Assessment of hepatitis B surface antibody in children after allogeneic peripheral blood stem cell transplantation and impact of donor/recipient immunity on hepatitis B surface antibody disappearance

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Abstract

After allogeneic peripheral blood stem cell transplantation (PBSCT), children are at high risk of hepatitis B virus (HBV) infection because of the potential loss of HBV immunity. The factors which can affect it are not fully understand. This study aimed to assess the probability of hepatitis B surface antibody (HBsAb) disappearance after PBSCT and to evaluate the impact of donor and recipient immunity on HBsAb disappearance. A total of 110 patients who underwent PBSCT between January 2016 and December 2018 and their paired donors were retrospectively enrolled in this study. Before transplantation, 87 (79.1%) patients were HBsAb seropositive, and 23 (20.9%) were HBsAb seronegative. Fifty-five (63.2%) patients with protective HBsAb titers before PBSCT lost their HBV immunity within one year after transplantation. Univariate analysis showed that the low recipient pretransplant HBsAb titer, antithymocyte globulin (ATG) administration, corticosteroid administration and graft-versus-host disease (GVHD) were significant risk factors for HBsAb disappearance ($P<0.05$). Multivariate analysis showed that only recipient pretransplant HBsAb titers lower than 207.5 IU/L ($P=0.022$, hazard ratio (HR): 1.925, 95% confidence interval (CI): 1.101-3.367) and the presence of GVHD ($P=0.033$, HR=1.921, 95% CI: 1.056-3.495) were risk factors for HBsAb disappearance one year after HSCT. In conclusion, most recipients lost previously acquired immunity to HBV after PBSCT. A high titer of HBsAb in the recipient before transplantation had a protective effect against posttransplant HBsAb disappearance, but the presence of donor immunity did not significantly influence the maintenance of recipient immunity to HBV.

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Short title: Assessment of hepatitis B surface antibody in children after PBSCT

Key words: Pediatric; Hepatitis B virus; Serologic immunity; Allogeneic peripheral blood stem cell transplantation

List of abbreviations:

AA: aplastic anemia; AL: acute leukemia; allo-HSCT: allohematopoietic stem cell transplantation; ATG: antithymocyte globulin; CML: chronic myeloid leukemia; GVHD: graft-versus-host disease; HBsAb: hepatitis B surface antibody; HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus; HLA: human leukocyte antigen; MAC: myeloablative conditioning; MDS: myelodysplastic syndrome; NHL: Non-Hodgkin's lymphoma; NMAC: nonmyeloablative conditioning; PBSCT: peripheral blood stem cell transplantation; ROC: receiver operating characteristic.

Abstract

After allogeneic peripheral blood stem cell transplantation (PBSCT), children are at high risk of hepatitis B virus (HBV) infection because of the potential loss of HBV immunity. The factors which can affect it are not fully understand. This study aimed to assess the probability of hepatitis B surface antibody (HBsAb) disappearance after PBSCT and to evaluate the impact of donor and recipient immunity on HBsAb disappearance. A total of 110 patients who underwent PBSCT between January 2016 and December 2018 and their paired donors were retrospectively enrolled in this study. Before transplantation, 87 (79.1%) patients were HBsAb seropositive, and 23 (20.9%) were HBsAb seronegative. Fifty-five (63.2%) patients with protective HBsAb titers before PBSCT lost their HBV immunity within one year after transplantation. Univariate analysis showed that the low recipient pretransplant HBsAb titer, antithymocyte globulin (ATG) administration, corticosteroid administration and graft-versus-host disease (GVHD) were significant risk factors for HBsAb disappearance (P <0.05). Multivariate analysis showed that only recipient pretransplant HBsAb titers lower than 207.5 IU/L (P =0.022, hazard ratio (HR): 1.925, 95% confidence interval (CI): 1.101-3.367) and the presence of GVHD (P =0.033, HR =1.921, 95% CI : 1.056-3.495) were risk factors for HBsAb disappearance one year after HSCT. In conclusion, most recipients lost previously acquired immunity to HBV after PBSCT. A high titer of HBsAb in the recipient before transplantation had a protective effect against posttransplant HBsAb disappearance, but the presence of donor immunity did not significantly influence the maintenance of recipient immunity to HBV.

Introduction

Allohematopoietic stem cell transplantation (allo-HSCT) has been commonly employed to treat hematologic malignancies, serious or refractory aplastic anemia and some congenital diseases. However, HSCT results in secondary immune deficiency that lasts for 1-2 years, including the loss of protective antibody levels against certain vaccine-preventable diseases, which predisposes transplant recipients to infections [1-5]. China remains one of the countries in the world with a high prevalence of viral hepatitis B according to WHO data, which estimates that approximately 86 million people in China are hepatitis B surface antigen (HBsAg) carriers [6-8]. Therefore, after allo-HSCT, children are at high risk of hepatitis B virus (HBV) infection due to secondary immune dysfunction and the need for multiple blood transfusions.

The primary objective of this study was to assess the probability of hepatitis B surface antibody (HBsAb) disappearance in children after allogeneic peripheral blood stem cell transplantation (PBSCT), and the second was to evaluate the impact of donor and recipient immunity on HBsAb disappearance.

Methods

Patients

Data from patients who underwent allogeneic PBSCT between January 2016 and December 2018 in Shanghai Children's Medical Center and their paired donors were reviewed retrospectively. Informed consent was obtained from each patient or their family, following the principles of the Declaration of Helsinki. Inclusion criteria included age under 18 years, sustained remission of the underlying disease after PBSCT, a follow-up period of more than 12 months, a complete set of clinical and medical records, and no medical history of HBV infection. Exclusion criteria included the failure to achieve remission or death during the study period, HBV infection prior to transplantation and missing complete records regarding HBsAb. Basic information about the patients, paired donors and transplant-related complications was extracted by retrospective review of the electronic medical records.

Antibody measurements

Serology tests for HBsAg, HBsAb, hepatitis B e antigen, hepatitis B e antibody, and hepatitis B core antibody were performed quantitatively using ELISA in all children before transplantation and at nearly 1 month, 3 months, 6 months and 12 months after transplantation. Blood samples were also collected to evaluate HBV serology status in donors before transplantation. HBsAb titers were defined as negative at levels <10 IU/L and positive at [?]10 IU/L; positive titers were considered to confer protective immunity against HBV.

Statistical analysis

The Statistical Program for Social Sciences (SPSS 23 for Windows) was employed for the statistical analysis. The Mann-Whitney-Wilcoxon rank-sum test was used to evaluate whether there was a significant difference in HBsAb levels between patient groups after transplantation. The χ^2 and Fisher exact tests were used to compare the difference in the HBsAb negative conversion rates between groups. The receiver operating characteristic (ROC) curve was used to evaluate the cutoff value of the pretransplant HBsAb titers for the prediction of HBsAb disappearance after HSCT. Multivariate analysis was performed using Cox regression. $P < 0.05$ (2-tailed) was identified as statistically significant.

Results

Patient characteristics

A total of 110 patients were enrolled in this study. The median ages of the 110 patients and donors were 5.5 years (range 0.8-16.7 years) and 26.5 years (range 1-46 years), respectively. Fifty-seven patients received myeloablative conditioning (busulfan plus cyclophosphamide, total-body irradiation plus cyclophosphamide, or total-body irradiation plus cyclophosphamide and etoposide), and 53 patients received nonmyeloablative conditioning (fludarabine plus cyclophosphamide). All patients received PBSCT. Ninety-eight of the 110 patients were treated with antithymocyte globulin (ATG) during conditioning, and 61 patients were offered corticosteroid therapy (Table 1).

HBsAb titers of recipients before and after transplantation

Before transplantation, 87 patients were HBsAb seropositive, and 23 were HBsAb seronegative. Twenty-one of the 23 patients who were HBsAb seronegative before transplantation became HBsAb seropositive after transplantation; 7 of the 21 patients had nonimmunized donors, and 14 had immunized donors. Within the first 3 posttransplant months, there was a trend toward higher HBsAb levels. The median HBsAb titers before the transplant and at 1 month, 3 months and 6 months after the transplant were 123.4, 437.3, 405.4 and 108.8 IU/L, respectively, and there were significant differences in HBsAb levels before and after transplantation (P <0.05). A decrease in HBsAb titers started from 6 months after the transplant, and 10 patients had

unmeasurable HBsAb levels. Finally, there were 55 (63.2%) patients with protective HBsAb titers before transplantation became HBsAb seronegative within one year after transplantation.

Impact of pretransplant donor HBsAb titers

Of the 110 patients, 72 patients (Group A, 65.5%, 72/110) received grafts from HBsAb-positive donors, and 38 patients (Group B, 34.5%, 38/110) received grafts from HBsAb-negative donors. There was no significant difference between patients in groups A and B in the levels of HBsAb at 1 month (P =0.278), 3 months (P =0.307) and 6 months (P =0.282) after transplantation or in the negative conversion rate of HBsAb within one year after transplantation (P =0.059).

Impact of pretransplant recipient HBsAb titers

Eighty-seven patients with protective immunity to HBV before transplantation were divided into two groups according to the status of HBsAb after transplantation: group A was composed of 55 (63.2%, 55/87) patients who became HBsAb negative, and group B was composed of 32 (36.8%, 32/87) patients who maintained HBsAb positivity one year after transplantation. The initial HBsAb titers in group B were significantly higher than those in group A (287.1 vs. 154.6 IU/L, P = 0.015).

The ROC curve was used to evaluate the cutoff value of the HBsAb titer before HSCT for the prediction of HBsAb disappearance within one year after HSCT. The area under the curve was 0.656 (P = 0.015), with a sensitivity of 62.5% and a specificity of 65.5%, and the cutoff value for the pretransplant HBsAb titer was 207.5 IU/L.

Impact of other pretransplant characteristics

There were significant differences in the negative conversion rate of HBsAb after transplantation between patients treated with and without ATG (P=0.047), patients treated with and without corticosteroids (P=0.009), and patients with and without GVHD (P<0.001). However, there was no difference in the negative conversion rate of HBsAb based on pretransplant characteristics, including patient age, sex and human leukocyte antigen (HLA) compatibility (P >0.05) (Table 2).

Multivariate analysis

Cox regression was performed to further evaluate the possible risk factors for HBsAb disappearance within one year after PBSCT. The following factors were included in the analysis: patient age, patient sex, recipient pretransplant HBsAb titer, donor pretransplant HBsAb titer, the administration of ATG, the administration of corticosteroids and the presence of GVHD. Of all the variables, only recipient HBsAb titers <207.5 IU/L before transplantation and the presence of GVHD were significantly associated with HBsAb disappearance within one year after HSCT (Table 2).

Discussion

In this study, we observed a significant increase in recipient HBsAb titers within the first 3 months after the transplant even in patients who were seronegative before the transplant, which can be explained as follows: (1) There may have been a transfer of antibody-producing cells from the donor. After myeloablation, the vast majority of specific helper T cells and B cells are transferred through allografts [9-12]. (2) There may have been persistent antigens and plasma cells in the recipients. The fact that administration of a recall protein antigen to recipients during conditioning can increase the posttransplant antibody levels shows the role of the antigen in recipients because it is not possible for recipient B cells to differentiate into plasma cells and produce antigen-specific antibodies at this time. In contrast, the antigens in recipients may stimulate donor B cells to differentiate into plasma cells [12].

Although HBsAb titers in recipients can remain positive or even show an upward trend in the early stage after transplantation, children who undergo PBSCT are inevitably at high risk of losing previously acquired immunity to HBV as a result of initially impaired humoral immunity because of the limited antibody repertoire and prolonged inadequate T cell-dependent B cell response [4]. Published studies with different

numbers of patients and follow-up periods have reported the incidence of HBsAb disappearance, which varies from 49.6%-100% [13-16]. Consistent with a previous study, we observed a decrease in HBsAb titers 6 months after the transplant, with 10 patients having unmeasurable HBsAb levels, and 63.2% of the patients in this study finally lost protective immunity to HBV within one year after transplantation.

It is theoretically feasible to transfer both cellular and humoral immunity from vaccinated donors to recipients through HSCT [12,17-21]. However, clinical studies assessing the impact of donor immunity status on the posttransplant HBsAb levels of recipients have produced inconclusive results [12,14,16,22-24]. One study conducted by Storek et al. [23] did not show substantial differences in posttransplant HBsAb titers between recipients who had vaccinated donors and those who had unvaccinated donors, and Kaloyannidis et al. [14] reported the same findings. Another study with a small sample size reported a benefit to the recipients of vaccinating donors with the hepatitis B vaccine [12], which was broadly in line with the finding from a study by Park et al. [16], which demonstrated that there was a protective effect of donor HBsAb on the maintenance of recipient HBsAb positivity. In our study, there was a significant correlation between recipient HBsAb titers before and after PBSCT, and initial HBsAb titers higher than 207.5 IU/L had a protective effect on the maintenance of posttransplant antibody levels. In contrast, the presence of donor immunity did not substantially influence the recipient HBsAb levels or the final HBsAb negative conversion rate after PBSCT. Hence, the evidence supporting the vaccination of donors to improve recipient antibody titers is weak.

ATG was widely administered in patients in this study during the conditioning regimen to decrease the occurrence of GVHD, and a considerable proportion of the patients received corticosteroids, which are associated with delayed immune recovery, due to severe GVHD [4]. As expected, these were significant factors that influenced the HBsAb titers according to the univariate analysis. In accordance with reported studies [14,16], the presence of GVHD was also a significant risk factor for HBsAb disappearance within one year after transplantation.

There were certain limitations of our study. The protocol was designed based on a single organization with a relatively small population of patients and used retrospective data. Furthermore, it is unlikely that we obtained comprehensive HBV vaccination data from the donors due to the nature of the research, so we could not analyze detailed information about the type of donor immunity.

In summary, in most recipients, pretransplant immunity to HBV is lost after PBSCT. A high pretransplant HBsAb titer in the recipient had a protective effect against HBsAb disappearance, but the presence of donor immunity did not significantly influence the posttransplant HBsAb titer. Consequently, careful monitoring of HBsAb status both before and after transplantation and providing booster hepatitis B vaccination to recipients who became HBsAb seronegative in a timely manner should be considered. In addition, vaccinating recipients before transplantation to achieve high HBsAb levels may be beneficial to the maintenance of HBsAb positivity after transplantation.

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Authors' contributions

CQ, FY, LCY and GYJ designed the study. All authors were involved in data collection, statistical analysis, interpretation of data, and drafting or revision of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Table 1. Patient characteristics

Characteristics	N
Patient sex	
Male	70
Female	40
Patient age at transplantation, years, median (range)	5.5(0.8-16.7)
Donor age at transplantation, years, median (range)	26.5(1-46)
Diagnosis	
AL or MDS	44
CML	1
NHL	2
AA	49
Thalassemia	2
Fanconi anemia	2
Mucopolysaccharidosis	5
Others	5
Donor-patient histocompatibility	
HLA-identical siblings	41
Partially mismatched family donors	69
Conditioning regimen	
MAC	57
NMAC	53
ATG administration	98
GVHD prophylaxis	
Cyclosporine plus mycophenolate mofetil	50
Cyclosporine plus methotrexate	60
Presence of GVHD	61
Steroids administration	61
Donor HBsAb status before transplantation	
HBsAb(+)	72
HBsAb(-)	38
Recipient HBsAb status before transplantation	
HBsAb(+)	87
HBsAb(-)	23

AL: acute leukemia; MDS: myelodysplastic syndrome; CML: chronic myeloid leukemia; NHL: Non-Hodgkin's lymphoma; AA: aplastic anemia; HLA: human leukocyte antigen; MAC: myeloablative conditioning; NMAC: nonmyeloablative conditioning; ATG: antithymocyte globulin; GVHD: graft-versus-host disease; HBsAb: hepatitis B surface antibody

Variable	HBsAb(+) Pretransplant	HBsAb(-) Posttransplant(%)	Univariate Analysis (*P* value)	Multivariate Analysis	Multivariate Analysis
				P value	*HR* (95% CI)
Age					
[?]8 years	59	35(59.3)	0.274	0.113	*
>8 years	28	20(71.4)			
Sex					
Male	54	32(59.3)	0.327	0.515	*
Female	33	23(69.7)			
ATG administration					
Yes	80	53(66.3)	0.047	0.204	*
No	7	2(28.6)			
Steroid administration					
Yes	51	38(74.5)	0.009	0.652	*
No	36	17(47.2)			
GVGD					
Yes	50	40(80.0)	<0.001	0.033	1.921 (1.056-3.495)
No	37	15(40.5)			
Donor HBsAb status before transplantation					
(-)	30	23(76.7)	0.059	0.698	*
(+)	57	32(56.1)			
Recipient HBsAb titer before transplantation					
<207.5IU/L	48	36(75.0)	0.011	0.022	1.925 (1.101-3.367)
[?]207.5IU/L	39	19(48.7)			

Table 2. Risk factors for HBsAb disappearance after transplantation

ATG: antithymocyte globulin; **GVHD:** graft-versus-host disease; **HBsAb:** hepatitis B surface antibody