

Prevalence of IgG antibodies to human parvovirus B19 in haemophilia children treated with recombinant factor (F)VIII only or with at least one plasma-derived FVIII or FIX concentrate: results from the French haemophilia cohort

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Summary. Human parvovirus B19 (B19) has been transmitted by some brands of virally attenuated plasma-derived factor VIII (FVIII) or IX (FIX) concentrates. To quantify the differences of human parvovirus B19 risk transmission between albumin-stabilized recombinant factor and plasma-derived factor, we studied the prevalence of IgG antibodies to B19 (anti-B19) in 193 haemophilic children between 1 and 6-years of age who had previously been treated with albumin-stabilized recombinant FVIII only ($n = 104$), and in children previously treated with solvent/detergent high-purity non-immunopurified and non-nanofiltered FVIII or IX concentrates ($n = 89$). Association between the prevalence of anti-B19 and the treatment group was analysed

using multivariate logistic regression. Age, severity and type of haemophilia, number of cumulative days of exposure to factor VIII or IX, previous history of red blood cells or plasma transfusion were considered as potential confounding variables. A higher prevalence of anti-B19 was found in children previously treated with solvent/detergent high-purity non-immunopurified and non-nanofiltered FVIII or IX concentrates than in children treated with albumin-stabilized recombinant FVIII only (OR: 2.2·3; CI: 7·9–62·8), independently of the other factors studied.

Keywords: haemophilia, clotting factor concentrates, recombinant FVIII, human parvovirus B19, viral safety.

Patients with haemophilia A or B are treated either with plasma-derived products or, more recently, with recombinant products. The plasma-derived products are prepared from large pools of plasma (usually more than 3000 donors), creating a risk of viral transmission. Viral inactivation of all factor concentrates, by pasteurization, wet-heating, or solvent-detergent treatment alone or in combination with dry-heat, has virtually eliminated the risk of human immunodeficiency virus (HIV) and hepatitis B or C virus transmission. However, several reports of human B19 parvovirus (B19) active infection or seroconversion after infusion of clotting factor concentrates (Bartolomei Corsi *et al.*, 1988; Lyon *et al.*, 1989; Azzi *et al.*, 1992), together with the identification of B19 DNA in various

virally inactivated products (Schwarz *et al.*, 1991; Lefrère *et al.*, 1994; Saldanha & Minor, 1996) and a higher-than-baseline seropositivity rate for this infection in patients treated with plasma-derived clotting factor concentrates (Laurian *et al.*, 1994a; Flores *et al.*, 1995; Peerlinck *et al.*, 1995; Eis-Hübinger *et al.*, 1996a), suggests that the risk of viral transmission (particularly for non-enveloped viruses such as B19) has not been totally eliminated using virucidal methods. Reported detection rates of B19 DNA in plasma-derived clotting factor concentrates range from 20% to 100% of batches (Zakrzewska *et al.*, 1992; McOmish *et al.*, 1993; Lefrère *et al.*, 1994; Saldanha & Minor, 1996; Eis-Hübinger *et al.*, 1999). In 1993, first-generation recombinant factor VIII (FVIII), generated from genetically engineered mammalian cells, was commercialized using human albumin as the stabilizer in the final product (Lusher *et al.*, 1993; Bray *et al.*, 1994). Albumin solutions are routinely pasteurized by heating at 60°C for 10 h in the liquid state to

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inactivate blood-borne viruses. Lefrère *et al* (1995) could not detect B19 DNA in any of the 29 albumin batches they tested, suggesting that the manufacturing process contained one or several steps able to remove B19 DNA. However, Saldanha & Minor (1996) showed that pasteurization of albumin may fail to remove B19 DNA, even if the level is greatly reduced. Eis-Hübinger *et al* (1996a) have demonstrated that recombinant coagulation FVIII products can contain B19 DNA; they speculated that differences in the albumin manufacturing process (alcohol fractionation, purification by chromatography or filtration) could explain the observed discrepancies. To our knowledge, few clinical studies of the risk of B19 transmission via recombinant products have been published. In a published pharmacovigilance study, 2 out of 16 susceptible patients treated with albumin-stabilized recombinant FVIII showed reliable B19 seroconversion (Aygören-Pürsün & Scharer, 1997); the authors ascribed these seroconversions to possible B19 contamination of the albumin excipient, although, in a letter, Prowse *et al* (1998) considered that this represented community-acquired infection.

Parvovirus B19 is important as a marker virus for potential transmission of more pathogenic viruses, even though B19 infection is generally asymptomatic, or causes very mild disease. However, HIV-infected haemophilia patients, who are frequently immunocompromised, run the risk of persistent infection (Musiani *et al*, 1995). Furthermore, various reports highlight the possibility of severe human B19 parvovirus infection transmitted by plasma-derived concentrates, even in immunocompetent haemophilia patients (Coumau *et al*, 1996; Yee *et al*, 1996). Studies of the prevalence of specific antibodies showed that B19 infection was often acquired in childhood and that 60% or more of adults are seropositive (Cohen, 1995). The French haemophilia cohort (Calvez *et al*, 2001) gave us the opportunity to study the prevalence of IgG antibodies to B19 (anti-B19) in two groups of children with haemophilia, who had previously been treated with albumin-stabilized recombinant FVIII only (group R) or with solvent/detergent high-purity non-immunopurified and non-nanofiltered FVIII or FIX concentrates (group P) considered to carry a risk of B19 transmission.

PATIENTS AND METHODS

Patients. From October 1994 to March 2000, a total of 1262 male haemophilia A and B patients were included in a prospective multicentre cohort study with active follow-up, created in 1993 under the auspices of the French Ministry of Health to monitor patients with haemophilia in France (Calvez *et al*, 2001). The patients were treated in 39 haemophilia centres. We selected children < 6 years of age for this analysis ($n = 265$) as B19 infection is also a community-acquired disease, called the fifth disease and usually observed at an early school age. We excluded 23 patients under 1 year of age, as transmission of maternal anti-B19 hinders the interpretation of serological results and may protect children from infection. If the serological result was missing at entry to the study, we used the first

result obtained between the ages of 1 and 6 years. Eighteen untreated patients and eight patients with missing data were excluded from further analysis. To obtain two homogeneous groups, we excluded from group R 10 patients who had previously been treated with only albumin-free recombinant FVIII or FIX, and from group P four patients previously treated only with FIX subjected to 15-nm nanofiltration and nine patients only treated with immunopurified FVIII or FIX concentrates. Thus, the prevalence of anti-B19 was studied in 193 haemophilia patients, 104 treated with albumin-stabilized recombinant FVIII only [Kogenate[®] (Bayer, Berkeley, CA, USA), Recombinate[®] (Baxter, Los Angeles, CA, USA), Helixate[®] (Aventis Behring, King of Prussia, PA, USA) or Bioclote[®] (Centeon, Kankakee, IL, USA)] and 89 treated with solvent/detergent high-purity non-immunopurified and non-nanofiltered FVIII or FIX concentrates [F VIII-THP/SD[®] and FIX-HP/SD[®] (LFB, Les Ulis, France)].

Methods. Sera were tested independently in each centre. The presence of B19 antibodies was detected by enzyme-linked immunosorbent assay (ELISA) using recombinant B19 coat protein VP1 and/or VP2 antigens expressed in an insect cell line (Baculovirus) (Koch, 1995). The diagnostic kits used by the virology laboratory of each centre were produced by several commercial manufacturers: Biotrin international (Dublin, Ireland) in most centres, Eurobio (Les Ulis, France), Euro-diagnostica (Paris, France) or DAKO (Zurich, Switzerland).

Statistical analysis. To describe the population we calculated the prevalence of anti-B19 in the two groups of children inside each age class and calculated the 95% confidence intervals using Miettinen methods (Exact method). To compare the prevalence we used Fischer's exact test.

Taking into account the difference between the two groups and in order to assess the roles of the product and potential confounding factors in the B19 transmission risk, we used univariate and multivariate logistic regression analyses on SAS software (version 6.12, SAS Institute, Cary, North Carolina). Anti-B19 was considered as a categorical variable (positive, negative). The confounding factors were the severity of haemophilia (< 1 U/dl, ≥ 1 U/dl), type of haemophilia (A, B), age (five 1-year classes), number of cumulative days of exposure (CDE) to FVIII or FIX (1–19, 20–99, ≥ 100), and previous red blood cell or plasma transfusions (yes, no). Multivariate analysis included only variables for which the *P*-value in the univariate analysis was ≤ 0.20. *P*-values below 0.05 were considered significant in multivariate analysis. All quoted confidence intervals are 95%. The validity of the model was tested using the Hosmer and Lemeshow goodness-of-fit test (Lemeshow & Hosmer, 1982).

RESULTS

The characteristics of the two groups of patients (R and P) are shown in Table I. The two groups were different for type of haemophilia, age and CDE to FVIII or FIX, and no difference was evidence concerning severity and previous

Table I. Characteristics of the two groups of patients.

| | Group R* (n = 104) | | Group P* (n = 89) | | P† |
|---|--------------------|---------|-------------------|--------|--------------------|
| | n | (%) | n | (%) | |
| Type of haemophilia | | | | | < 10 ⁻⁴ |
| A | 104 | (100.0) | 73 | (82.0) | |
| B | 0 | (0.0) | 16 | (18.0) | |
| Severity | | | | | 0.86 |
| Severe (< 1 U/dl) | 68 | (65.4) | 59 | (66.3) | |
| Mild (1–5 U/dl) | 25 | (24.0) | 19 | (21.3) | |
| Moderate (> 5 U/dl) | 11 | (10.6) | 11 | (12.4) | |
| Age (years) | | | | | < 10 ⁻⁴ |
| 1 to < 2 | 43 | (41.3) | 10 | (11.2) | |
| 2 to < 3 | 31 | (29.8) | 21 | (23.6) | |
| 3 to < 4 | 11 | (10.6) | 14 | (15.7) | |
| 4 to < 5 | 13 | (12.5) | 18 | (20.2) | |
| 5 to < 6 | 6 | (5.8) | 26 | (29.2) | |
| Number of cumulative days of exposure to FVIII or FIX | | | | | 0.002 |
| 1–19 | 61 | (58.7) | 30 | (33.7) | |
| 20–99 | 25 | (24.0) | 40 | (44.9) | |
| ≥100 | 18 | (17.3) | 19 | (21.4) | |
| Previous red blood cell or plasma transfusion | | | | | 0.38 |
| No | 89 | (85.6) | 72 | (80.9) | |
| Yes | 15 | (14.4) | 17 | (19.1) | |

*Group R: children previously treated with albumin-stabilized recombinant FVIII only; Group P: children previously treated with solvent/detergent high-purity non-immunopurified and non-nanofiltered FVIII or IX concentrate.

† χ^2 test.

red blood cell or plasma transfusion. The prevalence of anti-B19 in the two groups of children stratified by age is represented in Fig 1. Prevalence of anti-B19 inside each age class is different between group R and group P ($P < 0.01$), except for the last age class, 5–6 years.

Prevalence of anti-B19, according to the type and severity of haemophilia, age, CDE, treatment group and previous red blood cells or plasma transfusions, are shown

in Table II. Univariate analysis showed an increase on prevalence of anti-B19 with age and increasing days of exposure to FVIII or FIX. A higher risk was also found in patients with haemophilia B and in those of group P. The severity of haemophilia and previous red blood cells or plasma transfusions were not significantly associated with the prevalence of anti-B19. In the multivariate analysis, the type of haemophilia was not an independent predictor

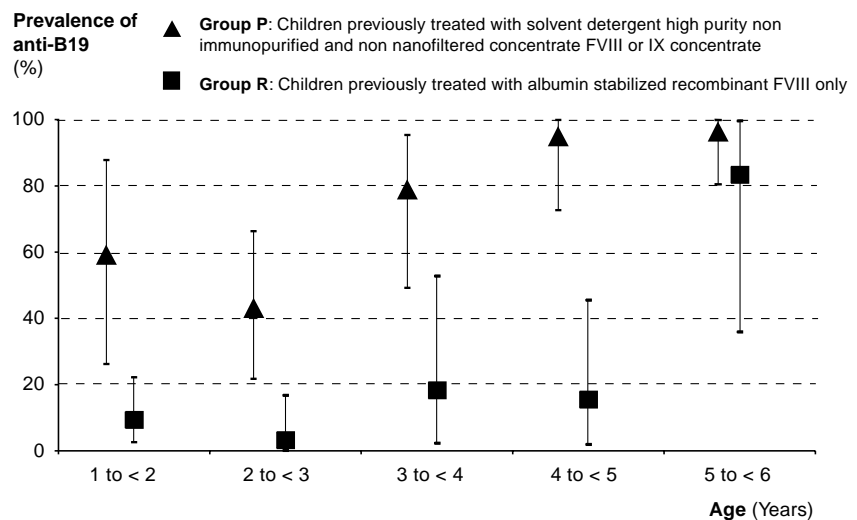


Fig 1. Prevalence of IgG antibodies to human parvovirus B19 by age in the two groups of children and 95% confidence intervals calculated using Miettinen methods (Exact method).

Table II. Factors associated with IgG antibodies to human parvovirus B19 in haemophilia patients aged between 1 and 6 years.

| | B19 seropositivity | | | Logistic regression | | | | | |
|---|--------------------|----------|--------|---------------------|---------------|------------------|--------------|--------------|------------------|
| | | | | Univariate | | | Multivariate | | |
| | <i>n</i> | <i>n</i> | (%) | OR | [CI] | <i>P</i> | OR | [CI] | <i>P</i> |
| Type of haemophilia | | | | | | | | | |
| A | 177 | 68 | (38.4) | 1 | | 0.002 | 1 | | 0.19 |
| B | 16 | 14 | (87.5) | 11.2 | [2.5, 50.9] | | 3.3 | [0.6, 19.2] | |
| Severity | | | | | | | | | |
| < 1 U/dl | 127 | 56 | (44.1) | 1 | | 0.53 | | | |
| ≥ 1 U/dl | 66 | 26 | (39.4) | 0.8 | [0.5, 1.5] | | | | |
| Age (years) | | | | | | | | | |
| 1 to < 2 | 53 | 10 | (18.9) | 1 | | 10 ⁻⁴ | 1 | | 10 ⁻⁴ |
| 2 to < 3 | 52 | 10 | (19.2) | 1.0 | [0.4, 2.7] | | 0.3 | [0.08, 1.19] | |
| 3 to < 4 | 25 | 13 | (52.0) | 4.7 | [1.6, 13.2] | | 1.8 | [0.4, 7.5] | |
| 4 to < 5 | 31 | 19 | (61.3) | 6.8 | [2.5, 18.5] | | 2.8 | [0.7, 11.3] | |
| 5 to < 6 | 32 | 30 | (93.7) | 64.5 | [13.2, 315.7] | | 21.8 | [3.3, 142.6] | |
| Number of cumulative days of exposure to FVIII or FIX | | | | | | | | | |
| 1–19 | 91 | 24 | (26.4) | 1 | | 10 ⁻⁴ | 1 | | 0.04 |
| 20–99 | 65 | 34 | (52.3) | 3.1 | [1.6, 6.0] | | 1.1 | [0.4, 3.4] | |
| ≥ 100 | 37 | 24 | (64.9) | 5.2 | [2.3, 11.7] | | 4.6 | [1.3, 16.1] | |
| Previous red blood cell or plasma transfusion | | | | | | | | | |
| No | 161 | 68 | (42.2) | 1 | | 0.87 | | | |
| Yes | 32 | 14 | (43.8) | 1.1 | [0.5, 2.3] | | | | |
| Group of patients* | | | | | | | | | |
| Group R | 104 | 14 | (13.5) | 1 | | 10 ⁻⁴ | 1 | | 10 ⁻⁴ |
| Group P | 89 | 68 | (76.4) | 20.8 | [9.9, 43.9] | | 22.3 | [7.9, 62.8] | |
| Total | 193 | 82 | (42.5) | | | | | | |

*Group R: children previously treated with albumin-stabilized recombinant FVIII only; Group P: children previously treated with solvent/detergent high-purity non-immunopurified and non-nanofiltered FVIII or IX concentrate.

of anti-B19 seropositivity, while the influence of age and the number of CDE were confirmed. The risk increased with age, particularly in children aged 5–6 years (OR: 21.8; CI: 3.3–142.6) compared with children aged 1–2 years. The odds ratio was 1.1 (0.4–3.4) for children who received 20–99 CDE and 4.6 (1.3–16.1) for those who received more than 100 CDE, compared with the group receiving less than 20 CDE. Furthermore, a higher prevalence of anti-B19 was observed in group P than in group R (OR: 22.3; CI: 7.9–62.8). The Hosmer and Lemeshow goodness-of-fit test showed good adequacy of the model ($P = 0.70$).

DISCUSSION

According to our results, a 22.3-fold lower risk of B19 transmission was found among patients previously treated only with albumin-stabilized recombinant FVIII relative to those having received blood-derived concentrates, independently of other selected factors. The prevalence of anti-B19 in children aged between 2 and 3 years who had previously been treated only with albumin-stabilized recombinant FVIII was 3.2% (CI: 0.1–16.7). At the same age, in children previously treated with solvent/detergent high-purity non-immunopurified and non-nanofiltered concentrate FVIII or

FIX concentrates, the prevalence of anti-B19 was 42.9% (CI: 21.7–66.3). The prevalence was high after age 5 years, even in the group receiving recombinant products only, but this result should be interpreted with care, as the number of patients in this age group was very small ($n = 6$). The other hypothesis is that this could be a consequence of starting school.

In this study, the age and CDE were also independent predictors of anti-B19 seropositivity. The major role of age, as the confounding variable in the B19 transmission risk, and the difference between the two groups for age was well considered in our analysis, as we selected children between 1 and 6 years and entered five 1-year classes in our model. As the sensitivity of the test for the presence of anti-B19 is good, we consider that the classification of patients for seropositivity is correct, even if the detection of IgG antibodies to B19 virus were determined independently in each centre.

The fact that none of the haemophilia B patients received only recombinant FIX explains why the type of haemophilia was significantly associated with B19 seropositivity in univariate analysis, but not in multivariate analysis. A previous history of red blood cell or plasma transfusion was not an independent predictor of seropositivity risk, as these labile products are mostly prepared from single donations

(red blood cells) or small plasma pools (solvent/detergent inactivated fresh-frozen plasma) (Mortimer *et al.* 1983).

None of the selected patients were HIV-infected, so we could not study this factor on the prevalence of anti-B19 in our series.

Previous studies in various countries (Grossebley *et al.* 1994; Laurian *et al.* 1994a; Flores *et al.* 1995; Peerlinck *et al.* 1995; Eis-Hübinger *et al.* 1996b; Ragni *et al.* 1996) show a higher prevalence of anti-B19 among haemophiliacs than in the general population or in control groups, and this difference is most marked during childhood. Other studies show that patients, who have previously received at least one non-inactivated plasma-derived clotting factor, have a high rate of B19 seropositivity at a young age compared with those who have received inactivated plasma-derived clotting factors (Bartolomei Corsi *et al.* 1988; Williams *et al.* 1990; Azzi *et al.* 1992). Nevertheless, none of the aforementioned reports presented information on anti-B19 prevalence in patients receiving only albumin-stabilized recombinant factors since birth (Laurian *et al.* 1994b). From our findings, it was not possible to conclude that albumin-stabilized recombinant FVIII does not transmit B19 because details of anti-B19 prevalence were not available from the control group. However, as shown in our multivariate analysis, the risk is 22.3-fold lower for patients previously treated only with albumin-stabilized recombinant FVIII.

The fact that B19 can still be transmitted by clotting factor concentrates indicates the possibility that other viruses or agents highly resistant to the virucidal and elimination steps may also be transmitted. Continuous efforts are made by manufacturers to improve the safety of their products. New methods of viral inactivation such as short-wavelength ultraviolet treatment (Chin *et al.* 1995) are being investigated for use in conjunction with solvent/detergent and heat treatment to eliminate the risk of transmitting non-enveloped viruses. Nanofiltration has already been implemented for a number of products (Burnouf-Radosevich *et al.* 1994), and also detection of B19 DNA in plasma pools. The first generation of recombinant factors contains human albumin and may thus carry a small risk of virus transmission. Recent second-generation recombinant is formulated without the addition of human proteins, in the aim of improving security against human transmissible agents.

Patients in this cohort treated only with nanofiltered-FIX were excluded from the analysis because of their small representation ($n = 4$); but further studies should compare the prevalence of IgG antibodies to parvovirus B19 in other subgroup of patients, such as those receiving plasma-derived products submitted to additional viral inactivation steps.

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APPENDIX

Suivi thérapeutique National des Hémophiles Group, as of March 2000

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