ORIGINAL ARTICLE

Significant loss of hepatitis A Ab after allogeneic hematopoietic SCT in pediatric patients

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Loss of specific immunity after hematopoietic SCT (HSCT) is well documented for polio, measles, mumps and tetanus. There are limited studies reporting the loss of Hepatitis A virus immunity and no reports evaluating the effect of donor immunity on Hepatitis A virus (HAV) immunity loss after HSCT. A total of 49 of the 81 patients who received HSCT at the Ankara University Pediatric HSCT Unit from January 1997 to December 2006 had HAV serology tested before HSCT and were evaluated for seroprevalence, and 30 of 49 patients were evaluated for the loss of Ab and for the effect of donor immunity on the loss of HAV Abs. The seroprevalence before HSCT was 75.5%. Loss of Ab was detected in 43.5% (10/23) of the patients. The median time to loss of Ab was 12 months (12–32 months), and 60% of these patients were seronegative at 12 months after HSCT. After HSCT, 46.7% of the patients were seronegative. Loss of Ab was higher in the seronegative donor group (75 vs 26%). The loss of HAV Ab is high after allogeneic HSCT for pediatric patients. Reimmunization should be considered for the continuation of individual and community immunity. Further studies with larger study groups are warranted to clarify the role of donor immunity on the loss of HAV immunity.

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Introduction

Hematopoietic SCT (HSCT) has been a treatment modality for many diseases over the last decade. Both the innate and specific immunity are impaired after HSCT. Loss of specific immunity against vaccinated pathogens has been well documented for measles, mumps, rubella,^{1,2} polio^{3,4} and

tetanus.^{5,6} However, there are very few studies reporting the loss of Hepatitis A virus (HAV) antibodies after HSCT.^{7,8} In addition, there are no reports showing the influence of donor immunity on the loss of HAV Ab after HSCT.

Hepatitis A is an RNA virus that is highly resistant and very contagious. It generally causes a self-limited mild-to-moderate hepatitis, but complications such as recurrent hepatitis and fulminant hepatitis occur in 3–20% and 0.4–2.5% of cases, respectively. Although it is rare, the mortality rate for fulminant hepatitis might be as high as 60%.⁹

In this study, we aimed at evaluating the seroprevalence of HAV before HSCT, the incidence of the loss of specific immunity against HAV, the influence of donor immunity on the loss of HAV antibodies and other possible risk factors, with a retrospective study on pediatric allogeneic HSCT recipients for both malignant and nonmalignant hematological disorders, at the Ankara University Medical Faculty, Pediatric Hematology HSCT Unit.

Materials and methods

Patients

The charts of the 81 consecutive patients who received allogeneic HSCT for malignant and nonmalignant hematological disease at the Ankara University Pediatric HSCT Unit from January 1997 to December 2006 were reviewed. Of these 81 patients, 49 had Hepatitis A serology recorded before HSCT and were included in the study group. All these 49 patients who had HAV serology tested before HSCT were evaluated for seroprevalence. A total of 30 patients within this group (30/49) who had pre- and post-HSCT HAV serology documented, and who also had donor serology tested, were evaluated for the loss of Ab after HSCT and for the transfer of donor immunity to the recipient. Hepatitis A was not on the routine immunization schedule at the time of this study and there was no history of Hepatitis A vaccination reported in the charts. All patients with positive HAV IgG were considered as being naturally immune.

Laboratory method

A qualitative commercially available chemiluminescent microparticle immunoassay (Architect system HAVAb

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IgG, Abbott Laboratories, Germany) was used to detect anti-HAV IgG. Results were reported as positive or negative.

Statistical method

Nonparametric variables were compared using the Mann-Whitney test. A comparison of the percentages between the two groups was carried out using the χ^2 -test or Fisher's exact test, where appropriate. The McNemar test was used to determine the significance of donor seropositivity on the outcome of HAV serology after HSCT. Differences with a *P*-value <0.05 were considered significant. SPSS 15.0 (Statistical Package for Social Sciences) statistical software version was used for analyses.

Results

Seroprevalence

In total, 49 patients were evaluated for HAV seroprevalence before HSCT. Table 1 summarizes the patient characteristics. The median age was 9 years (1–18 years) and the M/F ratio was 25:24. The diagnoses were thalassemia major in 51%, Fanconi aplastic anemia in 14.3% and acute myeloid leukemia in 10.5%. Hepatitis A seropositivity was 75.5% before HSCT. Seroprevalence increased by age and was 46, 71.4 and 95% at ages 1–4.9 years, 5.0–9.9 years and 10–15.9 years, respectively. The two patients above the age of 16 years were seropositive.

Loss of Ab

Table 2 presents the patient characteristics of the 30 patients evaluated for the loss of Ab. The median age was 7 years (2–17 years) and the M/F ratio was 12:18. Thalassemia major was the diagnosis in 46% of the patients. Stem cell source was BM in 16 (53.3%), peripheral blood in 13 (43.3%) and both BM and cord blood in 1 (3.3%) patient. Myeloablative conditioning regimen was used in all patients, and the GVHD prophylaxis consisted of CYA

and MTX in 46.6% of the patients. All patients received i.v. Ig at a dose of 400 mg/kg, weekly for the first 4 weeks and monthly thereafter, until 6 months after HSCT. Acute GVHD and chronic GVHD occurred in six (20%) and three (10%) patients, respectively. Of these, five had acute, two had chronic and one had both acute and chronic GVHD. The median time to discontinue immunosuppression was 6 months (5–32 months). The median follow-up was 56 months (21–123 months). A total of 86 samples were tested for HAV antibodies and the median number of sample collection per patient after HSCT was three (1–5 samples/patient). In total, 23 patients (76.6%) and 20 donors (66.6%) were seropositive before HSCT. Of the 23 patients who were seropositive before HSCT, 10 (43.5%) had lost HAV Ab after HSCT. The median time to loss of Ab was 12 months (12–32 months). Ab loss was detected by 12 months after HSCT in 60% (6 of 10) of the patients. Three of the seven patients who were seronegative before HSCT had become seropositive after HSCT, and two of these three patients had seropositive donors. There was no documented clinical hepatitis in these patients. After HSCT, 14 patients (46.7%) were seronegative.

Risk factors for the loss of hepatitis A Ab

None of the factors evaluated for the loss of Ab after HSCT, including age, sex, diagnosis, donor HAV serology,

Table 1 Characteristics of the patients evaluated for seroprevalence

	n	%
Patients	49	
Age-median (range)	9 (1–18)	
Sex		
Male	25	51
Female	24	49
Diagnosis		
Thalassemia major	25	51
Fanconi aplastic anemia	7	14.3
Acute myeloid leukemia	5	10.5
Severe aplastic anemia	3	6.1
Myelodysplastic syndrome	3	6.1
Chronic myeloid leukemia	2	4
Hemophagocytic	2	4
Lymphohistiocytosis		
Sickle cell disease	1	2
Juvenile myelomonocytic leukemia	1	2

Table 2 Characteristics of the patients evaluated for the loss of Ab

	n	%
Patients	30	
Age-median (range)	7 (2–17)	
Sex		
Male	12	40
Female	18	60
Diagnosis		
Thalassemia major	14	46.6
Fanconi aplastic anemia	5	16.6
Acute myeloid leukemia	4	13.3
Myelodysplastic syndrome	3	10
Severe aplastic anemia	2	6.6
Hemophagocytic	1	3.3
Lymphohistiocytosis		
Sickle cell anemia	1	3.3
Stem cell source		
BM	16	53.3
Peripheral blood	13	43.3
Cord blood + BM	1	3.3
HLA match		
HLA full-match family Donor	30	100
Conditioning regimen		
Myeloablative	30	100
GVHD prophylaxis		
Cyclosporin + MTX	21	70
Cyclosporin	9	30
GVHD		
Acute	5	20
Chronic	3	10

GVHD and follow-up time, were found to be statistically significant. For the patients who were HAV seropositive before HSCT, more patients lost Ab in the seronegative donor group compared with those in the seropositive donor group (4 of 15 (26%) vs 6 of 8 (75%)), but this was not found to be statistically significant.

Discussion

Hepatitis A remains a significant problem in developing countries. There are no reports indicating that the clinical course of Hepatitis A is more severe in patients after HSCT but, although rare, the complications might cause significant morbidity and mortality even in an immunocompetent host. There is very little data in the literature regarding the loss of HAV Ab after HSCT.^{7,8}

This is the first study reporting seroprevalence and the incidence of loss of Ab for HAV in pediatric HSCT recipients. Moreover, it is the first study showing the relevance of donor immunity in the loss of HAV Ab after HSCT. The loss of HAV Ab was high at 43%. The results were remarkable, showing that 46.7% of the patients were seronegative after HSCT, making these patients vulnerable for the disease in an environment in which contact with the virus is almost inevitable.

The seroprevalence of HAV before HSCT was high at 75.5%. This was slightly higher compared with that in the same age groups reported in Turkey. Sidal *et al.*¹⁰ reported the seroprevalence as 15.1, 26.7 and 49.6% between ages 1.0–4.9 years, 5.0–9.9 years and 10.0–15.9 years, respectively, in Turkey. Seroprevalence in these patients was closer to the adult HAV seroprevalence in Turkey, reported as 74.3% at 25–29 years of age.¹¹

In our study, loss of HAV Ab after HSCT was higher than that reported in the literature. The reported Ab loss for Hepatitis A was 14 and 11.2% by Dignani *et al.* and Godoi *et al.*, respectively.^{7,8} Table 3 summarizes these two studies. In both groups, the majority of the patients were adults, and in the first study, 86% of the patients had an autologous HSCT. The median follow-up time was 12 months, with the minimum follow-up of 1 month in the first study, and 644 days with a minimum of 82 days in the second. Both of these studies reported a considerably lower percentage of loss of antibodies than did our study. The possible reasons for the higher percentage found in our study are the following: (1) In the first group, the majority of patients received autologous HSCT, and it has been reported that the loss of specific Ab is higher after allogeneic HSCT than after autologous, and all the patients in our study group received allogeneic HSCT.¹² (2) In both studies, the follow-up time was shorter than that in this report. Godoi *et al.* reported that the loss of HAV Ab was significantly associated with a longer follow-up, and the probability of loss of antibodies had reached 23.4% ($\pm 9.6\%$) at 4 years after HSCT. The minimum follow-up time in this report was 21 months, which was longer compared with the 1 month and 82 days follow-up for the earlier two reports. The median time for the loss of Ab was 12 months in our report and all of our patients were followed up beyond this time point, which might increase

Table 3 Comparison of the studies reporting loss of HAV antibodies after HSCT

Study	Country	# of patients evaluated for loss of HAV IgG Ab	Median age (years)	Type of HSCT	Median f/u	Test used to detect HAV IgG Ab	% of Ab loss	Median time to loss of Ab
Dignani <i>et al.</i> ⁸	Argentina	36	47 y (14–66 y)	Autologous PSCT: 86% Allogeneic PSCT: 14%	12 mo (1–51 mo)	Qualitative enzyme immunoassay (cut-off value 100 mIU/ml)	14	358 days
Godoi <i>et al.</i> ⁷	Brazil	71	26 y (3–58)	Autologous HSCT: 91.6% Allogeneic HSCT: 8.4%	644 days (82–1530 d)	Qualitative enzyme immunoassay (cut-off value 20 mIU/ml)	11.2	NA
This report	Turkey	30	7 y (2–17)	Allogeneic HSCT: 100%	56 mo (21–123 mo)	Qualitative chemiluminescent microparticle immunoassay	43.5	12 mo

Abbreviations: HAV = hepatitis A virus; HSCT = hematopoietic SCT; PSCT = PBSC transplantation.

the possibility of detecting loss of Ab. (3) The patient population in this study was younger compared with that in the earlier reports, with a median age of 7 years (2–17 years), and younger age was one of the risk factors associated with the loss of HAV antibodies as reported by Godoi *et al.* This pediatric population as the study individuals in this report might be another reason for a higher percentage of loss of HAV Ab after HSCT.

The lower limit of Ab needed to confer immunity has not been defined for HAV. A commercially available qualitative chemiluminescent microparticle immunoassay was used to detect HAV IgG. The sensitivity and the specificity of the test were reported as 99.17% and $\geq 98\%$, respectively. Although not having quantitative measures of IgG level makes it hard to make a comparison between studies, the high sensitivity and specificity is convincing for the accurate detection of HAV IgG.

Although the loss of HAV Ab was significantly associated with longer follow-up, younger age and acute GVHD in the literature,⁷ none of the risk factors evaluated, including age, sex, diagnosis, donor HAV serology, GVHD and follow-up time, were found to be statistically significant in our study. The study group in this report was small, and larger patient groups might be necessary to evaluate these risk factors.

The influence of donor HAV serology on the loss of Ab has not been reported in the literature before. The transfer of immunity from the seropositive donor to the recipient had been shown for different diseases. Parkkali *et al.*¹³ had reported that patients transplanted from donors with high tetanus, diphtheria and *Haemophilus influenzae* type B Ab concentrations had higher respective Ab concentrations after HSCT than those transplanted from donors with low Ab concentrations. The transfer of donor immunity after allogeneic HSCT has been reported for Hepatitis B by earlier reports as well.¹⁴ In this study, strikingly, 75% of the patients who were seropositive before HSCT lost HAV Ab after HSCT if the donor was seronegative. In contrast, only the 26% of patients with a seropositive donor lost HAV Ab after HSCT. This was not found to be statistically significant, but further studies with larger patient groups are warranted. Furthermore, three patients who were seronegative before HSCT had become seropositive after HSCT. There was no history or documented hepatitis. The minimum time elapsed from the last dose of i.v. Ig was 1.5 years for these three patients, which made i.v. Ig an unlikely possible cause for seropositivity. Although a silent infection could be possible, two of these patients had an HAV-seropositive donor, and the possibility of the transfer of HAV immunity deserves further research.

Hepatitis A remains a problem for a large population of patients around the world. Although there are no reports of severe Hepatitis A after HSCT, these patients are vulnerable to the disease and its complications as is the general population. Morbidity and mortality are high for a vaccine-preventable illness. Currently, Hepatitis A is on the routine immunization schedule for healthy children in the United States, but revaccination for Hepatitis A after allogeneic HSCT is only recommended for patients with an underlying liver disease, or for patients who are traveling to

endemic areas or who are living in endemic areas. For developing countries in which the Hepatitis A infection is endemic, it is recommended to vaccinate patients for Hepatitis A after HSCT. In addition, for the continuation of community immunity, immunization/reimmunization with Hepatitis A after HSCT may be considered in countries in which Hepatitis A is on the routine pediatric immunization schedule to restore the immunity of a post-HSCT patient to that of the general population. The Turkish Society of Hematology recommends vaccination with an inactivated Hepatitis A vaccine at two doses starting at 6–12 months after HSCT. The response to the inactivated HAV vaccine, the optimal timing for the vaccination and the role of donor immunity/vaccination are still unknown, and more data from larger study groups are necessary to make further recommendations for the clinical applications of the HAV vaccine.

Conflict of interest

The authors declare no conflict of interest.

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