Seroconversion rate to positivity for antibodies against core antigen of hepatitis B virus and duration of renal replacement therapy

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Abstract

Background. Prevalence of total antibodies against core antigen (anti-HBc) of hepatitis B virus (HBV) is greater in longer dialysed patients, but there are no data indicating a relationship between the higher seroconversion rate to anti-HBc positivity and longer renal replacement therapy (RRT) vintage prior to seroconversion. Our aim was to evaluate the association between RRT duration and seroconversion to anti-HBc positivity.

Methods. An incidence of anti-HBc was evaluated in 425 anti-HBc-negative intermittent haemodialysis (IHD) patients: Group I included patients who underwent first anti-HBc testing 31 days from first IHD session, and Group II or III included patients with RRT duration <3 or ≥3 years, respectively. Anti-HBc testing was repeated every 6–12 months. Sex, age, RRT duration, anti-HCV, HCV RNA, ALT, ASP, GGT, full vaccination series against HBV with developed anti-HBs titre >10 IU/L, hepatitis history and underlying kidney diseases were used as independent variables predicting seroconversion to anti-HBc positivity.

Results. Seroconversion rate to anti-HBc positivity was 2.59, 2.12 and 2.44 episodes/100 patient-years in Group I (n = 174), II (n = 170) and III (n = 80), respectively. In the entire group, there were 15 seroconversions to anti-HBc and one seroconversion to HBsAg positivity. The only variable predicting seroconversion in all HBsAg-negative patients (n = 424) was the lack of full vaccination series against HBV with developed anti-HBs titre >10 IU/L maintained during the study (β = 0.112, P = 0.04).

Conclusions. Seroconversion rate to anti-HBc positivity is not related to duration of RRT treatment but to ineffective vaccination against HBV.

Keywords: anti-HBc; anti-HBs; haemodialysis; renal replacement therapy vintage; seroconversion

Introduction

After introduction of vaccination against the hepatitis B virus (HBV), cross-sectional examinations indicate a decreasing relative number of intermittent haemodialysis (IHD) patients with positive surface antigen of HBV (HBsAg). The risk for HBV infection was estimated to be 70% lower in vaccinated dialysis patients [1]. In the USA, the prevalence of HBV infection in IHD patients, defined as the percentage of all IHD patients who tested positive for HBsAg, progressively fell from 7.8% to 1.0% between 1976 and 2002 [2]. It has to be noted that during 1997–2002, the percentage of patients vaccinated against HBV infection in the USA increased from 7.8% to 1.0% between 1976 and 2002 [2]. In Poland, the percentage of IHD patients with positive HBsAg also decreased: from 17.6% in 1995 to 4.2% in 2007 [3]. In 2007, the prevalence of HBsAg-positive IHD patients in the Wielkopolska region of Poland was 3.4% [4].

HBsAg is usually eliminated during recovery after HBV infection, especially when acute HBV infection is associated with positive testing for the hepatitis B envelope antigen (HBeAg) [5]. However, IHD patients suffering from acute HBV infection (HBsAg positive) were shown to seroconvert to HBsAg negativity in only 8.5–38% of cases [6–8].

Total antibodies against core antigen of HBV (anti-HBc) are the established marker for previous or current infection with HBV. HBsAg-positive patients who become HBsAg negative during recovery from acute hepatitis B may develop anti-HBc, but anti-HBc may also occur in IHD patients who never suffered from acute hepatitis B. However, occurrence of anti-HBc indicates HBV transmission. Thus, prevalence of anti-HBc may reflect epidemiological status in regard to HBV infection. The prevalence of anti-HBc in the chronic dialysis population ranges from 0% to 54% [9–12].

In 2000, Vladutiu et al. [13] reported that the anti-HBc-positive patients had been dialysed for longer than the non-infected ones. Our data have confirmed results of their studies [14]. According to the aforementioned cross-sectional studies, prevalence of anti-HBc positivity

Keywords: anti-HBc; anti-HBs; haemodialysis; renal replacement therapy vintage; seroconversion
is greater in longer dialysed patients, but there are no prospective data indicating higher seroconversion rate to anti-HBc in patients with longer vintage of renal replacement therapy (RRT). It is not clear whether the greater number of positive anti-HBc in the group with longer RRT duration is due to cumulative effect or increasing susceptibility to HBV infection during RRT follow-up.

To show a casual relationship between higher anti-HBc positivity and duration of RRT, we have undertaken a prospective, observational study, including HBsAg/anti-HBc-negative patients starting IHD as the first method of RRT and already being treated with RRT. Factors predictive for seroconversion were assumed to be delivered from demographic, clinical and laboratory data of IHD patients included into the study. Special attention was paid for possible association between seroconversion to anti-HBc positivity and duration of RRT.

Materials and methods

Patients and dialysis settings

Twenty-one IHD centres participated in the study: 17 from the Wielkopolska region of Poland and 4 from other regions. In accordance to the European recommendations [15], in dialysis units cooperating in this study, HBsAg-positive patients were dialysed in separate rooms with dedicated machines in addition to patients with positive antibodies against hepatitis C virus (anti-HCV) being in separate rooms or areas with dedicated machines. However, dialysers from HBsAg-negative, anti-HCV-negative and HCV ribonucleic acid (HCV RNA)-negative patients were reused in one centre.

In all IHD centres, patients were assumed to be vaccinated against HBV according to standard rules [16]. A hepatitis B vaccine made by recombinant HBV deoxyribonucleic acid (DNA) technology was in use. In brief, all stable HBsAg-negative patients showing antibodies against surface antigen of HBV (anti-HBs) titre <10 IU/L were vaccinated or revaccinated.

The study protocol

HBsAg/anti-HBc-negative patients requiring RRT were enrolled for prospective, observational study, independently on anti-HBsAg status.

Three groups of patients were established basing on the relation between the date of anti-HBc testing and duration of RRT. Group I included patients who underwent first anti-HBc testing 31 days from the first session of IHD, being their first and only method of RRT. Patients not fulfilling inclusion criterion for Group I were included into Group II or III when duration from the start of RRT to anti-HBc testing performed for the study was <3 or ≥3 years, respectively. All patients of Group II and III were currently treated with IHD, but total duration of their RRT included also peritoneal dialysis (7 cases), lifetime with functioning renal graft (19 cases) or both (1 case).

All patients had routinely determined HBsAg, anti-HCV and antibodies against human immunodeficiency virus (anti-HIV1/HIV2) at the start of IHD. HBsAg and anti-HCV estimations were repeated every 3 months, and anti-HIV1/HIV2 every year. Anti-HBs were tested at the start of IHD, 1–2 months after the last dose of vaccine against HBV and every 6–12 months [16]. In all patients, anti-HBc testing was repeated after 8–12 months from the entry determination and after every further 6–12 months. Other viral markers were routinely determined as aforementioned. Incidence of anti-HBc and HBsAg positivity was calculated by subtraction of the percentage of patients remaining free of infection within the study period from 100%. Seroconversion rate to anti-HBc or HBsAg positivity was expressed in episodes of seroconversion per 100 patient-years.

Statistical analysis was performed only for data of patients who underwent at least one anti-HBc testing after the study entry. Patients’ age and RRT duration were calculated at the day of entry anti-HBc testing. Study duration was calculated for each individual patient from the entry anti-HBc testing to the last anti-HBc-negative testing or the first confirmed anti-HBc-positive testing.

The results of HBV seromarkers (HBsAg, anti-HBc, anti-HBs and HBV DNA) and HCV seromarkers (anti-HCV and HCV RNA) were collected and analysed. The results of serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltranspeptidase (GOT) were used as biochemical signs of liver disease. Analysed demographic and clinical data included race, gender, age, diagnosis of end-stage renal disease (ESRD), duration of RRT, history of acute hepatitis, and undergoing full vaccination series against HBV with developed anti-HBs titre ≥10 IU/L before anti-HBc testing at the study entry and maintained during the study.

Laboratory methods

HBsAg and anti-HBc were determined by the Microparticle Enzyme Immunoassay (MEIA) technology (AxSYM, Abbott Laboratories, Abbott Park, IL, USA). For anti-HBs and anti-HCV detections, the MEIA technology was also used (ABBOTT, Wiesbaden, Germany).

HBV DNA was determined using a qualitative test COBAS AMPLI-COR HBV MONITOR; HCV RNA was tested using COBAS AMPLI-COR Hepatitis C Virus Test, version 2.0 (both Roche Diagnostics Ltd., Rotkreutz, Switzerland). In selected cases, HBV DNA and HCV RNA were determined using a quantitative real-time polymerase chain reaction (PCR) with a lower limit of detection of 200 IU/mL (RoboGene® Quantification of HBV Genomes, AJ Roboscreen GmbH, Leipzig, Germany, and RoboGene® Quantification of HCV Genomes, AJ Roboscreen GmbH, Leipzig, Germany, respectively).

Serum activities of ALT, AST and GGT were determined by routine laboratory methods.

Results

In the dialysis centres cooperating in the study, the total number of patients treated with IHD was 1379 persons ageing over 18 years. The distribution of results of HBV seromarkers in IHD patients is presented in Figure 1. The study was carried out in 425 patients enrolled from 1048 HBsAg-negative and anti-HBc-negative patients. Among HBsAg-negative and anti-HBc-positive patients (n = 296, 21.5%), there was a single patient who tested HBV DNA positive in several consecutive examinations using quantitative real-time PCR testing. In the entire group, a single patient was anti-HIV1/HIV2 positive.

Amongst all patients who were enrolled for the study (n = 425), there were 15 seroconversions to anti-HBc positivity and one seroconversion to HBsAg positivity during 18.1 ± 6.5 months between the first and the last anti-HBc examination. None of the patients showed, independently on seroconversion occurrence, any clinical manifestation of acute infection with hepatotropic viruses in the course of examination. All patients, who seroconverted, were HBV
DNA negative. A woman, who became HBsAg positive, had a positive testing for HBeAg, but negative for anti-HBe and anti-HBc. During 18.1 ± 6.5 months of the study, the incidence of anti-HBc positivity was 3.53%, while the incidence of HBsAg positivity was 0.24%. In the entire group, seroconversion rate to anti-HBc positivity was 2.35 episodes/100 patient-years, and to HBsAg positivity 0.16 episodes/100 patient-years (P = 0.001 for comparison of both seroconversion rates).

Table 1 shows the demographic, clinical and laboratory data of the entire group (n = 425) and of patients grouped by duration of RRT. A patient who converted to HBsAg positivity was excluded from Group I. Patients of Group I (n = 174) were treated with IHD up to 31 days; patients of Group II (n = 170) were on RRT for 1.16 ± 0.80 years, and patients of Group III (n = 80) for 5.93 ± 3.28 years before the study entry. The last anti-HBe testing was done after 13.1 (8.5–40.9), 17.1 (12.2–48.0) and 15.8 (11.3–37.2) months from the first anti-HBe testing in Group I–III, respectively. Seroconversion rate to anti-HBc positivity was 2.59, 2.12 and 2.44 episodes/100 patient-years in Group I, II and III, respectively. Differences in seroconversion rate were not significant (Group I vs II, P = 1.0; Group I vs III, P = 0.8; and Group II vs III, P = 0.9).

Patients, who converted to anti-HBe positivity, did not differ significantly in the examined parameters from patients without seroconversion (Table 2).

Data (sex; age; RRT duration; the results of anti-HCV, HCV RNA, ALT, ASP and GGT; full vaccination series against HBV with developed anti-HBs titre >10 IU/L before negative anti-HBe testing at the study entry and maintained during the study; hepatitis history; and the four main causes of ESRD) of all patients who remained HBsAg negative (n = 424) were used as independent variables possibly predictive for seroconversion to anti-HBc positivity. The only variable predicting seroconversion to anti-HBc positivity was the lack of full vaccination series against HBV with developed anti-HBs titre >10 IU/L (β = −0.112, P = 0.04). Seroconverted patients showed an anti-HBs titre >10 IU/L before seroconversion in 66.7% of cases as compared with 80.7% in non-seroconverted group (Table 2). Eight from 15 seroconverted patients (53.3%) had anti-HBs <90 IU/L.

**Discussion**

The incidence of HBV infection during IHD treatment, defined as the percentage of all patients receiving IHD during the data collection period who seroconverted from HBsAg negative to HBsAg positive, decreased remarkably during the last decades of the 20th century mainly due to implementation of vaccination against HBV in the majority of IHD patients [2,3]. Among not vaccinated IHD patients susceptible to HBV, the 2-year risk of seroconversion for HBV infection was 38.9%, accounting for 19 seroconversions to HBsAg positivity per 100 patient-years [17]. Recent annual Thai incidence of HBsAg was 0.4%, indicating 0.15 seroconversions per 100 patient-years [6,18]. The latter data are similar to our incidence of HBsAg positivity (0.16 episodes/100 patient-years). However, HBV transmission to IHD patients is higher than indicated only by incidence of HBsAg positivity. True infection rate should also include incidence of anti-HBc positivity. In a recently published paper [12], seroconversion to anti-HBc positivity was not shown during a 12-month follow-up of 123 IHD patients, although prevalence of anti-HBc positivity in their IHD centres was 34.1% compared with 21.5% in our centres. In the presented study, incidence of anti-HBc positivity was 2.35 episodes/100 patient-years.

Our prospective studies did not show differences in seroconversion rate to anti-HBe positivity in IHD patients with different RRT duration. Vintage of RRT was also not a significant predictor for this seroconversion rate. Therefore, higher prevalence of anti-HBe positivity in patients being longer on RRT, shown in the study of Vladutiu et al. [13] and in our earlier study [14], seems to depend on the cumulative effect. Total duration of RRT was a variable predictive for prevalence of anti-HBe positivity in IHD centres [14], but is not predictive for seroconversion to anti-HBe positivity.

A question arises whether differences shown in the examined three groups with different RRT duration (Table 1) could be important for acquiring infection with HBV. We have compared patients who seroconverted to anti-HBe positivity with those without seroconversion in respect to their characteristics, and we did not find significant differences (Table 2).

In the study of Cendorogo Neto et al. [17], dialysis patients, who seroconverted to HBsAg positivity, also did not differ from patients with no seroconversion in respect to age, gender and duration of RRT, but in the study of...
Seroconversion to anti-HBc on renal replacement therapy

Table 1. Demographic, clinical and laboratory data of the entire group and of patients grouped by duration of renal replacement therapy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All patients n = 425</th>
<th>Group I n = 174</th>
<th>Group II n = 170</th>
<th>Group III n = 80</th>
<th>P-value for analysis among Group I–III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian/non-Caucasian</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>I vs II, I vs III, II vs III</td>
</tr>
<tr>
<td>(n, % of all)</td>
<td>422/3</td>
<td>173/1</td>
<td>169/1</td>
<td>79/1</td>
<td>0.5, 0.8, 0.8</td>
</tr>
<tr>
<td>Men/women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n, % of all)</td>
<td>228/197</td>
<td>94/80</td>
<td>99/71</td>
<td>35/45</td>
<td>0.4, 0.1, 0.03</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60±5/14</td>
<td>50.7/46.3%</td>
<td>54.0/46.0%</td>
<td>58.2/41.8%</td>
<td>43.8/56.2%</td>
<td>0.6, 0.6</td>
</tr>
<tr>
<td>Age &gt;30/≤50 years</td>
<td>407/18</td>
<td>163/11</td>
<td>164/6</td>
<td>79/1</td>
<td>0.3, 0.1, 0.5</td>
</tr>
<tr>
<td>(n, % of all)</td>
<td>95.8%/4.2%</td>
<td>93.7%/6.3%</td>
<td>96.5%/3.5%</td>
<td>98.8%/1.2%</td>
<td></td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n, % of all)</td>
<td>105/4</td>
<td>43</td>
<td>49</td>
<td>13</td>
<td>0.4, 0.1, 0.03</td>
</tr>
<tr>
<td>Hypertensive nephropathy</td>
<td>75</td>
<td>32</td>
<td>30</td>
<td>13</td>
<td>0.9, 0.7, 0.8</td>
</tr>
<tr>
<td>(n, % of all)</td>
<td>216/17</td>
<td>171/12</td>
<td>171/12</td>
<td>171/12</td>
<td>0.1, 0.1, 0.003</td>
</tr>
<tr>
<td>Chronic glomerulonephritis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n, % of all)</td>
<td>76/5</td>
<td>32</td>
<td>21</td>
<td>22</td>
<td>0.1, 0.1, 0.003</td>
</tr>
<tr>
<td>Chronic tubulointerstitial nephritis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n, % of all)</td>
<td>46/13</td>
<td>12</td>
<td>23</td>
<td>11</td>
<td>0.04, 0.08, 0.9</td>
</tr>
<tr>
<td>History of acute hepatitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n, % of all)</td>
<td>5/2</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0.07, 0.3, 0.3</td>
</tr>
<tr>
<td>Full vaccination series against HBV</td>
<td></td>
<td></td>
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<tr>
<td>against HBV with developed anti-HBs titre &gt;10 IU/L (n, % of all)</td>
<td>341/125</td>
<td>125</td>
<td>144</td>
<td>71</td>
<td>0.004, 0.005, 0.4</td>
</tr>
<tr>
<td>Positive/negative anti-HCV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n, % of all)</td>
<td>53/372</td>
<td>9/165</td>
<td>28/142</td>
<td>15/65</td>
<td>0.001, 0.001, 0.7</td>
</tr>
<tr>
<td>Positive/negative HCV RNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n, % of all examined)</td>
<td>30/381</td>
<td>5/168</td>
<td>16/144</td>
<td>8/69</td>
<td>0.01, 0.03, 0.9</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>15.7 ± 9.9</td>
<td>15.6 ± 8.3</td>
<td>16.2 ± 11.6</td>
<td>14.8 ± 9.0</td>
<td>0.5</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>17.0 ± 9.1</td>
<td>16.3 ± 7.1</td>
<td>17.4 ± 10.6</td>
<td>17.6 ± 9.1</td>
<td>0.7</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>23.0</td>
<td>24.0</td>
<td>24.0</td>
<td>21.0</td>
<td>0.6</td>
</tr>
<tr>
<td>(3.0–462.0)</td>
<td>(3.0–245.0)</td>
<td>(8.0–323.0)</td>
<td>(8.0–462.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation or median and range. ALT, alanine aminotransferase; anti-HBs, antibodies to surface antigen of hepatitis B virus; anti-HCV, antibodies to hepatitis C virus; AST, aspartate aminotransferase; GGT, gamma-glutamyltranspeptidase; HBV, hepatitis B virus; HCV RNA, ribonucleic acid of hepatitis C virus.

*10 cases with diabetic nephropathy due to type 1 diabetes and 95 cases due to type 2 diabetes.

Thanachartwet et al. [6], seroconversion from HBsAg negativity to HBsAg positivity in IHD patients was significantly associated with male gender [6]. In our study, not indicating gender difference in IHD groups with and without seroconversion to anti-HBC positivity, gender was also excluded as a significant predictive factor for this seroconversion.

Among the causes of ESRD, prevalence of diabetic nephropathy was the lowest, but prevalence of chronic glomerulonephritis and chronic tubulointerstitial nephritis was the highest in the IHD group with the longest RRT duration. Patients with or without seroconversion to anti-HBC positivity did not differ significantly in these four main causes of ESRD. In previous studies, diabetes mellitus and hypertension were not associated with probability of acquiring HBsAg positivity [6].

Prevalence of anti-HCV and HCV RNA positivity was the lowest in patients starting IHD than in those already being on RRT. Increasing prevalence of anti-HCV positivity with IHD duration was already documented in the previous studies [13,19,20]. However, there were no differences in this respect between groups with or without seroconversion to anti-HBC positivity. Results of anti-HCV and HCV RNA testing were also not predictive for seroconversion to anti-HBC positivity, although anti-HCV positivity was associated with prevalence of anti-HBC in various groups of population [21,22]. In IHD patients, results of such coincidence are not uniform [11,14,23].

Our IHD patients grouped by RRT duration before the study entry had significantly different rate of full vaccination series against HBV with developed anti-HBs titre >10 IU/L considered as protective for HBV infection. The longer the RRT duration, the higher the frequency of patients with satisfactory immunization (an anti-HBs titre >10 IU/L). In dialysis patients, receiving vaccine for the first time, antibody response was positively associated with the length of time on dialysis prior to receipt of vaccine [24]. Fabrizi et al. [25] reported that responder vaccines showed a length of time on IHD slightly longer than non-responders, whereas Sorkhi et al. [26] have found that duration of IHD had no significant effect on response to vaccination, although 80.0% patients with duration <2 years responded compared with 100% of those with longer durations. Our patients, being longer on RRT, underwent usually two full vaccination series against HBV and were given booster doses of vaccine. Finally, it could result in an anti-HBs titre >10 IU/L. Patients being long on RRT underwent natural selection with elimination of weaker patients, usually suffering from multiple co-morbidities. Surviving patients could have a greater ability for im-
munological response to vaccination. These findings are not contradictory to the difference between response to vaccination in early stages of chronic kidney disease and in patients on RRT, being the best in deep pre-dialysis period [27].

Incidence of HBV infection was higher at centres, in which <50% of patients received hepatitis B vaccine [28]. In our study, the only variable predicting seroconversion to anti-HBc positivity was the lack of full vaccination series against HBV or vaccination with failed immunization. Vaccination with an anti-HBs titre conferring protection was reported in 54–82% of patients using a recombinant vaccine [29,30]. In our study, seroprotection titre was obtained in 80.2% of all patients, and there was not a significant difference in frequency of an anti-HBs titre >10 IU/L proceeding seroconversion to anti-HBc positivity between patients with or without seroconversion (66.7% vs 80.7%, respectively). Occurrence of an anti-HBs titre >10 IU/L in 67% of seroconverted patients suggests that such anti-HBs titre was not protective against HBV infection in all patients. Fabrizi et al. [31] have listed a few circumstances under which a level of 10 IU/L might not completely exclude HBV infection. These would include exposure to an overwhelming HBV dose [32], production of antibody recognizing an HBsAg determinant different from that common to all subtypes [33,34], or infection by an HBV mutant producing HBsAg with determinants not neutralized by anti-HBs [35,36]. As a level of an anti-HBs titre close to 10 IU/L might not completely exclude HBV infection, Lombardi et al. [37] postulated that in dialysis patients, higher anti-HBs titre should be considered as protective (>50 IU/L). In some groups of patients, like HBsAg-negative children awaiting liver transplantation, an anti-HBs titre >200 IU/L was advised to be sufficient to prevent de novo HBV infection [38].

As the full vaccination series resulting in development of an anti-HBs titre >10 IU/L (with relatively low titres included) was a significant negative predictor for seroconversion to anti-HBc positivity, our patients already on RRT (Group I and II) benefited from remarkably higher immunization rate due to vaccination against HBV. Despite this fact, in these groups, seroconversion rate to anti-HBc positivity was not lower than that in patients starting RRT, showing a satisfactory result of vaccination in ~70% of cases (compared with 85% and 89% in Group II and III, respectively). Thus, it is very probable that there are other important predictors of seroconversion to anti-HBc positivity. Hospitalizations, co-morbidities and their severity, medical or cosmetic procedures with interruption of skin/mucosa integrity, accidental needle stick exposure from a HBV carrier, contamination from environmental surfaces, internally

Table 2. Demographic, clinical and laboratory data of examined patients grouped by occurrence of seroconversion to anti-HBc positivity

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group without seroconversion n = 409</th>
<th>Group with seroconversion n = 15</th>
<th>P-value for differences between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian/non-Caucasian</td>
<td>406/3 (99.3%/0.7%)</td>
<td>15/0 (100%/0%)</td>
<td>0.2</td>
</tr>
<tr>
<td>Men/women</td>
<td>221/188 (45.6%/0.0%)</td>
<td>78/188 (46.7%/53.3%)</td>
<td>0.8</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60.5 ± 14.8</td>
<td>61.0 ± 16.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Duration of RRT (years)</td>
<td>0.42 (0.00–16.9)</td>
<td>1.57 (0.00–10.9)</td>
<td>0.4</td>
</tr>
<tr>
<td>Full vaccination series against HBV with developed anti-HBs titre &gt;10 IU/L (n, % of all)</td>
<td>330 (80.7%)</td>
<td>10 (66.7%)</td>
<td>0.3</td>
</tr>
<tr>
<td>Positive/negative anti-HCV (n, % of all)</td>
<td>50/359 (12.2%/87.8%)</td>
<td>2/13 (13.3%/86.7%)</td>
<td>0.8</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>15.6 ± 10.0</td>
<td>16.7 ± 8.2</td>
<td>0.4</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>17.0 ± 9.2</td>
<td>17.2 ± 6.0</td>
<td>0.4</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>24.0 (3.0–462.0)</td>
<td>18.5 (7.0–323.0)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation or median and range. ALT, alanine aminotransferase; anti-HBs, antibodies to surface antigen of hepatitis B virus; anti-HCV, antibodies to hepatitis C virus; AST, aspartate aminotransferase; GGT, gamma-glutamyltranspeptidase; HBV, hepatitis B virus; HCV RNA, ribonucleic acid of hepatitis C virus; RRT, renal replacement therapy.
contaminated dialysis equipment, and individual IHD centre management protocols were excluded from the analysis, and their influence on seroconversion, although possible as indicated by other studies [31,39], remained unidentified.

All patients, who converted to anti-HBc positivity, had negative HBV DNA testing. In anti-HBc-positive dialysis patients, HBV DNA seems to be seldom found [11,40]. In our cross-sectional study, we were able to show one HBV DNA-positive patient in the group of 164 anti-HBs-negative and anti-HBc-positive IHD patients [14]. In the study of Fabrizi et al. [11], among dialysis patients seropositive for anti-HBc, none (zero of 123) had detectable HBV DNA. Out of 116 HBsAg-negative patients awaiting renal transplantation, 13% had anti-HBc, but none had detectable HBV DNA [40]. HBV DNA remains detectable more so in the liver than in the serum of anti-HBc-positive persons [41]. HBV DNA was also detected in peripheral blood mononuclear cells of HBsAg-negative and anti-HBc-positive IHD patients [42], but not in all studies [9]. These data indicate that anti-HBc-positive patients may be a source of HBV infection under circumstances favourable for HBV replication.

Although there are concerns regarding anti-HBc positivity, HBsAg negativity has been somewhat allayed by the belief that anti-HBc-positive patients underwent HBV infection and generally do not replicate HBV, and seroconversions to anti-HBc positivity indicate transmission of HBV to IHD patients. It requires a need for very strict realization of rigorous HBV vaccination programme and stricter isolation/separation policies for in-centre IHD patients. Seroconversions to anti-HBc positivity also indicate the limitations of infection control policies, the need for full implementation of appropriate infection control procedures and ongoing audit of HBV transmission rates for eradicating HBV among IHD patients.

Monitoring of seroconversion rate to anti-HBc positivity should be strongly advocated because HBV transmission resulting in anti-HBc positivity without detectable HBsAg may have clinical implications, which need diagnosis. It is already concluded that all anti-HBc-positive and HBsAg-negative dialysis patients on the transplant waiting list should be regarded as at risk of hepatitis B reactivation following immunosuppression due to renal transplantation [40]. Blanpain et al. [43] estimated the risk of reactivation on 5%. HBV can be also transmitted from anti-HBc-positive and HBsAg-negative donors to organ recipients [44]. Frech et al. [45] showed that 2% of women with isolated anti-HBc positivity acquired HBsAg positivity at a median of 7.5 years. In addition to the aforementioned clinical implications, the relationship between anti-HBc positivity and hepatic and pancreatic carcinogenesis should be mentioned, although it is still not clear [46–48].

In conclusion:

- the IHD patients are still under significant exposure on HBV infection which results in seroconversion to anti-HBc and HBsAg positivity;
- seroconversion rate to anti-HBc positivity is remarkably higher than that to HBsAg positivity (2.35 patient-years vs 0.16 episodes/100 patient-years);
- a lack of effective vaccination against HBV is a significant predictor of seroconversion to anti-HBc positivity, which can be influenced by a revised vaccination strategy;
- the duration of RRT seems not to be a risk factor for seroconversion to anti-HBc positivity;
- and a periodical determination of anti-HBc in dialysis centres with the evaluation of seroconversion rate to anti-HBc positivity may be helpful in recognition of epidemiological status and risk for HBV infection, and useful in making a decision on commencement of more effective prophylactic measures.

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Conflict of interest statement. None declared.

References

34. Koziol DE, Alter HJ, Kirchner JP et al. The development of HBsAg-positive hepatitis despite the previous existence of antibody to HBsAg. *J Immunol* 1976; 117: 2260–2262
40. Roberts RC, Lane C, Hatfield P et al. All anti-HBc-positive, HBsAg-negative dialysis patients on the transplant waiting list should be regarded as at risk of hepatitis B reactivation post-renal transplantation—report of three cases from a single centre. *Nephrol Dial Transplant* 2006; 21: 3316–3319
41. Lok AS, Mcmahon BJ. Chronic hepatitis B. *Hepatology* 2007; 45: 507–539
44. Wachs ME, Amend WJ, Ascher NL et al. The risk of transmission of hepatitis B from HBsAg(−), HBeAb(+), HBcAb(−) organ donors. *Transplantation* 1995; 59: 230–234

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