Nosocomial transmission of hepatitis B virus associated with endomyocardial biopsy

Michel ROSENHEIM (1), Jean-François CADRANEL (2), Lieven STUYVER (3), Richard DORENT (4), Franck GOLLIOIT (5), Pascal ASTAGNEAU (5), Vincent DI MARTINO (6), Annick DELCOURT (7), Irajid GANDJBAKHCH (4), Jean-Marie HURAUX (8), Françoise LUNEL (9)

(1) Service de Santé Publique, Groupe Hospitalier Pitié Salpêtrière, Paris ; (2) Service d’Hépatogastroentérologie et de Diabétologie, Centre Hospitalier Laennec, Creil ; (3) Innogenetics, Gent, Belgium ; (4) Service de Chirurgie Thoracique et Cardiovasculaire, Groupe Hospitalier Pitié Salpêtrière, Paris ; (5) CLIN Paris Nord, Paris ; (6) Service d’Hépatologie, Hôpital Jean Minjoz, Besançon ; (7) Service d’Anatomie Pathologique, Paris ; (8) Service de Virologie, Groupe Hospitalier Pitié Salpêtrière, Paris ; (9) Service de Virologie, Centre hospitalier Universitaire Angers.

SUMMARY

Objectives — A high prevalence of chronic hepatitis B has been previously reported in heart transplant recipients in our center. Nosocomial transmission of hepatitis B has been therefore suggested. The aim of the present study was to investigate an outbreak of hepatitis B infection in heart transplant recipients and to look for nosocomial acquisition of hepatitis B in these patients.

Methods — In a retrospective case-control study, review of transvenous endomyocardial biopsy (TEB) procedure, line probe assay and DNA sequencing for characterization of the outbreak isolate genotypes were performed in order to assess the possible risk of nosocomial transmission of hepatitis B in the setting of heart transplantation. Case was defined as a patient negative for HBsAg before heart transplantation and positive after. Controls were matched with cases by date of transplantation and time-interval of HBV infection occurrence in the cases patients.

Results — Transmission of HBV was associated with the number of HBsAg positive patients undergoing TEB the same day and in the same ward (OR = 1.17, per additional encounter; 95%CI = 1.01-1.37, P = 0.02) and with the total number of TEB undergone after a HBsAg positive patient (OR = 1.43 for additional encounter, 95%CI = 0.97-2.1, P = 0.056) but not with the number of biopsies. The virological study identified eight different strains. No common devices nor gloves, drapes, or medical solution were shared among patients during TEB. One staff member, but no surgeon, was HBsAg positive. No further case occurred after implementation of control measures.

Conclusions — Patient-to-patient transmission during TEB sessions was demonstrated by the virological and the case-control studies. This transmission occurred without evidence of blood contact through vials or devices. There is strong evidence that this transmission may be due to the spread of infective blood droplets on the environmental surfaces and the material during the TEB procedure.

RÉSUMÉ

Transmission nosocomiale du virus de l’hépatite B chez les malades après transplantation cardiaque :
mise en évidence du rôle des biopsies endomyocardiques

Michel ROSENHEIM, Jean-François CADRANEL, Lieven STUYVER, Richard DORENT, Franck GOLLIOIT, Pascal ASTAGNEAU, Vincent DI MARTINO, Annick DELCOURT, Irajid GANDJBAKHCH, Jean-Marie HURAUX, Françoise LUNEL

(Gastroenterol Clin Biol, 2006;30:1274-1280)

Buts — Une prévalence élevée d’hépatite chronique B a été rapportée après transplantation cardiaque dans notre centre. Des travaux de notre groupe ont émis l’hypothèse d’une transmission nosocomiale de l’infection par le virus de l’hépatite B chez ces malades.

Méthodes — Lors de cette étude rétrospective cas contrôles la procédure des biopsies myocardiques trans-veineuses (BTB) a été revue. Une analyse moléculaire avec caractérisation des isolats et des génotypes a été effectuée dans le but d’évaluer la possibilité d’une transmission nosocomiale de l’infection par le virus de l’hépatite B dans le contexte de la transplantation cardiaque. Les cas index étaient définis comme des malades antigène HBs positif avant la transplantation et positif après. Les malades contrôles ont été apparus avec les cas index pour la date de la transplantation cardiaque et l’intervalle entre la greffe et l’apparition de l’antigène HBs chez les cas index.

Résultats — La transmission de l’infection par le virus de l’hépatite B était associée de façon significative avec le nombre de patients Antigène HBs positif ayant eu une BTB le même jour et dans la même salle de cathétérisme (OR = 1,17; IC 95 % 1,01-1,37, P = 0,02) et avec le nombre total de BTB réalisées après un malade antigène HBs positif (OR = 1,43; IC 95 % 0,97-2,1, P = 0,056) mais pas avec le nombre de biopsies. L’analyse virologique a identifié 8 souches différentes. Aucun partage de linge, de matériels solidus ou de perfusion n’a eu lieu entre les malades pendant les BTB. Un membre de l’équipe soignante était positif pour l’antigène HBs mais aucun chirurgien ne l’était. Après l’institution de mesures d’hygiène drastique aucune nouvelle contamination n’est apparue.

Conclusion — Une transmission de malade à malade de l’infection par le virus de l’hépatite B survenant lors des BTB a été documentée par l’analyse virologique et l’étude de cas témoins. Cette transmission a eu lieu en l’absence de contact sanglant direct ou indirect. Il existe une forte présomption d’une contamination liée à la dissémination de particules contaminantes sur les surfaces ou le matériel durant les BTB.
Introduction

Since the screening of blood donors for hepatitis B virus (HBV) surface antigen (HBsAg) and the use of disposable injection devices in the mid-1970's, nosocomial transmission of HBV has occurred in very peculiar conditions. Direct patient-to-patient blood borne transmission was related to the use of capillary blood sampling device in diabetic patients [1, 2], jet injectors in weight reduction clinics [3] and reusable acupuncture needles [4, 5]. Indirect transmission was associated with storage of frozen bone marrow in a contaminated liquid nitrogen tank [6] and use of common medication vials [7].

In hemodialysis patients, incidence of HBV infection has decreased since the screening of blood donors, the isolation of HBsAg chronic carriers and the anti-HBV immunization program. However, HBV infection outbreaks have been reported in this population. In some cases, the route of cross-contamination was clearly identified as being medication vials of heparin or local anesthetic products shared by patients [8], venous pressure gauge of dialysis monitor machine [9] or blood leaks in dialysis machines [10]. Other reports [11] suggest that HBV transmission resulted from failure to identify and isolate HBV infected patients, and from sharing of staff among HBV susceptible and non-susceptible patients.

Transmission of HBV from surgeons to patients have been documented in gynecologic [12], oral [13], and orthopedic surgery [14]. In cardiac surgery, most outbreaks of HBV infection were also demonstrated to be transmitted by a HBsAg chronic carrier surgeon [15, 16]. We observed a high prevalence (16.5%) of HBV infection in heart transplant recipients (HTR) in a retrospective study of patients receiving grafts between 1982 and 1985 [17, 18]. All but one of the patients (positive before grafting) acquired HBV infection within 2 years after grafting. This suggests that HBV infection was not contracted during transplant or during the post transplantation period and that factors other than blood transfusion or infected organs donors (i.e., nosocomial transmission) may be responsible for HBV infection [19, 20]. Our study reports an outbreak of HBV infection occurring in at least 86 heart transplant recipients and related to transversal endomyocardial biopsies (TEB), an invasive procedure performed during the post-operative period for surveillance of graft rejection.

Methods

Descriptive epidemiology

The cardiac surgery unit of Pitié-Salpêtrière Hospital, a 2300-bed university hospital, is one of the main reference centers in heart transplantation in France. From 1968 to May 1994, 906 heart transplantations have been performed.

A study designed to assess the cause of long-lasting dysfunction in eighty patients who underwent heart replacement between 1982 and 1985, found eleven cases of post-operative hepatitis B [17, 18]. A prospective serological surveillance has then been implemented including serum collections before and every 6 months after heart transplantation. The transmission was suspected to occur during transversal endomyocardial biopsies (TEB) performed for early diagnosis of graft rejection. TEB were performed weekly the first two months; each other week during the two following months, every three weeks during month five and six, every four weeks until month twelve, every two months until month eighteen, every three months until month twenty-four and then every six months. For any rejection, a follow-up biopsy was done within three weeks after initiation of anti-rejection therapy.

TEB sessions were organized daily in specific catheterization room to avoid cross contamination. The biopsy forceps is usually introduced through an internal jugular catheter and three successive tissue specimens are collected. A procedure assessment identified reusable biopsy forceps as the only device shared among patients. Therefore, in June 1991, the reusable forceps were changed for disposable devices.

In May 1994, as new cases still occurred, a case-control study was undertaken and practices in the TEB ward and HBV serology of hospital staff involved in HTR care were reviewed. New control measures were implemented, consisting mainly of separating positive HBsAg patients from their negative counterparts during TEB sessions and anti-HBV vaccination of HTR candidates and their household contacts was reinforced.

Epidemiological investigations

To describe the epidemic, the case-patient definition was a HTR positive for HBsAg which has been HBsAg negative before transplantation. Data about HBsAg status before and after transplantation were obtained from computerized files of the laboratory of virology of the hospital.

For the case-control study, as data concerning TEB had been computerized since 1986 and given the time-interval between transplantation and HBV infection, cases and controls were selected among patients transplanted between January 1, 1986 and December 31, 1992. The cases were defined based on the epidemiic case-patient definition. The controls were the HTR who were negative for HBV markers (i.e., HBsAg, HBs and Hbc antibodies) before and after transplantation. The controls were matched with cases by date of transplantation and time-interval of HBV infection occurrence in the cases.

Demographic and clinical data were retrospectively collected from the individual clinical charts in the cardiac surgical unit, including age, sex, underlying cardiac disease, surgeon who performed the transplantation, duration of extra-corporeal circulation, graft rejections and immunosuppressive therapy. The graft rejection events were assessed by the total number of graft rejection and on the mean rejection grade (expressed as the ratio of the cumulated grade of rejection by the number of graft rejections). Immunosuppressive regimen as well as treatment of acute rejections have evolved over the period of analysis. However, all patients received anti-thymocyte globulin as induction therapy. For maintenance immunosuppression patients were given cyclosporine, prednisolone and azathioprine. There was no difference in immunosuppressive treatment between HBs antigen positive patients and control patients. The treatment of acute graft rejection depended on the histologic grade and the presence of hemodynamic compromise. This treatment might include increase oral dose of prednisolone, IV corticosteroids or antibody therapy. The level of immunosuppression was assessed by the mean daily dose of cyclosporine (expressed as the ratio of the cumulated dose by the number of in-study days) and the mean cyclosporine blood level (expressed as the ratio of the cumulated cyclosporine blood level by the number of in-study days). The histologic grading of rejection severity was made from grade 0 to 3 [21].

Data on TEB and virology were obtained from routine computerized files of the hospital laboratories. A specific computer program, that linked those two files, was written to determine the number of TEB per patient, the number of patients undergoing a TEB in the same session (contacts) and the number, among them, of HBsAg carriers. Clinical, biological, virological, histological characteristics and natural history of patients with HBV infection has been reported elsewhere [22].

Virological methods

Testing for HBsAg, anti-HBsAg and anti-Hbc antibodies was made using a commercial enzyme immunoassay (Abbott Laboratories, North Chicago, Ill.). HBV DNA was searched for using molecular hybridization (Hybaid plc., Middlesex, England). HBV DNA typing was performed using polyclonal antibodies and counter-electrophoresis as previously described [23]. A line probe assay (LPA) for simultaneous determination of HBV genotypes A to F, of the clinically important pre-Core mutations and of the HBsAg codon 143 (ACG to ATG; indicated as preCore inner sense: 5'-bio-GTTCC(T/G)GAACTGGAGCCACCAG-3'
pree3 nested sense: 5'-bio-GAACAGAAGTCAGCACTG-3'
pree3 nested antisense: 5'-bio-TCCGATCATGCTTCGAGTATG-3'
preCore outer sense: 5'-bio-ACAATAAGGACCTTGGAC-3'
preCore outer antisense: 5'-bio-GGCGAGAAGCATACG-3'

preCore nested sense: 5'-bio-TACTCCAGACTGTGTTTTA-3'
prefCore nested antisense: 5'-bio-CTCCACAGT/T/AAGCTTCAATTC-3'.

The amplification products were analysed on HBV genotyping, HBV pre-Core wild type/mutant, and on HBsAg codon 143 wild type/mutant research LiPA strips. To confirm LiPA, the above indicated preS1, pre-Core, and HBsAg amplification products were sequenced in a direct PCR sequencing protocol (dye terminator protocol, ABI, Foster City, CA).

Statistical methods

Incidence density of HBV infection was the number of new HBV infections divided by the sum of the time intervals between heart transplantation and end of follow-up, including death, HBV infection and loss of follow-up, expressed for 100 HTR-year.

Categorical variables were compared between cases and controls using paired Mac Nemar test for dichotomous variables, paired Stuart-Maxwell test for variables with more than two categories [25]. Quantitative variables were compared using paired Wilcoxon test. A conditional paired logistic regression [26] was used to estimate the odds ratio and its 95% confidence interval (95%CI) using the maximum likelihood method. Data were computed using SAS release 6.01 [27].

Results

Descriptive study

The first case of post-transplantation HBV infection was retrospectively found in 1984. Between July 1st, 1984, and May 1st 1994, 770 heart transplantations were performed in the cardiac surgery unit. During this period, 86 HTR became HBsAg positive after transplantation (overall attack rate: 11.5%; overall incidence density: 3.45 HBV infections per 100 HTR-year) (figure 1).

The number of patient cases steadily increased until 1990 (1990-incidence density: 7.17 per 100 HTR-year; 21 cases) and then decreased. Ninety percent of cases occurred after 217 days following transplantation (10th percentile: 217 days, median: 718 days, range: 30 to 2585 days). Four patients were found to be HBsAg chronic carriers before transplantation.

The survey among the medical and non-medical staff members identified one HBV chronic carrier. This person was a woman in charge of washing the TEB forceps until the use of disposable device in June 1991. She was HBs antigen positive; however HBeAg and HBV DNA were undetectable through hybridization tests. HBV DNA was detectable only through qualitative PCR with a threshold of detection of 1000 copies per ml PCR.

Case-Control study

The case control study took place between 1st January 1986 and 31 December 1992 (see paragraph epidemiological evaluation before this period data concerning TEB was not computerized). Among 649 patients transplanted during the study period, 57 (8.8%) responded to the case-patient definition. Data were available for 39 cases matched to 39 controls. As shown in table I, cases and controls did not differ significantly according to sex, age, cardiac underlying diseases, surgeon, duration of extra-corporeal circulation, immunosuppressive therapy after transplantation, number, grade and treatment of graft rejections. As shown in table II, the risk of acquiring HBV infection increased with the number of HBsAg positive HTR encountered during TEB procedure (OR = 1.17, per additional encounter, 95%CI = 1.01-1.37, P = 0.02), and with the total number of TEB undertaken after a HBsAg positive HTR (OR = 1.43 for one additional encounter, 95%CI = 0.97-2.1, P = 0.056). The odds ratio was 2.25 (95%CI = 1.05-4.82) for a difference of 5 encounters and 5.04 (95%CI = 1.10-23.29) for difference of 10. The total number of encounters and the total number of biopsies did not differ significantly between cases and controls.

Virological study

HBV serotype results were analyzable in 58 patients. Among them 36 (62%) were infected with serotype adw2 and 22 (38%) with serotype ayw3. Molecular analysis could be performed in 76 out of 86 patients (table III). Among these 76 patients, HBV DNA could be amplified for 73 out of 86 patients who became HBsAg positive after transplantation, for 3 out of 4 patients that were HBsAg positive before transplantation, and for the HBsAg positive health-care worker. For the remaining 14 samples, HBV DNA could not be amplified because of bad storage conditions.

Using the combination of the following conditions preCore wild type (tryptophan at codon 28, glycine at codon 29) versus a translational stop at codon 28, or an asparagine at codon 29, or random mutations; HBsAg genotype A versus genotype D, HBsAg wild type (threonine at codon 143) versus mutant (methionine at codon 143), we were able to organize the epidemic in 8 groups (table III). The occurrence of the detected infections according to this grouping is visualized in figure 2.

One isolate of genotype B was found only in a HBsAg positive patient before transplantation. HBV belonging to group D2 was present in one patient before transplantation and in 7 patients who became HBsAg positive after transplantation. Similarly, HBV of group D2 was present in one patient before transplantation and in 2 patients who became HBsAg positive after transplantation. Among the remaining cases, 9 belonged to group A1, 24 to group A2, 5 to group A3, 5 to group A4, 3 to group A5, 19 to group D1. The isolates of the health-care worker contained HBV of genotype A and genotype D.

Impact of control measures

Since May 1994, the HBsAg positive HTR have been separated from the HBsAg negative during the TEB procedures in giving specific days. Control measures were focused on TEB procedures in addition to the use of disposable TEB device implemented since 1991. As blood droplets contact was suspected as the mode of transmission of HBV during TEB, recommendations consisted of i) not purging the syringe full of blood during catheterization; ii) use of catheter with anti-reflow device; iii) unwrap
Nosocomial transmission of hepatitis B virus associated with endomyocardial biopsy

TEB material at the onset of TEB procedure to avoid contamination of the opened material. In addition, the standard measures of central catheterization were suggested to be reinforced according to the recommendations of the Society for Cardiac Angiography and Interventions Laboratory Performance Standards Committee [28]. A double-dose HBV vaccination was recommended for all HTR candidates. All medical and non medical staff members were advised to control their HBs antibody level and to be revaccinated if the antibody level was lower than 50 IU. Since implementation of these control measures, no additional case-patient has been identified among 80 new heart transplantations performed between May 1994 and December 31, 1995.

Discussion

To our knowledge, this study reports the largest and longest outbreak of HBV infection observed in HTR. Over a ten-year period, HBV was increasingly transmitted to 86 HTR. As reported during the same period in another heart transplant center in Europe, TEB was demonstrated as the vehicle of HBV transmission [29].

In contrast to other outbreaks of HBV infection reported in cardiac surgery [15,16], the transmission was unlikely to occur during operation, as the surgeons were negative for HBsAg and the time interval between transplantation and HBV infection was

Table I. – General characteristics of heart transplant recipients: comparison of cases and controls.

<table>
<thead>
<tr>
<th>Characteristic/risk factor</th>
<th>No pairs</th>
<th>Cases</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>39</td>
<td>45.7 ± 11.2 (13-63)</td>
<td>46.2 ± 11.1 (16-62)</td>
<td>0.99*</td>
</tr>
<tr>
<td>Sex ratio M/F</td>
<td>39</td>
<td>34/5</td>
<td>35/4</td>
<td>0.50†</td>
</tr>
<tr>
<td>Cardiopathy</td>
<td>38</td>
<td>13</td>
<td>18</td>
<td>0.60‡</td>
</tr>
<tr>
<td>ischemic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>idiopathic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>other</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgeon</td>
<td>30</td>
<td>5</td>
<td>3</td>
<td>0.50†</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extra Corporeal Circulation (minutes)</td>
<td>33</td>
<td>96.7 ± 21.4 (50-156)</td>
<td>100.2 ± 28.3 (55-182)</td>
<td>0.63*</td>
</tr>
<tr>
<td>Ciclosporine</td>
<td>34</td>
<td>7.2 ± 2.8 (3.2-14.8)</td>
<td>7.2 ± 2.7 (4.3-15.8)</td>
<td>0.84*</td>
</tr>
<tr>
<td>mean dose (mg/kg/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean cyclosporine blood level (ng/ml)</td>
<td>36</td>
<td>248 ± 52 (153-357)</td>
<td>258 ± 77 (130-507)</td>
<td>0.42*</td>
</tr>
<tr>
<td>Graft rejections</td>
<td>34</td>
<td>4.2 ± 3.3 (1-14)</td>
<td>4.2 ± 3.4 (1-14)</td>
<td>0.97†</td>
</tr>
<tr>
<td>number</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cumulated grade</td>
<td>34</td>
<td>4.9 ± 4.1 (1-16)</td>
<td>5.3 ± 5.2 (1-24)</td>
<td>0.72‡</td>
</tr>
<tr>
<td>mean grade</td>
<td>34</td>
<td>1.1 ± 0.3 (1-3)</td>
<td>1.2 ± 0.3 (1-3)</td>
<td>0.41*</td>
</tr>
<tr>
<td>Treatment of graft rejections</td>
<td>34</td>
<td>12</td>
<td>15</td>
<td>0.63†</td>
</tr>
<tr>
<td>increased ciclosporine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>increased oral corticosteroids</td>
<td>29</td>
<td>33</td>
<td>33</td>
<td>0.22†</td>
</tr>
<tr>
<td>IV corticosteroids</td>
<td>17</td>
<td>14</td>
<td>14</td>
<td>0.64†</td>
</tr>
<tr>
<td>anti-thymocyte globulin</td>
<td>10</td>
<td>11</td>
<td>11</td>
<td>1.0†</td>
</tr>
<tr>
<td>OKT3</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>1.0†</td>
</tr>
</tbody>
</table>

For quantitative variables: mean ± standard deviation (range).
* Wilcoxon paired test.
† Mac Nemar paired test.
‡ Stuart-Maxwell paired test.

Table II. – Risk factors for HBV transmission during transvenous endomyocardial biopsy: comparison between heart transplanted patients and controls.

<table>
<thead>
<tr>
<th>Characteristic/risk factor</th>
<th>No pairs</th>
<th>Cases</th>
<th>Controls</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>total contacts</td>
<td>39</td>
<td>178 ± 62 (39-327)</td>
<td>178 ± 55.7 (50-286)</td>
<td>0.98†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>number biopsies</td>
<td>39</td>
<td>21.7 ± 7.0 (6-39)</td>
<td>21.7 ± 5.9 (8-33)</td>
<td>0.98*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBs Ag+ patients encountered</td>
<td>39</td>
<td>10.3 ± 5.8 (1-29)</td>
<td>8.5 ± 5 (1-23)</td>
<td>1.17</td>
<td>1.01-1.37</td>
<td>0.02†</td>
</tr>
<tr>
<td>biopsies after an HBs Ag+ patient [estimated]</td>
<td>39</td>
<td>4.2 ± 2.1 (0.5-9.8)</td>
<td>3.6 ± 1.9 (0.5-7.8)</td>
<td>1.43</td>
<td>0.97-2.1</td>
<td>0.056‡</td>
</tr>
</tbody>
</table>

For quantitative variables: mean ± standard deviation (range), OR: odds ratio, 95% CI: 95% confidence interval.

† Wilcoxon paired test.
* estimated using the formula: probability of having a biopsy after an AgHBs positive patient = number of AgHBs positive patients during the biopsy session / total contacts.
† conditional paired logistic regression.

longer than the standard incubation period. Among the other staff members, one health care worker, in charge of washing the TEB forceps after each procedure, was found to be HBsAg positive. This woman was positive with presence of HBV DNA through a qualitative PCR with a cutoff of 1000 copies per ml. However, she did not present with detectable HBV DNA according to hybridization test with a cutoff of 1 pg of DNA per ml representing 2.85 × 10^5 copies per ml. The low HBV DNA level therefore posed a very low risk of transmission. However transmission from this care worker, although not probable cannot formally be ruled out. In addition, she was carrier of two different HBV genotypes, suggesting she was likely to be a secondary case rather than the source of the epidemic. Transmission through blood transfusion and a reactivation of a latent pretransplantation HBV infection were unlikely in this population, as hypothesized in previous published study of our group [19, 20].

Moreover all transplanted patients had received before, during and after heart transplantation many blood products without any differences between postoperative HBs antigen patients and post operative negative positive patients (data not shown). In addition immunosuppressive treatment was not different between HBs antigen positive patients and HBs antigen negative patients. It was not possible to look for the presence HBV DNA in patients with positive HBC antibodies at time of transplantation; even if very unlikely the possibility of some cases of reactivation of occult HBV infection present at time of the transplantation cannot totally be ruled out. However this study clearly shows that no case of HBV infection was found when universal hygiene rules were improved, therefore occult HBV reactivation can only be marginal. The assumption of a patient-to-patient transmission is supported by epidemiological evidences and demonstrated by the molecular biology study. Eight different clusters of patients derived from eight HBV groups belonging to genotype A or D. This pattern of transmission is not compatible with the hypothesis of occult HBV reactivation. There is strong evidence that HBV was transmitted during TEB. The risk of HBV infection increased with the number of TEB following an HBsAg positive patient in the same TEB session. This transmission was first favored by impairment of the TEB procedure rather than the TEB material or the
operator. Indeed, the total number of TEB performed during the epidemic period was not associated with HBV infection. In addition, new cases still occurred after replacement of reusable for disposable TEB device.

The most striking issue is that this transmission occurred without evidence of blood contact through vials or devices used during TEB. The observation of TEB practices in the catheterization room showed that neither common devices nor gloves, drapes, or medical solution such as heparin or anesthetic solution were shared among patients. The usual procedure generated spread of blood droplets, especially during purging of syringes and the withdrawal of catheter leader. Those droplets might contaminate the TEB material unwrapped when patients consecutively underwent TEB. The presence of HBV particles in the environment surrounding a HBsAg positive patient undergoing vascular procedure is supported by several other studies. Dreschler et al. [29] demonstrated that viral particles could be yielded one meter beyond the biopsy site during a simulated TEB. In 1971, a report suspected an airborne spread of HBV within a hemodialysis unit. As an enteric transmission was excluded for HBV in further publications [31], airborne transmission was suspected an airborne spread of HBV within a hemodialysis unit beyond the biopsy site during a simulated TEB. In 1971, a report suspected an airborne spread of HBV within a hemodialysis unit [30], but the authors suggest that transmission occurred by ingestion of infective droplets. As an enteric transmission was excluded for HBV in further publications [31], airborne transmission was then ruled out but contamination of medical devices by droplets remains consistent with the data of the 1971 outbreak. Other reports [32-35] demonstrated the presence of HBsAg on environmental surfaces in hemodialysis units, even without evidence of visible blood contamination, suggesting a droplet contamination. In contrast, no HBsAg was found in air sampling of an hemodialysis unit [36], suggesting that blood droplets deposited on the surfaces within a short time. In our study, the implementation of specific preventive measures resulted in the disappearance of the outbreak, supporting the assumption of a cross-contamination through blood droplets.

The risk of HBV transmission may be increased by the high level of viremia observed in our cases, yielding blood droplets with high infectivity. This high viremia is related to impairment of immune response owing to highly immunosuppressive therapy given for prevention of graft rejection. In addition, impairment of immune response also results in an incomplete vaccine efficacy in HTR during the period of deep immunosuppression following transplantation, despite a double dose vaccine.

Although the route of HBV transmission was identified in this study, the source of the outbreak remains unclear. Multiple sources could be involved as molecular virological study identified eight different groups of HBV among cases. For two groups, transmitted to nine patients, it seems clear that the source is two pre-transplantation HBsAg chronic carriers. For the remaining strains, the source remains unclear.

In conclusion, this study highlights a very peculiar pathway of HBV patient-to-patient transmission through infective blood droplets, generated during an intra-vascular procedure. This pathway deserves to be considered in other populations with a high HBV infection incidence rate, such as hemodialysis patients. Since other blood-borne viruses might be transmitted through the same pathway, control efforts should be made in all patients undergoing intra-vascular procedure. These measures should focus on the control of blood droplets production, respect of the guidelines for the use of intra-vascular devices [28], and of isolation precautions based on the Centers for Disease Control recommendations [37].

ACKNOWLEDGMENTS - This work has been supported in part by a grant of the Délégation à la Recherche Clinique, Assistance Publique-Hôpitaux de Paris.
We are indebted to Anne-Marie Couroucé for HBV serotyping and to Françoise Maillot, Madeleine Tacnet and Michèle Perrin for technical help in retrieving clinical, pathological and virological data, Jerry Bram for rewriting English and Myriam Lombard for excellent secretarial assistance.

REFERENCES


