Evidence for the transmission of parvovirus B19 in patients with bleeding disorders treated with plasma-derived factor concentrates in the era of nucleic acid test screening

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BACKGROUND: Parvovirus B19 (B19V) is a small, nonenveloped virus that typically causes a benign flu-like illness that occurs most frequently in childhood. The virus is resistant to current viral inactivation steps used in the manufacture of antihemophilic factor concentrates and B19V transmission through these products has been documented. Since 2000, B19V nucleic acid test (NAT) screening of plasma pools has been implemented to further decrease the viral burden in these products, but no study has examined populations using these products to assess the impact of the screening on B19V transmission.

STUDY DESIGN AND METHODS: Blood specimens obtained from participants of a surveillance system established in federally supported specialized bleeding disorders clinics were used in a B19V seroprevalence study.

RESULTS: A total of 1643 specimens from 1043 participants age 2 to 7 years born after B19V NAT screening was implemented were tested. Age-specific prevalence rates were generally higher for subjects exposed to either plasma-derived products alone or in combination with other products compared to subjects with no exposure to antihemophilic products. Overall, compared to participants unexposed to blood or blood products, those exposed to plasma-derived products alone were 1.7 times more likely to have antibodies to B19V ($p = 0.002$).

CONCLUSION: These results are consistent with continued B19V transmission through plasma-derived factor concentrates. Effective viral inactivation and detection processes are needed to protect users of these products from infection with B19V or other new or emerging viruses.

Parvovirus B19 (B19V) is a ubiquitous small nonenveloped DNA erythrovirus and infects up to 50% of persons by the age of 15 years.[1] Most persons have only a mild rash as a child or young adult (erythema infectiosum), and complications are rare. However, infection during pregnancy can cause fetal death.[2,3] In addition, the infection can produce severe anemia in persons with hemolytic anemias as well as acute and subacute joint swelling in as many as 3% to 10%

ABBREVIATIONS: B19V = parvovirus B19; BMI = body mass index; HTC(s) = hemophilia treatment center(s); ROM = range of motion; UDC = Universal Data Collection.

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of children and up to 50% to 60% of adults, particularly women.[1,4] The development of anti-B19V immunoglobulin (Ig)M may be associated with elevated serologic variables characteristically associated with rheumatoid arthritis[5] and up to 17% of patients may develop a chronic arthritis after the infection that is clinically similar to rheumatoid arthritis.[4,6] In fact, B19V has been proposed as a causative agent for rheumatoid arthritis by some authors.[7-9] One study reported fluctuating levels of antiparvovirus IgM in conjunction with disease flare-ups in children with juvenile rheumatoid arthritis.[10]

The relatively high prevalence of B19V infection in the general population combined with the large number of blood donations used in the manufacture of plasma-derived factor concentrates virtually assures some contamination.[11,12] Resistance to inactivation and elimination techniques by available virucidal processes has led to the transmission of B19V by plasma-derived factor replacement products used to treat hemophilia.[13,14]

In an earlier report, we found that the seroprevalence of IgG antibodies to B19V was much higher among very young (age 2-7 years) hemophilia patients exposed to plasma-derived products compared to those not exposed.[15] B19V seroprevalence among those using only recombinant products was not elevated above the baseline rate. In addition, joint range-of-motion (ROM) loss was greater among subjects who were seropositive compared to those who were seronegative, suggesting that at least some of the joint disease may have resulted from B19V delivered intravenously (IV) from contaminated product. This association was significant after controlling for the effect of independent variables that included the number and severity of bleeding events, the total exposure to factor concentrates, and body mass index (BMI).

The contribution of B19V to the pathogenesis of hemophilic arthropathy remains unknown. Zakrzewska and colleagues[16] detected B19 DNA in the synovium of 20 of 65 (31%) patients with hemophilic arthropathy ages 34 to 76 years and 5% of controls who underwent synovectomy. Among the 38 patients with hemophilia for whom serum specimens were available, all showed serologic evidence of B19V infection. The authors postulated that bleeding in the joints allows the virus to reach the synovial tissue. Whether the presence of the virus in the joint incites an inflammatory response that exacerbates hemophilic arthropathy remains unknown.

In 2000, manufacturers began voluntary "in-process" nucleic acid test (NAT) screening of plasma pools for B19V to ensure the quality of source plasma and solvent/detergent-treated pooled plasma.[17] Plasma minipools containing more than a threshold concentration of B19V were withheld from fractionation. There is some evidence that this process has been effective in reducing transmission of B19V. For example, in a careful investigation of a patient with a factor-transmitted infection resulting from an infusion of product from two lots, B19V was found only in product from the lot not subjected to NAT screening.[18] A recent Food and Drug Administration (FDA) study comparing B19V prevalence in various plasma-derived products before and after the institution of minipool B19V NAT screening concluded that the process effectively lowered levels of B19V to fewer than 10^3 genomic equivalents in all products and to undetectable levels in 81% of tested lots.[19] However, to date there has not been a systematic study of the effectiveness of this strategy in reducing B19V transmissions among recipients of these products.

The continuing availability of blood specimens from young children with bleeding disorders participating in a Centers for Disease Control and Prevention (CDC)-sponsored national surveillance project provided the opportunity to repeat the earlier seroprevalence study to determine whether B19V NAT screening has decreased the risk of B19V transmission in plasma-derived antihemophilic factor products. We hypothesized that after institution of B19V NAT screening, children with bleeding disorders enrolled in our surveillance project and exposed to plasma-derived factor concentrate would have a similar rate of B19V infection and measurable antibodies compared to those not exposed to factor concentrate or those exposed only to recombinant factor concentrates.

MATERIALS AND METHODS

Since 1998, in collaboration with specialized hemophilia treatment centers (HTCs) in the United States, CDC has sponsored a surveillance system called the Universal Data Collection (UDC) project.[20] People with bleeding disorders who receive care in these centers are eligible to participate in the surveillance. Demographic, treatment, and outcome data are collected on all participants by care providers and a sample of blood is collected for infectious disease testing and storage for blood safety investigations at CDC. The surveillance has been approved by the CDC and all local investigational review boards. All participants (or parents of minor children) gave informed consent. Only participants born after January 1, 2001, were eligible for this study so that any treatment with factor concentrates would occur after the implementation of B19V NAT screening.

Data collection

Data were collected on standardized forms according to definitions and guidelines provided by CDC. Pertinent to this study, data collected at enrollment included the month and year of birth, race/ethnicity, sex, type of bleeding disorder, and whether there had been any previous exposure to factor concentrates or to blood products (includes blood plasma or cellular fractions and factor concentrates prepared from human plasma). At enroll-

ment and at each subsequent clinic visit, the following additional data were collected: height, weight, highest inhibitor titer obtained since the last visit and whether immune tolerance therapy for an inhibitor was being used, the number of bleeding episodes in the 6-month period before the visit, treatment schedule (the last three items reflective of treatment intensity), names of treatment products used since the last visit, and measurements of the ROM of 10 joints (shoulders, elbows, hips, knees, and ankles) as a potential indicator of B19V contribution to arthropathy.

For the analysis, age was calculated as of the date of the blood specimen. Parent-reported race/ethnicity was categorized as white, black, or other for those not of Hispanic ethnicity; those of Hispanic ethnicity were categorized as Hispanic regardless of race. The type of bleeding disorder was categorized as hemophilia A, hemophilia B, von Willebrand disease (VWD), or other (i.e., other factor deficiencies such as Factor [F]VII, FXI, FXIII).

Height and weight measures were converted to BMI by dividing weight in kilograms by height in meters squared. Participants with hemophilia whose highest inhibitor titer was 1 or more Bethesda unit or who were receiving immune tolerance therapy were defined as having a current inhibitor. Patients were categorized as receiving either episodic (factor infusions in response to a bleed) or prophylactic (factor infusions on a regular basis to prevent bleeding) therapy. As a measure of disease severity, the number of bleeds (according to infusion logs or parent recall) was categorized as none, one to three, or four or more bleeds in the previous 6 months. ROM measures were obtained by trained physical therapists or other care providers trained and certified in the use of standard methods detailed in a CDC-provided manual and video. These measures were used to calculate for each participant an overall summary measure of joint motion that reflects the subject's proportion of normal ROM based on published reference data.[21]

Blood samples and testing

Blood specimens were obtained at each UDC visit for eligible subjects. Since subjects could participate annually in UDC, some participants had multiple specimens available. Most participants with hemophilia had been exposed only to recombinant factor concentrates. All specimens obtained from children unexposed to blood or antihemophilic factor products and from those exposed to plasma-derived products were tested. Because the number of available samples from children who had been exposed only to recombinant products was far greater than the number from children with no product exposure (the reference group), we maximized testing efficiency by randomly selecting from the recombinant-only group approximately the same number of samples available in the age group with the largest number of unexposed patients (n = 125).

Because acute-phase markers of infection are relatively transient, they were not considered appropriate for this study. Therefore, all available serum samples from eligible participants were tested for the presence of IgG anti-B19V using an enzyme-linked immunosorbent assay test (Biotrin International Ltd, Dublin, Ireland). The test uses purified recombinant VP2 protein coated wells to detect the IgG antibodies in human serum or plasma. The high degree of homology of the VP2 capsid protein between genotypes results in a high level of antigenic cross-reactivity between the genotypes; however, the test does not discriminate between the different genotypes. All testing was performed according to instructions provided by the manufacturer and all readings were within manufacturer-supplied quality control guidelines.

Statistical analysis

Data on lifetime exposure to factor concentrates and blood products along with the list of all products used before each clinic visit were used to categorize participants into one of four exposure groups: 1) subjects with no exposure to either factor concentrates or blood products, 2) subjects exposed to recombinant factor concentrates only, 3) subjects exposed to both recombinant and plasma-derived factor concentrates, and 4) subjects exposed to plasma-derived factor concentrates only.

Relations between patient demographic and clinical characteristics and both product exposure category and B19V seroprevalence were examined using chi-square tests. The seroprevalence of B19V for each risk subgroup defined by age and product exposure category was calculated by dividing the number of patients who tested positive by the total number of patients tested in each subgroup. Confidence intervals (CIs) for the prevalence estimates were calculated according to the efficient score method (corrected for continuity) described by Newcombe.[22] Prevalence estimates for the exposed patient subgroups that were outside the 95% CIs for the prevalence in the unexposed patient subgroup were considered to be significantly different.

The independent effect of exposure category on B19V prevalence was assessed for statistical significance using logistic regression. Prevalence odds ratios (ORs) were calculated for each exposure category relative to the subgroup unexposed to factor or blood products from a regression model that also included variables for age, sex, race/ethnicity, bleeding disorder, bleeding frequency, inhibitor status, and year of blood sample.

Finally, the independent effect of B19V positivity on joint ROM was assessed using a multiple linear regression model that used the summary ROM measure as the dependent variable and included all of the terms included

in the logistic model as well as BMI as independent variables. All analyses were performed using computer software (SAS, Version 9.2, SAS Institute, Cary, NC) and p values of not more than 0.05 were considered significant.

RESULTS

As of August 2010, there were 1863 eligible UDC participants who had donated a blood specimen at each of 3970 visits. We excluded specimens from subjects with missing data on either current product exposures or lifetime exposure to blood or blood products (n = 311) as well as specimens from subjects with exposure to blood or blood component transfusion only (n = 4). After random selection of specimens from participants who had been exposed to recombinant products only, the final study sample consisted of 1643 specimens from 1043 participants (Fig. 1). Among the subjects, 61% had one specimen, 26% had two specimens, 9% had three specimens, and 4% had four or more specimens tested.

The demographic and clinical characteristics of the participants are shown in Table 1. Approximately two-thirds of participants had hemophilia, nearly one-third had VWD, and a small proportion had other factor deficiencies. The majority of subjects were male, reflective of the hemophilia population, while the distribution of race and ethnicity was similar to that of the population receiving care in the US HTCs. Two-thirds of the study subjects were using treatment products only in response to a hemorrhage, nearly one-half of the subjects had not experienced a bleeding event in the 6 months before the clinic visit, and just under 6% had a current hemophilia inhibitor.

As expected, the distributions of product exposures varied across most of the patient characteristics (Table 1). For example, most factor-exposed patients with hemophilia used recombinant products whereas those with VWD used plasma-derived products since recombinant products contain no von Willebrand factor. This difference is also reflected in the markedly higher use of plasma-derived concentrates by females, most of whom had VWD.

The prevalence of B19V antibodies according to patient characteristics is also shown in Table 1. As expected, B19V prevalence increased with age but was not associated with any of the other demographic or clinical characteristics.

Figure 2 shows the prevalence of B19V antibodies by age and product exposure category. CIs for the prevalence among subjects unexposed to factor concentrates indicate the values within which the true prevalence of the unexposed population lies with a certainty of 95%. The B19V prevalence values for each age group exposed only to recombinant products fall within the CI for each corresponding unexposed age group (Fig. 2). On the other hand, the prevalence values for subjects in four of the six age groups exposed either to both plasma-derived and recombinant products or to plasma-derived products alone are higher than the upper CI boundary for unexposed subjects. Furthermore, among 7-year-olds exposed to plasma-derived products only, the prevalence is near the upper CI boundary for unexposed subjects (Fig. 2).

Using logistic regression, we assessed the independent effect of product exposure on the likelihood of B19V positivity. After the effects of all of the patient characteristics as well as for the year in which the blood sample was obtained to account for trends in prevalence rates over time were adjusted for, the odds of positivity were 70% higher (OR, 1.7; p = 0.002) among subjects exposed to plasma-derived products alone relative to those unexposed (Table 2). There was no significant difference in the odds of positivity between either of the other product

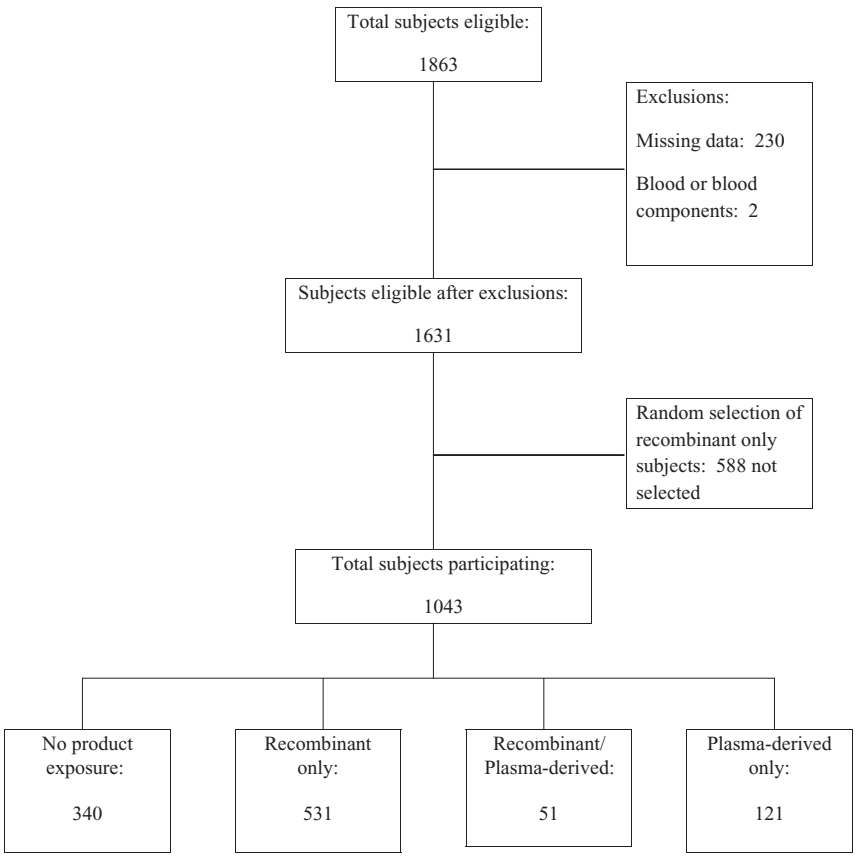

Fig. 1. Subject selection and exclusion algorithm.

TABLE 1. Characteristics at enrollment of 1043 subjects with bleeding disorders and relations with products used and B19V serostatus*

Characteristic	Total	Product type				Human B19V positive
		No product	Recombinant	Recombinant and plasma derived	Plasma derived	
Age (years)						
2	129 (12.4)	40 (31.0)	75 (58.1)	5 (3.9)	9 (7.0)	27 (20.9)†
3	159 (15.2)	54 (34.0)	79 (49.7)	5 (3.1)	21 (13.2)	41 (25.8)
4	167 (16.0)	62 (37.1)	77 (46.1)	6 (3.6)	22 (13.2)	65 (38.9)
5	174 (16.7)	63 (36.2)	77 (44.3)	11 (6.3)	23 (13.2)	74 (42.5)
6	192 (18.4)	67 (34.9)	94 (49.0)	7 (3.6)	24 (12.5)	94 (49.0)
7	222 (21.3)	54 (24.3)	129 (58.1)	17 (7.7)	22 (9.9)	121 (54.5)
Race/ethnicity						
White	634 (60.8)	222 (35.0)	318 (50.2)	23 (3.6)	71 (11.2)	247 (39.0)
Black	125 (12.0)	33 (26.4)	69 (55.2)	9 (7.2)	14 (11.2)	54 (43.2)
Hispanic	194 (18.6)	63 (32.5)	94 (48.4)	12 (6.2)	25 (12.9)	82 (42.3)
Other	90 (8.6)	22 (24.4)	50 (55.6)	7 (7.8)	11 (12.2)	39 (43.3)
Sex						
Male	870 (83.4)	216 (24.8)‡	528 (60.7)	49 (5.6)	77 (8.9)	355 (40.8)
Female	173 (16.6)	124 (71.7)	3 (1.7)	2 (1.2)	44 (25.4)	67 (38.7)
Bleeding disorder						
Hemophilia A	537 (51.5)	58 (10.8)‡	413 (76.9)	48 (8.9)	18 (3.4)	226 (42.1)
Hemophilia B	141 (13.5)	21 (14.9)	107 (75.9)	3 (2.1)	10 (7.1)	50 (35.5)
VWD	322 (30.9)	233 (72.4)	0	0	89 (27.6)	122 (37.9)
Other§	43 (4.1)	28 (65.1)	11 (25.6)	0	4 (9.3)	24 (55.8)
Treatment type						
Episodic	713 (68.4)	338 (47.4)‡	246 (34.5)	32 (4.5)	97 (13.6)	285 (40.0)
Prophylaxis	330 (31.6)	2 (0.6)	285 (86.4)	19 (5.8)	24 (7.2)	137 (41.5)
Bleeds in past 6 months						
None	462 (44.3)	223 (48.3)‡	181 (39.2)	15 (3.2)	43 (9.3)	181 (39.2)
1 to 3	363 (34.8)	69 (19.0)	229 (63.1)	14 (3.9)	51 (14.0)	151 (41.6)
4+	218 (20.9)	48 (22.0)	121 (55.5)	22 (10.1)	27 (12.4)	90 (41.3)
Inhibitor‖ status						
Current inhibitor	60 (5.8)	0‡	26 (43.3)	26 (43.3)	8 (13.4)	28 (46.7)
No inhibitor	983 (94.2)	340 (34.6)	505 (51.4)	25 (2.5)	113 (11.5)	394 (40.1)

* Data are reported as number (%). Total does not sum to 100% due to missing data.
† $p < 0.05$ for differences in the proportion of persons positive for human B19V at baseline across levels of the characteristic by chi-square test.
‡ $p < 0.05$ for differences in the distribution of products used across levels of the characteristic by chi-square test.
§ Rare factor deficiencies.
‖ Hemophilia patients only.

exposure groups compared to the unexposed group. As expected, the prevalence increased significantly among older subjects relative to the youngest participants. The increasing prevalence ORs for subjects tested in later years of the study provide evidence for population trend of increasing B19V prevalence in 2008 to 2010 that was significant independent of the characteristics of the bleeding disorders population.

Finally, linear regression was used to evaluate whether subjects with hemophilia who were B19V positive had less joint function than those who were B19V negative. After adjustment for age, number of bleeding episodes, BMI, and prophylaxis status, there was no significant difference in mean summary joint function by B19V status.

DISCUSSION

Because screening of blood donors can miss infections during the period between exposure and development of detectable antibodies, the safety of plasma products rests on effective viral detection and inactivation or elimination. In the case of B19V, blood donors can have a high viremic load without overt signs of infection. In addition, there is evidence that B19V viremia can persist for years in healthy individuals.[23] These characteristics combined with a prevalence of B19V of approximately 1% among blood donors lead to a high probability that plasma pools will be contaminated.[11,12] Because B19V capsid is resistant to the viral inactivation processes currently used in the manufacture of plasma derivatives, the strategy of screening plasma pools for B19V and rejecting from fractionation plasma pools containing a concentration higher than 1×10^4 genome equivalents per milliliter of B19V DNA has been implemented by product manufacturers as a voluntary in-process control step.

Compared with the results from our previous study,[24] our current findings suggest that this strategy appears to have led to decreased transmission of B19V through these products; however, some transmission continues to occur.

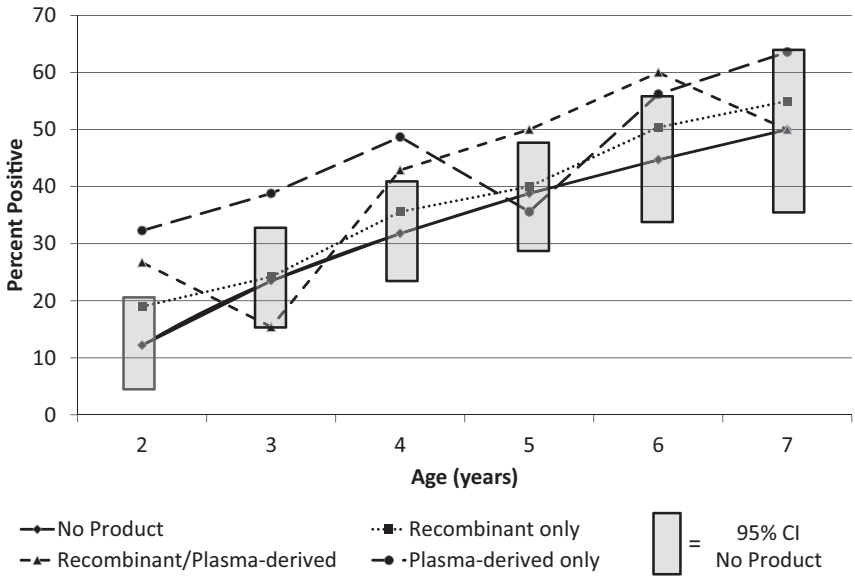

Fig. 2. B19V seroprevalence by age and product exposure category.

TABLE 2. Characteristics independently associated with B19V positivity among 1043 subjects with bleeding disorders

Characteristic	OR* (95% CI)	p value
Exposure group		
None	Reference	
Recombinant only	1.1 (0.7-1.6)	0.7
Recombinant and plasma derived	1.0 (0.6-1.8)	0.9
Plasma-derived only	1.7 (1.2-2.4)	0.002
Age (years)		
2	Reference	
3	1.3 (0.8-2.0)	0.001
4	2.0 (1.3-3.1)	0.8
5	2.3 (1.5-3.6)	0.2
6	3.0 (1.9-4.8)	<0.001
7	3.2 (2.0-5.2)	<0.001
Year specimen drawn		
2003	Reference	
2004	3.3 (0.7-15.3)	0.7
2005	3.7 (0.8-17.0)	0.9
2006	3.3 (0.7-15.1)	0.6
2007	3.3 (0.7-15.0)	0.6
2008	5.6 (1.2-25.0)	0.005
2009	5.2 (1.2-23.3)	0.03
2010	8.3 (1.8-39.3)	<0.001

* ORs from a logistic model that also accounted for differences in the distribution of sex, race, treatment type, bleeding frequency, and hemophilia inhibitor status.

We found that young children with bleeding disorders exposed only to plasma-derived factor concentrates were 70% more likely to have antibodies to B19V than those unexposed to any products.

While lower than the 7.6 times increased likelihood of B19V antibodies that we reported among children with hemophilia who had received plasma-derived products during the period 1993 to 2001,[15] the reason for the increased seroprevalence among similarly exposed children in this cohort born after implementation of NAT screening of plasma pools is not clear. An FDA study found that NAT screening of plasma lowers B19V levels but does not completely eliminate the virus.[19] The same study noted that B19V IgG, also present in plasma pools and shown in other studies to protect susceptible individuals from infection when exposed to product containing B19V DNA,[24] is virtually eliminated from the final product, apparently by steps in the manufacturing process. It is possible that multiple exposures to plasma-derived products contaminated with low levels of B19V but depleted in B19V IgG may have caused infections in B19V-naïve subjects that led to the higher prevalence that we observed among users of these products.

Because B19V NAT screening was not initiated at the same time for all products, it is possible that some patients may have received product that had not been screened. For example, investigation of the product-associated case identified among UDC participants in 2001 revealed the source of this infection was product from a lot manufactured from unscreened plasma.[18] Also, in the FDA study,[19] approximately 50% of the tested lots of recovered plasma used to make one product during the period 2001 to 2004 had excessive viral loads leading the investigators to conclude that screening was not being performed. However, if delayed implementation of screening alone was responsible for our findings, we would have expected to see higher prevalence rates among persons exposed during the earlier period of the current study. In fact, the prevalence was highest for the years 2008 to 2010 even after adjustment for patient age and other characteristics (Table 2). The latter finding is most likely explained by the fact that small epidemic outbreaks of B19V typically occur in the general population at intervals of a few years.[25]

In a previous study we demonstrated a decreased overall level of joint ROM for boys with hemophilia who were B19V antibody positive compared to those who were negative.[15] We hypothesized that IV infusions of B19V-contaminated factor during active bleeding in a joint

could have provided a route for direct inoculation and synovial infection. Other studies have demonstrated B19V DNA in the synovial tissues of patients with arthritis but it is not known whether the virus is causal, exacerbating, or opportunistic in these cases.[26] The pathogenesis of blood-induced arthropathy in hemophilia includes invasion and destruction of cartilage by hyperplastic activated synoviocytes. In vitro studies have demonstrated that the B19V capsid mediates fibroblast-like synoviocyte activation[27] and that B19V-containing sera induce invasive properties in normal synoviocytes.[28] We found no evidence of decreased joint ROM among B19V-exposed compared with unexposed boys with hemophilia in this study. However, the earlier patient cohort had among its members a much higher proportion of product-associated infections that would be more likely to lead to synovial seeding than infections acquired via the normal respiratory route. In addition, only approximately 15% of the boys with hemophilia in the current cohort had been exposed to plasma-derived products versus 30% in the previous study.[15] Therefore, the lack of difference in joint mobility between exposed and unexposed hemophilia patients in the current study might be expected given the much lower rate of presumed product-associated infections found in the current patient cohort.

Our study had some limitations that should be considered when evaluating these results. First, since we had no information on clinical illness among UDC participants, it is unknown whether the serologic evidence of B19V that we measured correlated with a symptomatic infection. B19V infections that occur during the young age period represented by the subjects in this study are usually quite mild and often are undiagnosed. In the absence of clinical signs of disease it might be questioned whether the serologic evidence of excess B19V exposure that we found represents immunologic response to noninfectious B19V viral particles that may have been present in these products. We are not aware of any reported evidence that this phenomenon has occurred with B19V but we cannot rule out the possibility.

Second, we relied on data collected by care providers to assign lifetime exposure to blood and blood products for the subjects in our study. It is possible that some exposures may have been missed or incorrectly recorded. For example, some patients may have been infected through undocumented transfusions with blood components. However, because the participants of this study were very young and receiving care in specialty care clinics it is likely that most had received all of their care since birth from the same care provider who would be likely to have complete records of these exposures. In any case, there is no reason to think that such undocumented transfusions would be more likely to occur in patients that received plasma-derived concentrates. As another example of exposure misclassification, if some patients in the unexposed group had actually used plasma-derived products, this error would have had a tendency to lower the effect that we observed. Therefore, to the extent that product exposures were misclassified, we may have underestimated the strength of the association between plasma-derived factor use and B19V exposure.

We were missing complete exposure information for 311 samples from 230 subjects. Based upon the information that we did have, 83% of these samples would have been classified as either unexposed to any product (47%) or exposed to recombinant product only (36%). When classified this way, the age-specific B19V prevalence rates were similar to those reported for subjects with complete exposure information (results not shown). Therefore, we do not believe that our results were biased by exclusion of these samples from the analysis.

The current strategy of B19V NAT plasma minipool screening may not be completely effective in preventing transmission of B19V and, more importantly, would not detect other new or emerging viruses with similar characteristics that could pose a hazard to the users of these products. For example, a recent study of a cohort of persons with hemophilia documented transmission through plasma-derived factor concentrates of a newly discovered human parvovirus (PARV4) that was most commonly associated with rashes and exacerbation of hepatitis.[29] This virus, along with other non–lipid-enveloped viruses such as hepatitis A and E, is also resistant to current viral inactivation steps and would not be detected by the current NAT screening. It is important to note that while hepatitis screening implemented in the 1970s resulted in some decreased transmission of HIV through factor concentrates, it was not until effective viral inactivation methods were applied to the manufacturing process in 1985 that the risk of transmission was eliminated.[30] Our findings add to the growing body of evidence that viral inactivation steps effective against these types of viruses are urgently needed.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to the manuscript submitted to **TRANSFUSION**.

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