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Hepatitis B Virus Reactivation following Allogeneic Hematopoietic Stem Cell Transplantation

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Reactivation of resolved hepatitis B virus (HBV) infection has been reported in allogeneic hematopoetic stem cell transplantation (HSCT) recipients, but its epidemiology is not well characterized. We performed a retrospective assessment of the timing and risk factors of HBV reactivation among patients with resolved HBV infection undergoing allogeneic HSCT between January 2000 and March 2008. HBV reactivation was defined as development of positive hepatitis B surface antigen after transplant. Among the 61 patients with resolved HBV infection before transplant (hepatitis B core antibody-positive, hepatitis B surface antigen-negative), 12 (19.7%) developed HBV reactivation. The cumulative probability of HBV reactivation 1, 2, and 4 years after transplant was 9.0%, 21.7%, and 42.9%, respectively. In a time-dependent Cox model, the adjusted hazard ratio (HR) of HBV reactivation for patients with pretransplant hepatitis B surface antibody levels <10 milli-international units per milliliter (mIU/mL) was 4.56 (95% confidence interval [CI] 1.23-16.9) compared to those with levels ≥ 10 mIU/mL; the adjusted HR among patients who developed extensive chronic graft-versus-host disease (cGVHD) was 7.21 (95% CI 1.25-41.5) compared to those who did not. HBV reactivation is a common late complication among allogeneic HSCT recipients with pretransplant resolved infection. Screening for HBV reactivation should be considered for at-risk HSCT recipients. In this cohort, HBV reactivation often developed in patients with cGVHD. Liver biopsy was useful in those patients with both to delineate the contribution of each to liver dysfunction.

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INTRODUCTION

Reactivation of latent viral pathogens after allogeneic hematopoetic stem cell transplantation (HSCT) is a significant clinical problem. Viral pathogens such as cytomegalovirus (CMV) predictably reactivate and cause disease at high rates following allogeneic HSCT. Hepatitis B virus (HBV) infection is of particular interest after allogeneic HSCT because it is a common infection; over 2 billion people worldwide have been infected with HBV, and it causes chronic infection in over 350 million people [1]. In the last 2 decades, several cancer centers in regions endemic for HBV have reported reactivation of HBV characterized by a significant rise in HBV virus load and development of hepatitis after HSCT in patients who were asymptomatic chronic carriers of HBV prior to transplantation [2-4].

HBV reactivation has also been reported after allogeneic HSCT in recipients with evidence of resolved HBV prior to transplantation, where resolved HBV infection is indicated by negative hepatitis B surface antigen (HBsAg), positive hepatitis B core antibody (HBcAb), and/or positive hepatitis B surface antibody (HBsAb) in the absence of prior HBV vaccination [3-17]. Historically, clearance of HBsAg after HBV infection, in conjunction with the appearance of HBsAb, signaled resolution of HBV infection. However, multiple studies have demonstrated that HBV can persist in the liver and in peripheral blood mononuclear cells (PBMCs) for years after serologic resolution of the infection [18-21]. These reservoirs are likely the source for HBV reactivation in this population, which is characterized by redevelopment of circulating HBsAg and HBV with or without

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associated hepatitis after transplantation. This reactivation phenomenon has also been termed "reverse seroconversion" in HSCT recipients who were HBsAb positive prior to transplantation and subsequently lose HBsAb positivity in conjunction with redevelopment of circulating HBsAg after transplantation [7].

Several case series and small studies have attempted to assess the incidence, risk factors, and timing of HBV reactivation in the allogeneic HSCT population. The reported cumulative incidence of HBV reactivation among allogeneic HSCT recipients with evidence of resolved HBV varies between studies, ranging from 6% to 86% [7,10-12,14-16]. Several small studies have suggested a variety of possible precipitating factors including HBsAb negative serologic status of the donor [7,10], development of chronic graft-versus-host disease (cGVHD) [5,11-13], corticosteroid exposure after HSCT [10], and loss of protective native HBsAb after HSCT [10,14-17]. In addition, small case series have suggested that the risk of reactivation persists for years after transplantation [10,14,16]. However, these findings are limited by the short duration of follow-up in many studies and the small number of patients included in most studies. Furthermore, there are no data or guidelines available to direct subsequent assessment and management of active HBV infection in this specific population [22-25].

In the present study, we assessed the potential precipitating factors and timing of HBV reactivation in a large cohort of patients with a history of resolved HBV infection who subsequently underwent allogeneic HSCT at our institution. We also described the clinical characteristics and treatment history of the subjects who developed HBV reactivation during follow-up.

MATERIALS AND METHODS

Patients

All adult patients who underwent allogeneic HSCT at the Dana-Farber Cancer Institute/Brigham and Women's Hospital (DFCI/BWH) between January 1, 2000, and March 31, 2008, were included in this analysis. Patients were identified as having resolved HBV infection if they were HBcAb positive, HBsAg negative, and HBV virus load negative on pretransplant evaluation. Patients who were HBsAb positive and HBcAb negative were not considered to have resolved HBV infection because DFCI/BWH is not in an HBV-endemic area, and, therefore, most patients who are HBsAb positive in the absence of other hepatitis B markers have been vaccinated for HBV and have not been previously exposed to HBV.

The DFCI/BWH stem cell transplant database and electronic medical records were reviewed for covariates and outcomes of interest including: age at the time of transplantation, sex, birthplace, reason for HSCT pretransplant infection status of chronic hepatitis C virus (HCV) and human immunodeficiency virus (HIV), transplant conditioning regimen, donor relatedness, human leukocyte antigen (HLA) donor matching, stem cell source, development and maximal grade of acute GVHD (aGVHD), development and grade of cGVHD, pretransplant HBsAb measurements, and use of posttransplant immunosuppressive therapy including corticosteroids and rituximab. Patient data were censored at death or on December 1, 2008. The Office for Human Research Studies at the DFCI/BWH approved this study.

Transplant conditioning regimens were classified as either myeloablative (MA) or reduced intensity (RIC) based on the conditioning agents used. During the study period, MA regimens at DFCI/BWH typically included cyclophosphamide (Cy) and total body irradiation (TBI). The standard RIC regimen at the DFCI/BWH during the study period typically included low-dose busulfan (Bu) and fludarabine (Flu). Donors were considered HLA matched if 6 of 6 HLA-A, -B, and -DRB1 were identical. aGVHD was graded according to the consensus grading system [26]. cGVHD was graded by the system proposed by Shulman et al. [27]. HBsAb was treated as a dichotomous variable; the groupings included patients who were HBV immune with HBsAb $\geq$10 milli-international units per milliliter (mIU/mL) and patients who were not immune with HBsAb $<$10 mIU/mL [28].

Among those cohort members who developed HBV reactivation after HSCT, information was collected regarding assessment and treatment of HBV including: liver biopsy results, alanine aminotransferase (ALT) results, HBV virus load and genotype, HBV antigen tests including hepatitis Be antigen (HBeAg) and HBsAg, HBV serologic tests, including hepatitis Be antibody and HBsAb, and duration and choice of antiviral therapy directed at HBV infection.

HBV Testing

All stem cell donors are routinely tested for HBsAg and HBcAb prior to donation, according to standard blood donation screening procedures in the United States [29]. HBsAb is not part of these standard screening procedures, and was not routinely measured in stem cell donors during the study period. All potential HSCT recipients are routinely tested for HBcAb and HBsAg prior to transplantation. Those potential recipients who are HBcAb positive on initial screen are also subsequently tested for HBsAb and HBV virus load at our institution.

HBV reactivation was defined in our cohort as development of positive HBsAg after transplantation in recipients who had negative results before

transplantation. HSCT recipients who were HBcAb positive before transplantation were assessed for HBV reactivation after transplant with HBsAg and HBV virus load testing on the basis of signs, symptoms, or clinical suspicion at the discretion of his or her primary transplant oncologist. In addition, beginning in the Fall of 2004, recipients who were HBcAb positive prior to transplantation were also intermittently screened for HBV reactivation after transplant with HBsAg and HBV virus load testing in the absence of signs or symptoms.

Serum measurements of HBsAb were performed using different assays during the study period. Most measurements of HBsAb during the study period were made by quantitative enzyme immunoassay (Quest Diagnostics, Cambridge, MA) and a few were made by the qualitative Vitros ECi immunometric assay (Ortho Clinical Diagnostics, Rochester, NY). Measurements of HBsAg and HBcAb were also performed using different assays during the study period. Measurements of HBcAb during the study period were made using the Vitros ECi immunometric assay (Ortho Clinical Diagnostics), chemiluminescent immunoassay (Abbott Diagnostics, Abbott Park, IL). or enzyme immunoassay (Abbott Diagnostics). Measurements of HBsAg during the study period were made using the Vitros ECi immunometric assay (Ortho Clinical Diagnostics) or by chemiluminescent immunoassay (Abbott Diagnostics).

HBV virus load was determined by real-time polymerase chain reaction (PCR) using Roche COBAS TaqMan HBV Test (Roche, Basel, Switzerland) between January 2000 and January 2008. After January 2008, HBV virus load was determined by the branched DNA method (Mayo Medical Laboratories, Rochester, MN). HBV genotyping of the S and POL genes (Quest Diagnostics Nichols Institute, San Juan Capistrano, CA) was performed in patients with HBV reactivation at the time reactivation was diagnosed and when suspected resistance developed during therapy.

Statistical Analysis

Baseline pretransplant and posttransplant characteristics were initially compared by 2-sided Fisher's exact test or Wilcoxon rank-sum test where appropriate. The incidence rate of HBV reactivation was determined, and its confidence interval was calculated by Fisher's method using OpenEpi version 2.2.1 (http://www.openepi.com; Atlanta, GA). Kaplan-Meier curves were calculated to determine the cumulative probability of HBV reactivation. Possible predictors of HBV reactivation identified in the initial analysis were evaluated in univariate Cox proportional hazard models. The hazard ratio (HR) and 95% confidence interval (CI) were determined for each candidate covariate. aGVHD and cGVHD were modeled as time-dependent covariates. Candidate covariates included in multivariate analysis were limited to those closely associated with HBV reactivation ($P \leq .10$) given the number of events in the cohort. Statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC).

RESULTS

Baseline Characteristics

Of 1386 patients who underwent allogeneic HSCT during the study period, 61 (4.4%) had evidence of resolved HBV infection with positive HBcAb prior to transplantation. Baseline pretransplant characteristics of the cohort of patients with resolved HBV infection prior to transplant are displayed in Table 1. There were more men than women in this cohort, and the majority of patients had underlying acute or chronic leukemia as the reason for transplantation. All patients with evidence of prior HBV infection were HIV negative and HBV virus load negative at baseline. One patient had chronic HCV infection prior to transplantation. Patients in this cohort were followed for a median of 17.0 months (range: 2.0-81.0 months).

HBV Reactivation

Twelve of the 61 patients in the at-risk cohort of patients who were HBcAb positive prior to HSCT developed HBV reactivation after transplantation (cumulative incidence 19.7%). The patient with chronic HCV infection prior to transplantation was not among the 12 who developed HBV reactivation. The incidence rate of HBV reactivation in this group was 0.11 episodes per patient-year after transplantation (95% CI 0.55-1.85). The cumulative probability of the HBV reactivation was 9.0% at 1 year, 21.7% at 2 years, 30.4% at 3 years, and 42.9% at 4 years after HSCT. HBV reactivation was diagnosed at a median time of 17.5 months after HSCT (range: 4.4 to 46.9 months; interquartile range: 11.2 to 26.4 months).

Risk Factors for HBV Reactivation

There was no difference in the cumulative incidence of HBV reactivation in the at-risk cohort based on most baseline characteristics (Table 1). Patients who developed HBV reactivation tended to be younger than those who did not ($P = .09$). In addition, recipients with HBsAb <10 mIU/mL before transplantation were significantly more likely to develop HBV reactivation during follow up than those who had baseline HBsAb levels ≥ 10 mIU/mL ($P = .02$).

The cumulative incidence of HBV reactivation in the at-risk cohort based on posttransplant characteristics was also assessed (Table 2). There was no difference

Table 1. Baseline Pretransplant Characteristics of HBcAb-Positive Allogeneic HSCT Cohor

Pretransplant Characteristics	Reactivation N = 12 (%)	No Reactivation N = 49 (%)	Total N = 61 (%)	*P*
Recipient age, years				.09
Median	45	52	51	
Range	32-62	19-71	19-71	
Recipient sex				1.00
Male	9 (75)	34 (69)	43 (70)	
Female	3 (25)	15 (31)	18 (30)	
Reason for transplant				.61
Acute leukemia or MDS	7 (58)	18 (37)	25 (41)	
Chronic leukemia	3 (25)	15 (31)	18 (30)	
Lymphoma or multiple myeloma	2 (17)	12 (24)	14 (23)	
Nonmalignant*	0 (0)	4 (8)	4 (7)	
Pre-transplant HBsAb†				.02
HBsAb ≥10 mIU/mL	7 (64)	45 (94)	52 (88)	
HBsAb <10 mIU/mL	4 (36)	3 (6)	7 (12)	
Conditioning regimen				.11
Reduced intensity‡	4 (33)	30 (61)	34 (56)	
Myeloablative§	8 (67)	19 (39)	27 (44)	
Donor relatedness				.53
Related donor	7 (58)	23 (47)	30 (49)	
Unrelated donor	5 (42)	26 (53)	31 (51)	
HLA match¶				1.00
Matched donor	10 (83)	41 (84)	51 (84)	
Mismatched donor	2 (17)	8 (16)	10 (16)	
Stem cell source				.73
Peripheral blood	12 (100)	42 (86)	54 (89)	
Bone marrow	0 (0)	5 (10)	5 (8)	
Umbilical cord blood	0 (0)	2 (4)	2 (3)	

MDS indicates myelodysplastic syndrome; HBsAb, hepatitis B surface antibody.
*Includes 3 patients with aplastic anemia and 1 with thalessemia major.
†Pretransplant HBsAb measurements were not available on two HSCT recipients.
‡All patients undergoing reduced-intensity allogeneic HSCT were conditioned with fludarabine (Flu) and low-dose busulfan (Bu) except: 1 patient conditioned with Flu, low-dose Bu, and antithymocyte globulin (ATG); two patients conditioned with Flu, melphalan (Mel), and ATG; and 1 patient conditioned with total body irradiation (TBI).
§All patients undergoing myeloablative allogeneic HSCT were conditioned with cyclophosphamide (Cy) and TBI except: 3 patients conditioned with Cy and ATG; 1 patient conditioned with cyclophosphamide (Cy), TBI, thiotepa, and ATG; and 1 patient conditioned with Cy and Bu.
¶Donors were considered HLA matched if 6/6 HLA-A, -B, and -DRB1 were identical.

in the cumulative incidence of HBV reactivation based on the duration of corticosteroid exposure or receipt of rituximab after transplantation. No patients who developed severe (grade II-IV) aGVHD went on to develop HBV reactivation. Significantly more patients with extensive cGVHD after HSCT developed HBV reactivation in comparison to those with limited or no cGVHD ($P = .003$).

A Cox proportional hazard model analysis was performed to assess risk factors for HBV reactivation in a time-dependent manner. Because pretransplant HBsAb measurements were not available for 2 patients (one of whom developed HBV reactivation and 1 of whom did not), these 2 patients were excluded from this analysis. Severe aGVHD and extensive cGVHD were modeled as time-varying covariates. A univariate HR for each covariate associated with HBV reactivation in the initial analysis (where $P < .2$) is shown in Table 3.

The covariates closely associated with HBV reactivation on univariate Cox modeling, including development of extensive cGVHD and pretransplant HBsAb <10 mIU/mL, were assessed on multivariate analysis. Both covariates were independently associated with development of HBV reactivation. Patients in this cohort who had HBsAb levels below 10 mIU/mL prior to transplant had an adjusted HR of 4.6 for developing HBV reactivation after transplantation when compared to those with pretransplant HBsAb levels ≥10 mIU/mL (95% CI 1.23-16.9; $P = .023$). In addition, those patients who developed extensive cGVHD after transplantation had an adjusted HR of 7.2 for developing HBV reactivation than those who did not (95% CI 1.25-41.5; $P = .027$).

Clinical Characteristics of HBV Reactivation

The clinical characteristics of the 12 patients who developed HBV reactivation are shown in Table 4. Of the 12 patients who developed HBV reactivation in the at-risk cohort, 11 developed chronic active HBV infection, including 1 who had fulminant hepatitis at the diagnosis of HBV reactivation (Patient 1). Only Patient 5 had transient HBV reactivation that cleared in <6 months after detection without antiviral treatment. In addition, only 1 patient developed a detectable HBV virus load that preceded the detection of HBsAg. Patient 5 developed a detectable HBV virus load 139 days prior to development of positive HBsAg.

Ten of the 12 patients who developed HBV reactivation had extensive cGVHD initially diagnosed 4 to

Table 2. Posttransplant Characteristics of HBcAb-Positive Allogeneic HSCT Cohort

Posttransplant Characteristics	Reactivation N = 12 (%)	No reactivation N = 49 (%)	Total N = 61 (%)	*P*
Acute GVHD				.10
Grades 0-I	12 (100)	37 (76)	49 (80)	
Grades II-IV	0 (0)	12 (24)	12 (20)	
Chronic GVHD				.003
None	2 (17)	25 (51)	27 (44)	
Limited	0 (0)	10 (20)	10 (16)	
Extensive	10 (83)	14 (29)	24 (39)	
Posttransplant corticosteroids, days				.54
Median duration	187	119	139	
Interquartile range	62-385	21-359	22-359	
Posttransplant rituximab*				.40
Yes	3 (25)	7 (14)	10 (16)	
No	9 (75)	42 (86)	51 (84)	

GVHD indicates graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; HBcAb, hepatitis B core antibody.
*No patient who received rituximab had developed hepatitis B virus (HBV) reactivation before or at the time of infusion. Rituximab was administered for the treatment of: extensive chronic GVHD, posttransplant lymphoproliferative disorder, immune-mediated hemolytic anemia, immune-mediated thrombocytopenia, or relapsed malignancy.

12 months after transplantation (Table 4). All patients who developed cGVHD were treated with corticosteroids initially. Adjunctive agents, including tacrolimus, sirolimus, mycophenolate mofetil (MMF), rituximab, or extracorporeal photopheresis, were added or the dose was increased as dictated by the individual response to steroids. The median time from diagnosis of cGVHD, when increased immunosuppression started, to HBV reactivation among these patients was 12.5 months (range: 2-37 months). The immunosuppressive regimens during the 100 days prior to the diagnosis of HBV reactivation are summarized in Table 4. No patient had evidence of CMV viremia or disease within 100 days of the diagnosis of HBV reactivation.

Although most patients in this group had HBsAb levels well above 10 mIU/mL prior to HSCT, the majority had HBsAb <10 mIU/mL by the time HBV reactivation occurred. At the time of diagnosis, a majority of patients had abnormal ALT levels ranging from just above the upper limit of normal to 10 times the upper limit of normal. Two patients had no elevation of ALT at the time of HBV reactivation, including Patient 5, who cleared HBV viremia within a few months during supportive care, and Patient 10, who was the only patient who underwent T cell-depleted transplantation in the cohort. With the exception of Patient 9, who had HBV infection with a precore mutant HBV and was HBeAg negative at the time of reactivation, the other patients in the group were all HBeAg positive at diagnosis. Ten of 12 patients underwent baseline genotyping of the reactivating HBV. Most patients in the cohort were genotype A or D.

Nine patients in the group underwent liver biopsy shortly after diagnosis of HBV reactivation. Four patients had evidence of liver fibrosis and 1 patient

Table 3. Proportional Hazards Modeling of Risk of HBV Reactivation after Allogeneic HSCT

Characteristic	Univariate HR (95% CI)	*P*	Multivariate HR (95% CI)*	*P*
Age	0.992 (0.946-1.040)	.73	—	—
Pretransplant HBsAb				
HBsAb ≥10 mIU/mL				
HBsAb < 10 mIU/mL	4.840 (1.357-17.260)	.015	4.558 (1.228-16.917)	.023
Conditioning regimen				
Reduced intensity				
Myeloablative	2.416 (0.704-8.284)	.16	—	—
Acute GVHD†				
Grade 0-I				
Grade II-IV	0 (0-∞)	1.00	—	—
Chronic GVHD				
None or limited				
Extensive	7.282 (1.341-39.549)‡	.022	7.210 (1.252-41.530)	.027

GVHD indicates graft-versus-host disease; HR, hazard ratio; CI, confidence interval; HBV, hepatitis B virus; —, not applicable; HSCT, hematopoietic stem cell transplantation; HBsAb, hepatitis B surface antibody.
Pretransplant HBsAb measurements were unavailable for two patients in the at-risk cohort, including 1 who developed HBV reactivation and 1 who did not. This analysis includes only the 59 patients for whom pretransplant HBsAb was available.
*Only characteristics with $P < .10$ on univariate analysis were included in multivariate analysis.
†There were no patients in the cohort who developed grade II-IV aGVHD and HBV reactivation.
‡Univariate analysis of chronic GVHD in the entire cohort (including the two patients without available pretransplant HBsAb) results in a similar and statistically significant HR: 7.977 (1.498-42.476), $P = .015$.

Table 4. Clinical Characteristics of HBcAb-Positive Allogeneic HSCT Recipients with HBV Reactivation

Patient Number	Age, Years	Sex	Reason for HSCT	Birthplace	Chronic GVHD Grade	Time to Chronic GVHD Diagnosis, Months*	Immuno suppression Preceding 100 days	HBsAb at Baseline, mIU/mL	Time to HBV Diagnosis, Months	Laboratories at HBV Diagnosis					HBV Genotype
										ALT, U/L†	HBsAb, mIU/mL	HBeAg	HBeAb	HBV VL, LogIU/mL	
1	37	F	AML	Brazil	Extensive	11	Tac, ritux	<10	27	532	<10	+	-	8.54	ND
2	49	M	ALL	France	Extensive	8	Pred, tac, sir	<10	26	102	<10	+	-	>8.70	A
3	33	M	CML	Mexico	Extensive	7	Pred, tac, sir	≥10	12	119	<10	+	-	7.29	A
4	43	M	NHL	Dominican Republic	Extensive	4	Pred taper	<10	15	83	<10	+	-	8.62	D
5	54	F	NHL	China	None	—	Tac taper	18	5	29	12	+	ND	3.81	ND
6	63	M	AML	USA	Extensive	7	Pred taper	≥10	21	52	<10	+	-	7.40	A
7	50	M	MDS	USA	Extensive	9	Tac taper	>150	20	160	10	+	-	7.38	D
8	58	M	CLL	USA	Extensive	7	Pred taper, tac	>150	11	57	14	+	-	7.43	D
9	42	M	MDS	China	Extensive	10	Pred, tac taper, mm, photopher	≥10	47	109	<10	-	+	>8.70	C‡
10	53	M	AML	USA	None	—	Ritux, TCD	<10	4	16	<10	+	-	7.14	A
11	40	M	MDS	USA	Extensive	10	Pred taper, tac	–	12	101	<10	+	-	>8.70	A
12	45	F	CML	USA	Extensive	7	Pred, sir	19	39	65	<10	+	-	8.69	D

GVHD indicates graft-versus-host disease; HBeAb, hepatitis B e antibody; VL, virus load; LogIU/mL, logarithm of international units per milliliter; F, female; AML, acute mylogenous leukemia; Tac, tacrolimus; ritux, rituximab; +, positive; -, negative; ND, not determined; M, male; ALL, acute lymphoblastic leukemia; pred, prednisone; sir, sirolimus; CML, chronic mylogenous leukemia; NHL, non-Hodgkin lymphoma; —, not applicable; MDS, myelodysplastic syndrome; CLL, chronic lymphocytic leukemia; mm, mycophenolate mofetil; photopher, extracorporeal photopheresis; TCD, T cell-depleted transplant.

*Patients 5 and 10 did not develop chronic GVHD during follow-up.

†Normal range for alanine aminotransferase (ALT): 7-52.

‡Precore mutant.

had hepatic necrosis (Table 5). In 6 cases (67%), the clinical diagnosis prior to biopsy differed from the diagnosis established by pathology. Patients 7 and 9 were empirically treated for cGVHD of the liver over the preceding months with increased immunosuppression until the diagnosis of HBV was established by serologic testing and liver biopsy. Four patients (Patients 1, 4, 6, and 11) had simultaneous evidence of GVHD and HBV infection in varying patterns on histology.

HBV Treatment

The majority of the patients with reactivation (11/12) were treated with antiviral medications directed at HBV during follow up (Table 5). Ten patients were treated with entecavir monotherapy. The first patient diagnosed with HBV reactivation in the cohort, Patient 1, was treated with adefovir and lamivudine, the 2 medications that were FDA approved for HBV treatment at the time of diagnosis in 2004.

One HSCT recipient with HBV reactivation, patient 5, did not develop chronic HBV infection and was not treated with any antiviral medications directed at HBV. Patient 5 had a normal ALT and, unlike the other cohort members with HBV reactivation, also had a relatively low HBV virus load (<10,000 international units per milliliter [IU/mL] or 4 logarithms of international units per milliliter [logIU/mL]) at the time of reactivation. This patient was monitored clinically and subsequently reconverted to HBsAg negative and HBV virus load negative within 4 months.

Response to HBV Treatment

Most patients in the cohort who were treated for HBV had a biochemical response to treatment with normalization of ALT shortly after initiation of treatment. Among the 11 patients who were treated with antiviral therapy, 9 started therapy at least 6 months before the end of follow-up. Three of 9 (33%) achieved undetectable HBV virus loads by 6 months after the start of therapy. Four of the 6 patients who had detectable HBV viremia at 6 months of therapy had low virus loads, under 2,000 IU/mL (3.30 logIU/mL) consistent with a partial virologic response [24].

Six patients with HBV reactivation in the cohort were started on antiviral therapy at least 12 months before the end of follow-up. Three of these 6 (50%) achieved undetectable HBV virus loads by 12 months after the start of antiviral therapy. Interestingly, 2 of the 3 patients with detectable HBV virus loads 12 months after the start of therapy (Patients 3 and 8) developed a complete response to therapy with clearance of HBV virus load, loss of HBeAg, and loss of HBsAg with continued therapy after 21 and 16 months. Conversely, Patient 6 who had an undetectable HBV virus load 12 months after starting therapy redeveloped a low detectable HBV virus load (<3.30 logIU/mL) despite unchanged antiviral therapy 14 months after therapy was initiated.

By the end of follow-up, 7 of 11 patients (64%) treated for HBV reactivation in this cohort achieved a virologic response to therapy with undetectable HBV virus loads and negative HBeAg in those treated for more than 6 months, and declining virus loads in those treated for <6 months. Most notably, 4 of the patients with virologic response (36% of those treated for HBV reactivation) also had a "complete" response to therapy with loss of HBsAg during follow-up [22]. Patient 1 lost HBsAg after 49 months of therapy with adefovir and lamivudine. Patients 3, 7, and 8 lost HBsAg after 21, 8, and 16 months of therapy with entecavir, respectively. Three of these 4 developed robust titers of HBsAb, ranging from 98 to 244 mIU/mL in association with clearance of HBsAg and circulating HBV. All 3 who redeveloped HBsAb currently remain HBsAg negative off of antiviral therapy.

No patient treated with antiviral medications directed at HBV developed serious side effects related to HBV treatment including lactic acidosis or hepatic flare during follow-up. Four patients had an incomplete response to entecavir therapy. Patients 2 and 6 had low (<3.3 logIU/mL), but detectable HBV virus loads at the end of follow-up, 8 and 23 months after starting therapy, respectively. Both were continued on entecavir monotherapy. Patient 4 had a persistent HBV virus load between 3.3 and 4.0 logIU/mL after 2 years of entecavir monotherapy despite an initial decline in HBV virus load. There was no indication of poor compliance with therapy and genotypic analysis revealed no drug-associated resistance mutations. The patient was continued on entecavir monotherapy. After 7 months of entecavir monotherapy, Patient 10 developed a significant increase in HBV virus load (>1 logIU/mL) after an initial response. Clinically, there was no indication of noncompliance with entecavir. HBV genotype revealed no medication-associated resistance mutations. The patient was continued on entecavir, and tenofovir was added to the antiviral regimen.

Survival

The 1-year survival in the HBcAb-positive cohort was 70.3%. Overall, 35 (57%) patients survived to the end of follow-up, 20 (33%) died, and 6 (10%) were censored (because of transfer of care from this center to a local facility). There were no HBV-associated deaths in the cohort.

DISCUSSION

These data demonstrate that reactivation of HBV is a frequent, but usually late complication of transplantation among patients with serologic evidence of resolved HBV infection prior to allogeneic HSCT.

Table 5. Histologic and Treatment-Associated Characteristics of HBcAb-Positive Allogeneic HSCT Recipients with HBV Reactivation

Patient Number	Diagnosis Preliver Biopsy*	Diagnosis Postliver Biopsy†	Fibrosis Stage	Treatment Regimen	HBV VL 6 Months after Treatment Started, LogIU/mL	HBV VL 12 Months after Treatment Started, LogIU/mL	Laboratory Values at Last Follow-up					Decrease in HBV VL at Last Follow- up, LogIU/mL	Duration Treatment, Months	Hepatitis Status at Last Follow-up
							ALT, U/L‡	HBsAb, mIU/mL	HBeAb	HBeAg	HBV VL, LogIU/mL			
1	HBV	HBV + GVHD	0§	Lamivudine + adefovir	< Assay	< Assay	14	244	—	—	< Assay	8.54	49	Complete response
2	HBV	HBV	0	Entecavir	3.14	—	25	<10	ND	+	3.14	5.56	8, on going¶	Incomplete response
3	—	—	—	Entecavir	2.94	2.22	51	98	ND	-	< Assay	7.29	21	Complete response
4	GVHD	GVHD + HBV	2	Entecavir	4.33	3.56	29	<10	—	+	3.72^	4.90	28, on going	Incomplete response
5	—	—	—	Supportive care	—	—	15	<10	ND	ND	< Assay	3.81	—	Resolution
6	GVHD	HBV + GVHD	2	Entecavir	3.12	< Assay	41	<10	—	+	3.04	4.36	23, on going	Incomplete response
7	GVHD	HBV	3	Entecavir	< Assay	< Assay	33	101	+	—	< Assay	7.38	8	Complete response
8	GVHD	GVHD	0	Entecavir	2.09	3.80	44	<10	+	—	<Assay	7.43	16, on going	Complete response
9	GVHD	HBV	3	Entecavir	< Assay	—	41	<10	+	—	< Assay	8.70	11, on going	Virologic response
10	—	—	—	Entecavir	6.04	—	48	<10	—	+	5.59^	1.55	8, on going	Incomplete response
11	GVHD	GVHD + HBV	0	Entecavir	—	—	100	<10	—	+	4.19	4.51	5, on going	Virologic response#
12	HBV	HBV	0	Entecavir	—	—	76	<10	—	+	6.23	2.46	3, on going	Virologic response

GVHD indicates graft-versus-host disease; HBeAb, hepatitis B e antibody; VL, virus load; LogIU/mL, logarithm of international units per milliliter; F, female; AML, acute mylogenous leukemia; Tac, tacrolimus; ritux, rituximab; +, positive; -, negative; ND, not determined; M, male; ALL, acute lymphoblastic leukemia; pred, prednisone; sir, sirolimus; CML, chronic mylogenous leukemia; NHL, non-Hodgkin lymphoma; —, not applicable; MDS, myelodysplastic syndrome; CLL, chronic lymphocytic leukemia; mm, mycophenolate mofetil; photopher, extracorporeal photopheresis; TCD, T cell-depleted transplant.

*Patients 3, 5, and 10 did not undergo liver biopsy.

†Where there are two diagnoses listed, the first is the predominant pattern appreciated on liver biopsy.

‡Normal range for alanine aminotransferase (ALT): 7-52.

§Wide-spread hepatic necrosis.

¶Patient transferred care to another institution in conjunction with a move 8 months into HBV therapy.

^HBV genotype performed for increased virus load after initial response; no medication-associated resistance mutations found.

#Virologic response determined by reduction in HBV virus load by over 1 logIU/mL after 3 months of therapy in these patients with <6 months of follow-up after initiation of antiviral therapy [24].

The cumulative risk of HBV reactivation rose by 9% to 13% per year of survival after transplantation in this cohort, to give a cumulative probability of 43% at 4 years after HSCT. Overall survival did not seem to be affected by pretransplant-resolved HBV infection when compared to other cohorts at our center [30].

We identify 2 significant independent risk factors for the development of HBV reactivation after HSCT: low pretransplant HBsAb levels and development of cGVHD after transplantation. HBsAb is a surrogate for HBV immunity; HBsAb of 10 mIU/mL is typically considered the threshold for protection from HBV infection [28]. Although most patients in the cohort had protective levels of HBsAb prior to HSCT, the risk for HBV reactivation among the few cohort members with pretransplant HBsAb below 10 mIU/mL prior to transplant was nearly 5 times greater than the risk for those with HBsAb at or above the threshold of 10 mIU/mL before HSCT.

The immunologic reason why recipient-derived humoral immunity would have a long-term protective effect against HBV reactivation after allogeneic HSCT is not completely clear. It is possible that development of high levels of HBsAb after the initial HBV infection in some patients reflects true clearance of the HBV virus, and thus some of HSCT recipients with high levels of HBsAb prior to transplant may not be truly at risk for HBV reactivation. Alternatively, high levels of HBsAb at the time of HSCT may prevent HBV reactivation in the initial posttransplant period, when the recipient is most immunocompromised, allowing the recipient to remain free of HBV reactivation until new donor-derived immunity to HBV can develop.

Development of cGVHD was also a strong predictor of HBV reactivation in this cohort, which confirms the findings of Seth et al. [11], who first reported a significant association between cGVHD and HBV reactivation. This study further delineates that the grade of cGVHD is predictive, as suggested in the case series by Knoll et al. [12]. In our cohort patients who developed extensive cGVHD were 7 times more likely to develop HBV reactivation than those with limited or no cGVHD.

Of the 12 patients who developed HBV reactivation during follow up, 10 had extensive cGVHD at the time of HBV diagnosis, many of whom were temporally clinically diagnosed with liver involvement of cGVHD before they were diagnosed with HBV reactivation. To clarify the contribution of cGVHD versus HBV to the liver disease present in these patients, 9 of 12 patients with HBV reactivation underwent liver biopsy shortly after HBV reactivation was diagnosed. Notably, the prebiopsy clinical diagnosis did not fully agree with the definitive diagnosis established by biopsy in 6 of 9 cases. Furthermore, 4 had evidence of both active HBV and active GVHD on biopsy. These findings suggest that there may be a complex relationship between active HBV infection and cGVHD of the liver after transplantation. Although the immune dysregulation associated with cGVHD and subsequent immunosuppressive therapy aimed at treating GVHD may lead to HBV reactivation, it is also possible that chronic active HBV replication stimulates an immunologic response that leads to localized GVHD. Further studies are needed to delineate this relationship. In addition, these findings highlight the important role that liver biopsy may play in the assessment and subsequent management of allogeneic HSCT patients with HBV reactivation. Similar to the present study, Ma et al. [31] also found that liver biopsy was useful for targeting therapy among patients with cGVHD in a cohort of HSCT patients with a high prevalence of HBV infection.

Although not all patients in this cohort met criteria for treatment of HBV based on the current treatment guidelines proposed for nonimmunocompromised hosts [22-24], given the potential synergistic effects of HBV and GVHD on the liver and the immunocompromised status of the patients in our cohort, treatment was pursued in most patients. Ten of 12 patients who developed HBV reactivation were treated with entecavir monotherapy, which resulted in virologic response in 6 of 10 patients (60%). Most notably, 3 of the patients treated with entecavir monotherapy (30%) developed a complete response to therapy, with loss of HBsAg and durable clearance of HBV virus load. An additional patient treated with adefovir and lamivudine also achieved a complete response. The overall rate of complete response in the patients who were treated in this cohort, including patients treated for <1 year, was 36% (4/11), which is high in comparison to the 5% rate reported with entecavir after 2 years of therapy and adefovir after 4 to 5 years of therapy [25]. This high overall rate of complete response to HBV therapy may be a reflection of the unique immunologic situation in allogeneic HSCT recipients in which the immune function of the recipient (who developed the initial HBV infection) is replaced by that of the donor. Donor immunity prior to HSCT may have an impact on the ability of HSCT recipients with HBV reactivation to regain immunologic control of the infection, but needs to be studied further.

Donor immunity may also have an impact on the overall risk of HBV reactivation. Because pretransplant HBsAb was not routinely checked in stem cell donors during the study period (and is not part of routine blood donor screening in the United States [29]) one of the limitations of this study is that we were unable to assess the impact of pretransplant donor HBsAb serologic status on the subsequent risk of HBV reactivation. Several studies have suggested

that donor immunity to HBV, acquired by donor vaccination or natural infection, may protect against HBV reactivation after HSCT [7,10]. This has led to the suggestion that vaccination of the donor prior to transplant may be a strategy to prevent later HBV reactivation in the recipient [7]. However, at least one group has reported HBV reactivation with an escape mutant in an HSCT recipient transplanted with stem cells from a vaccinated donor [16]. Further studies are needed to determine the role that donor immunity plays in recipient development of HBV reactivation and to determine if HBV vaccination of the donor prior to HSCT would be helpful to prevent this complication.

We were also limited in this study because serial screening measurements of HBsAb, HBsAg, and HBV virus load were only performed in a subset of patients during posttransplant follow-up. Others have shown that HBV viremia may precede frank reactivation with development of positive HBsAg [12], but in the absence of serial HBV virus load measurements after HSCT in the entire cohort, we were unable to systematically assess for this. Furthermore, as demonstrated in a few patients in this cohort, HBV reactivation can occur in the absence of ALT abnormalities and can resolve without intervention. Thus, it is possible we underestimated the frequency of HBV reactivation in this cohort, as some, but not all, patients were routinely screened for HBV in the absence of ALT elevation or clinical symptoms.

In addition, without serial HBsAb measurements after transplantation, no assessment of the trend in HBsAb levels and its impact on the risk of HBV reactivation could be performed. Several groups have reported that levels of HBsAb decline after HSCT and have suggested this that may lead to increased susceptibility to HBV reactivation [14,17]. In response to this finding, one group assessed HBV vaccination in 12 patients previously exposed to HBV after HSCT and reported that posttransplant vaccination may prevent HBV reactivation [32]. However, from the criteria used for vaccination, patients with extensive cGVHD, who are most susceptible to HBV reactivation, would have been excluded from vaccination in that study and postvaccination follow-up was short. Further studies that include patients with extensive cGVHD are needed to clarify the impact of loss of HBsAb after transplantation and recipient HBV vaccination after transplantation on the risk of HBV reactivation.

Last, this analysis was also limited by the size of the cohort. Although this is the largest study to date assessing HBV reactivation in HSCT recipients with resolved HBV infection prior to transplantation, like other studies, there was a relatively small number of outcomes in the cohort. In addition, in this small cohort, there was only 1 patient who underwent a T cell-depleted transplant, so we were unable to assess the impact of this type of GVHD prophylaxis on the risk of HBV reactivation.

In summary, HBV reactivation is a common late complication of allogeneic HSCT. Low levels of HBsAb prior to transplantation and development of extensive cGVHD are strong independent predictors of increased risk for HBV reactivation. Based on these findings, periodic post-HSCT screening for HBV reactivation with HBsAg, HBsAb, and HBV virus load should be considered in patients with evidence of resolved HBV prior to transplant, particularly those who have low HBsAb levels prior to transplantation and those who develop extensive cGVHD after transplantation. In patients who do develop HBV reactivation after transplant and have suspected or proven cGVHD, liver biopsy can be useful to delineate the degree of liver disease caused by each process. Further studies in this population are needed to determine when therapy should be initiated, particularly in the setting of liver GVHD, and which regimen is optimal. In this cohort, entecavir monotherapy was effective in most patients to achieve a biochemical and virologic response and 30% of patients treated with entecavir achieved complete response to therapy with loss of HBsAg.

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