

Human Parvovirus B19 Detection in Asymptomatic Blood Donors: Association with Increased Neopterin Concentrations

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Serum neopterin concentrations were determined in 20,000 blood donations. For the 400 donations with neopterin concentrations above the 98th percentile and another 1200 donations with neopterin concentrations in the lower range, results of human parvovirus (HPV) B19 tests were compared. Infectious specimens were identified by dot blot hybridization assay and polymerase chain reaction (PCR) that used the outer primers and detected 1 pg of HPV B19 DNA, corresponding to $\sim 10^5$ copies of the genome, in the specimens and by a nested PCR that detected 1–10 fg of DNA, corresponding to 10^2 – 10^3 copies of the genome. Of 400 specimens with neopterin concentrations ≥ 12 nmol/L (98th percentile, current cutoff), 10 tested positive by dot blot hybridization assay (9 of these were confirmed by nested PCR). Among 1200 specimens with low neopterin concentrations (< 12 nmol/L), no specimen containing HPV B19 DNA was detected. These findings suggest an association between elevated neopterin concentrations and HPV B19 infectivity.

Human parvovirus (HPV) B19 is a small, nonenveloped, single-stranded DNA virus [1]. The virus causes a variety of human diseases, including erythema infectiosum (fifth disease) in children, acute or chronic arthritis in adults, hydrops fetalis and spontaneous abortion in early pregnancy, aplastic crisis in patients with hemolytic disorders, and persistent pure red blood cell aplasia in immunocompromised persons. HPV B19 exhibits a high degree of cell tropism. The replication of HPV B19 is restricted to differentiating and proliferating erythroid precursors in human bone marrow or fetal liver. A globoside, the carbohydrate portion of the red blood cell P antigen, is essential for virus absorption [2]. HPV B19 is regularly transmitted via the respiratory tract, and infection results in a brief viremic period 7–10 days after infection, with titers as high as 10^{14} viruses/mL [3].

Several studies have evaluated the risk of transmission of HPV B19 by blood products, because current virus-inactivating steps, such as solvent detergent treatment and heat treatments, seem

to be ineffective [4]. Two recent studies of plasma donations and serum samples of asymptomatic blood donors demonstrated a prevalence of HPV B19 DNA by polymerase chain reaction (PCR) of $\sim 1:800$ and $1:3915$, respectively [5, 6]. Anti-HPV B19 antibodies are found in 40%–60% of adults. Although the accidental transmission of HPV B19 will not be of concern in most cases, severe clinical manifestations can occur in certain risk groups, such as pregnant women, patients with hemophilia, persons with chronic or acquired immunodeficiency, and persons with elevated red blood cell production. A recent study [7] showed that HPV B19-associated infections might be more common in immunocompromised persons than previously anticipated. For their cohort of 60 bone marrow graft recipients, the authors reported an incidence of B19 infections of 15% and a B19-associated mortality rate of 7%.

Neopterin, a product of interferon- γ -activated monocytes/macrophages, is a sensitive indicator of cell-mediated immune activation; thus, acute viral infections accompany increased neopterin concentrations [8]. Neopterin concentrations can be measured by various methods, including high-performance liquid chromatography, RIA, and ELISA [9]. The last method is commonly used for screening blood donations, which has been mandatory in Austrian blood transfusion centers since 1994. Among healthy blood donors, higher neopterin concentrations are associated with older age, higher diastolic blood pressure, and higher body mass index. Smokers have lower neopterin concentrations than nonsmokers [10]. However, these differences are detectable within the normal range of neopterin levels

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and, therefore, are far below the increase of neopterin concentrations commonly seen during viral infections. In the present study, we sought to determine the associations between the detection of HPV B19 DNA and elevated neopterin concentrations in blood donor specimens.

Materials and Methods

Sample collection. HPV B19 was examined in 400 specimens with elevated neopterin concentrations that were found among 20,000 consecutive blood donations (women, 40.1%; men, 59.9%) in spring and summer of 1997. Of the 20,000 donations, 1200 specimens (same seasonal distribution) from donors of similar ages and sex that had normal neopterin levels served as controls. There were 400 whole-blood donations (taken in batches of ~50 from the same period) and 800 consecutive platelet donations.

DNA hybridization assay. The hybridization probe was generated using the plasmid pGEM2-B19 (donated by J. P. Clewley, Public Health Laboratory Service, Colindale, UK). A 4.5-kb DNA insert containing ~80% of the HPV B19 genome was amplified from pGEM2-B19 with the Expand TM Long Template PCR System (Boehringer Mannheim). A digoxigenin-labeled probe for hybridization was prepared by random-primed labeling (digoxigenin DNA labeling kit; Boehringer Mannheim). Sample preparation and hybridization were done as described by Hicks et al. [11]. In brief, 10- μ L serum samples were denatured in alkali in a microtiter plate for 15 min at room temperature and spotted with a blotting apparatus to a positively charged nylon membrane.

Hybridization was done overnight at 68°C. The membranes then were incubated with anti-digoxigenin alkaline phosphate antibody conjugate, and hybridization signal was visualized by addition of a chemiluminescent substrate (CSPD; Boehringer Mannheim). Serial dilutions of the plasmid pGEM2-B19 and HPV B19 DNA-positive serum samples served as positive controls; HPV B19 DNA serum samples that were negative by nested PCR served as negative controls. Detection limits of this method were 1 pg of HPV B19 DNA, corresponding to ~10⁵ copies of the genome, which could be detected after a 15-min exposure to x-ray film.

PCR. Total viral DNA was extracted and purified from 200 μ L of human serum by QIAamp Blood Kit (Qiagen) and eluted in 200 μ L of aqua dest. PCR was done as described by Clewley [12]. Primer sequences were chosen from the NS1 protein of HPV B19: P1 (1417–1434), 5'-GGG CCG CCA AGT ACA GGA-3' and P2 (2160–2141), 5'-AGG TGT GTA GAA GGC TTC TT-3' for outer PCR; and P3 (1498–1525), 5'-AAT GAA AAC TTT CCA TTT AAT GA-3', and P4 (2088–1965), 5'-TCC TGA ACT GGT CCC GGG GAT GGG-3' for internal (nested) PCR, respectively. PCR was performed with a solution containing 100 mM Tris-HCl (pH 8.8), 15 mM MgCl₂, 500 mM KCl, 1% Triton X-100, 10 mM each dNTP, 5 μ M primers P1 and P2 for outer PCR, 5 μ M primers P3 and P4 for nested PCR, respectively, and 1 U/ μ L taq polymerase (Boehringer Mannheim). Ten microliters of purified DNA were added to a final PCR volume of 50 μ L.

As positive controls, we used 1 pg/ μ L pGEM-B19 plasmid DNA standard and 10 μ L of DNA purified from an HPV B19-positive serum pool. Negative controls were 10 μ L of DNA purified from an HPV B19-negative serum pool and master mix without DNA.

Outer PCR conditions were as follows: 35 cycles of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C. Amplification with internal (nested) primers was performed in a reaction mix identical to that described above, except that 2 μ L was used if the initial reaction mixture was added as the template. Conditions for nested PCR were as follows: 35 cycles for 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C. Standard measures to minimize and detect contamination were followed. The amplification products were detected by electrophoresis of 5 μ L of the primary and 5 μ L of the secondary reaction mixture together in 1 lane in an ethidium bromide-stained 2% agarose gel and were 744 bp (outer PCR) and 591 bp (nested PCR) in size. The sensitivity of the outer PCR was determined by amplifying serial dilutions of the cloned HPV B19 DNA (derived from pGEM2-B19) and corresponded to the sensitivity of the DNA hybridization assay, whereas the nested PCR was ~100–1000 times more sensitive.

ELISA. For detection of HPV B19-specific IgG and IgM antibodies, we used an EIA based on a recombinant VP2 protein (Biotrin). This IgG EIA detects the World Health Organization HPV B19 IgG international standard (IS), in which the cutoff value corresponds to an IS level of 3–5 IU/mL.

Neopterin testing. For neopterin measurement, we used a commercial ELISA (ELitest neopterin; BRAHMS) and followed the manufacturer's instructions in the automated version (interassay coefficient of variation, 6.54%; limits of detection without dilution, 2–50 nmol/L). The normal range of neopterin concentrations was chosen as the 98th percentile of the distribution of neopterin concentrations of all donations (~100,000) for 1997 and 1998 (12.0 nmol/L).

Statistical analysis. We used Fisher's exact test to compare frequencies of HPV B19-positive results in specimens with elevated and normal neopterin concentrations.

Results

Of 400 specimens with elevated neopterin levels, 10 were positive by dot blot hybridization and, in all but one case, were confirmed by nested PCR (table 1). Among 1200 specimens with normal neopterin levels, no HPV DNA-positive specimens were detected. All positive HPV B19 DNA results were found within the group with elevated neopterin concentration ($P < .001$). Thus, by using the 98% cutoff value of the distribution of neopterin concentrations, all 10 HPV B19 DNA-positive specimens were detected. There were no DNA-positive results within the group of neopterin-negative specimens.

Discussion

In the present study, we evaluated a method for detection of HPV B19 that has been used by several investigators and was found to be reliable for screening blood products. We evaluated the samples by the nonspecific marker neopterin and correlated the results with the DNA dot blot hybridization technique. As a reference method for specificity of HPV B19 DNA, we chose a nested PCR technique. Whether PCR reactivity indicates infectivity in all cases is unknown at present, and performing

Table 1. Human parvovirus (HPV) B19 DNA dot blot hybridization, nested polymerase chain reaction (PCR), IgG and IgM antibodies, and neopterin concentrations in asymptomatic blood donors.

Specimen	HPV B19 DNA dot blot hybridization	Nested PCR ^a	IgG antibodies	IgM antibodies	Neopterin concentration, nmol/L
1	+	+	+	-	18.7
2	+	+	W+	+	14.0
3	+	++	+	-	14.8
4	+	++	+	-	13.3
5	+	+	BL	-	13.1
6	+	+	+	-	14.3
7	+	++	+	-	14.6
8	W+	++	+	-	20.8
9	W+	-	BL	-	22.8
10	W+	+	+	-	12.9

NOTE. -, Negative; +, positive (i.e., >3–5 IU/mL); BL, borderline (range of values, cutoff value minus 10%); W+, weak positive (i.e., 3–5 IU/mL).

^a For nested PCR, “+” denotes a sample that was negative in the first-round PCR and positive in the nested PCR; “++” denotes a sample that was positive in both the first-round and nested PCRs.

PCR for all blood donations increases the costs of blood products and demands large logistic efforts.

We found significant association of DNA dot blot hybridization results and neopterin levels. The sensitivity of the DNA dot blot hybridization method allows for detection of 1 pg of serum (corresponding to $\sim 10^5$ copies of the genome/10 μ L). All dot blot–positive samples also clearly had elevated neopterin levels, whereas no HPV B19 PCR positivity was found in samples with normal neopterin concentrations. This observation demonstrates a coincidence between presence of HPV and increased neopterin concentration, likely because of immune stimulation by HPV. In a previous study [13], we found a statistically significant relationship between neopterin elevation and the occurrence of IgM antibodies against HPV B19. These findings corresponded with results obtained for cytomegalovirus, Epstein-Barr virus, and other viral infections in humans and animal model systems [13, 14].

Neopterin concentrations increase within days after virus inoculation before specific IgM and IgG antibodies can be detected [14, 15]. Thereafter, neopterin concentrations decrease simultaneously with the decrease of viremia and the increase of specific IgG antibodies that may occur within days to weeks. In case of persistent infections like human immunodeficiency virus (HIV), neopterin concentrations follow a similar pattern; however, after seroconversion, neopterin concentrations, in most cases, do not return to the normal range. In contrast, about three-quarters of asymptomatic HIV-seropositive persons present with increased concentrations that correlate with degree of viremia [9]. Until now there has been little knowledge about neopterin in the late state of infections or in persistent infections in patients who are not immunocompromised.

In a previous study [13], we used IgM antibody positivity as the only marker for infectivity and compared it with neopterin concentrations. Because of the study design, it was not possible to identify donors in the pre-IgM state who were viremic and

had high neopterin concentrations or with persistent HPV infection with or without IgM antibodies, neutralizing or non-neutralizing IgG antibodies, and viremia.

In the present study, we obtained additional information about the late course of infection by identifying viral DNA with the use of PCR. IgM antibodies were detected in 1 of 10 and IgG antibodies in all specimens. This pattern suggests late state or persistence of infection with disappearing IgM antibodies, although viral DNA was still detectable. Increased neopterin concentration may be a sign of infection activity. One specimen showed only reactivity in dot blot hybridization, whereas it remained negative by nested PCR. In this case, we cannot exclude a nonspecific cross-reaction. However, the highly elevated neopterin concentration may suggest another cause of immune activation.

From our results, we conclude that measurement of neopterin concentrations provides additional immunologic information about the state of HPV B19 infection. The nucleic acid–amplifying testing (NAT) alone may also detect noninfectious immunologic silent DNA, in addition to infectious viruses. By combining neopterin and NAT, one should be able to identify HPV B19 infectious patients. Regarding screening strategies for identifying HPV B19 infectious blood donations, neopterin testing alone may be adequate to minimize the risk of HPV B19 transmission via blood products.

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