurs in the bone marrow within erythroid precursor cells. These cells are destroyed because of the lytic nature of the virus, which leads to anemia. Therefore, B19 DNA should be present within the bone marrow prior to its systemic release and development of anemia. During B19 infection, viral titers within serum can be found as high as 10^{12} particles per ml (1). Our PCR-based assay can consistently detect 10 to 100 virus particles within a serum sample (data not shown). The patients with iron-deficiency anemia could be distinguished from the other anemic patients on the basis of the reduced iron levels. In the absence of reticulocyte counts and specific tests for B19 viremia, the patients with B19 infection could not be differentiated from other anemic patients on clinical grounds. Furthermore, anemia was present prior to the detection of viremia in five of the six cases; this suggests that the anemia in these patients may not be entirely attributable to B19 infection.

Secondly, B19-specific antibody studies were not consistently performed at the time of the initial detection of the anemia. Moreover, it is unlikely that serological assays would have been very helpful given the fact that IgM antibodies were not found in any transplant recipient and IgG antibodies were noted in two PCR-positive individuals.

The role of myelotoxic drugs in the production of the anemia in these patients cannot be minimized (Table 2). The many drugs that liver transplant recipients take daily induce myelosuppression, hemolytic anemia, or gastrointestinal blood loss and include tacrolimus, carboplatin, and sulfamethoxazole (myelosuppression), dapsone (hemolytic anemia), and prednisone (gastrointestinal bleeding). Other commonly prescribed agents include ceftizoxime and furosemide. Additionally, many recipients are given erythropoietin, which has been reported to outstrip the reticuloendothelial system's ability to deliver iron for hemoglobin production, resulting in a unique form of iron-deficiency anemia.

TABLE 2. Causes of anemia in liver transplant patients

Adverse reactions to prescription drugs
Myelosuppression
Tacrolimus
Trimethoprim-sulfamethoxazole
Alpha interferon 2b
Carboplatin
Doxorubicin
Azathioprine
Gastrointestinal bleeding
Prednisone
Fludrocortisone
Hemolysis
Dapsone
Furosemide
Other
Erythropoietin
Ceftizoxime
Viral infections
B19
CMV
EBV
Hepatitis B virus
Hepatitis C virus
Other
Nutritional deficiency
Coagulopathy
Gastrointestinal blood loss (e.g., varices)
Decreased erythropoietin (renal disease)

An important component missing in this retrospective study is the concomitant analysis of bone marrow samples from the anemic patients who had no detectable B19 DNA within their blood. Had bone marrow samples been available to analyze for B19 DNA, our assay would have been able to detect it within these marrows, if the patient's anemia was due to this virus and not to drugs. However, bone marrow was not available, and since many patients were on anemia-inducing drugs, it is reasonable to assume that a portion of these anemias from patients with B19-negative serum could have been due to drugs, and not to B19 infection, as our assay has the necessary sensitivity to detect low-level virus.

Recently, Langnas et al. (12) have suggested a role for B19 in the development of fulminant liver failure and aplastic anemia. In this study, liver and serum samples from pediatric patients presenting with both fulminant liver failure and aplastic anemia were screened for the presence of B19 DNA by PCR. An indirect association between B19 and fulminant liver failure was found which would require corroboration. Using PCR alone to demonstrate the presence of B19 DNA within liver tissue, without the use of in situ hybridization analysis to localize B19 DNA within hepatocytes, is inconclusive, as the liver is a highly vascular organ, rich in hematopoietic cells, the major target for B19 function.

The retrospective nature of this study was limiting in several respects. First, it was likely that all of the patients' medical care

was not captured in the available electronic and hard copy medical records since many of these individuals lived in the surrounding communities or abroad. The effect of this was minimized by restricting the period of observation to 1 month prior to, and until 5 months after, the date of the sample. It would have been especially useful to corroborate active parvovirus B19 infection in the viremic persons with their reticulocyte counts. As it stands, the data do not differentiate sufficiently anemia due to B19 infection from anemia with other viral or nonviral causes. Lastly, this study lacks a control population to test the hypothesis that B19 viremia was present to the same extent or not in liver transplant patients irrespective of the hemoglobin level. In other words, the change in hemoglobin level from the baseline to nadir may be more important than the presence of anemia per se. Note is made of the one B19 DNA-positive patient (S) whose loss of viremia was documented by PCR; his hemoglobin plot showed a steady, then a precipitous, drop in hemoglobin which persisted well beyond the negative sample, 18 days later. This rapid decline in hemoglobin demonstrates the vulnerability of these patients to decreased erythropoiesis from any cause. It is not possible to state in this case the duration of B19 viremia prior to the observed drop in hemoglobin. Obviously, any control population would have to be matched for the causes of liver failure, to characterize the effect of preexisting viral infection versus ethanol-induced bone marrow suppression on the posttransplant anemia. Future studies will correct these deficiencies to better document the course of B19 infection and viremia in liver transplant recipients.

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