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Naturally acquired hepatitis A antibodies after haematopoetic stem cell transplantation

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SUMMARY

Haematopoietic stem cell transplant (HSCT) recipients lose immune memory of exposure to infectious agents and vaccines accumulated throughout their lifetime and therefore need to be revaccinated. We aimed to evaluate the influence of different factors on hepatitis A virus (HAV) immunity in both child and adult HSCT recipients living in an intermediate endemic region, Turkey. Eighty patients (age range 2·5–57 years) who had HAV serology prior to HSCT were evaluated. The prevalence of HAV seropositivity was 85% ($n=68$) before HSCT. There was no history of HAV vaccination before HSCT in children and HAV vaccine was not available in Turkey 10 years ago, so it was assumed that all seropositive patients reflected natural immunity. After the exclusion of six patients with autologous HSCT, the remaining 62 seropositive and allogeneic patients were included in this retrospective study. The duration of HAV seropositivity was estimated using the Kaplan–Meier method, log-rank analysis and Cox regression models. Estimated mean time to loss of HAV seropositivity was 48·6 months after transplantation. Patients who were older ($\geqslant$18 years) at transplantation and who had older ($\geqslant$18 years) donors became seronegative later ($P<0{\cdot}05$). Cox backward-stepwise regression confirmed that older age of recipient at transplantation was the only significant parameter for HAV seropositivity ($P<0{\cdot}05$). HAV-inactivated vaccine might be recommended later to older HSCT recipients in intermediate endemic regions.

Key words: Hepatitis A, follow-up serology, HSCT, seroprevalence.

INTRODUCTION

Hepatitis A characteristically is an acute, self-limiting illness associated with fever, malaise, jaundice, anorexia, and nausea, but some patients can develop fulminant hepatitis with fatality rates around 60–80% or extrahepatic complications [1]. Fulminant hepatitis is rare but is more common in people with underlying liver disease [1–3]. There are differences in hepatitis A virus (HAV) prevalence and outcome, particularly between developed and developing countries. In Turkey anti-HAV prevalence in different age groups and regions ranges from 7·8% to 98·0% [4–8]. The reported rates of HAV seropositivity were

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19·9–87·4% in children [4–8] and 71·3% in people aged <30 years [5]. There is a shift in the age of hepatitis A seropositivity from childhood to adulthood in intermediate endemic regions of world like Turkey, and the severity of the disease increases progressively with age [4, 9]. While HAV infection during childhood is often asymptomatic, occurrence during adulthood is symptomatic and associated with a mortality rate of up to 2% in industrialized countries in patients aged >40 years [1]. However, hepatitis A was the most commonly detected cause in cases with fulminant hepatic failure in Turkish children [2, 3]. HAV infection also can cause haematological complications [10]. Wünschmann *et al*. reported a maturational defect of human peripheral blood monocytes upon challenge with HAV *in vitro*, which might contribute to functional abnormalities of the bone marrow stroma and which may lead to mild perturbations of immunoregulation and haematopoiesis observed during acute HAV infections *in vivo* [11]. In order to protect haematopoietic stem cell transplant (HSCT) recipients against HAV infection it is important to determine the loss of HAV antibodies, especially in endemic countries. The pre-conditioning regimen, viral infections, graft *vs*. host disease (GVHD), and veno-occlusive disease are responsible for the unwanted effects of HSCT on liver. Hepatic complications including viral infections can be observed as causes of morbidity and mortality in HSCT recipients [12]. Because of the above-mentioned reasons, even though complications or fulminant course of HAV infection in HSCT recipients has not been reported, patients should be protected against HAV infection.

Hepatitis A vaccine is inactivated. According to the Infectious Diseases Working Party of the European Group for HSCT, routine administration of hepatitis A vaccine is not recommended but can be considered at ⩾12 months after HSCT for people who have chronic liver disease or people from areas with endemic infection or outbreaks of hepatitis A [13]. In this study, we aimed to evaluate the duration of HAV seropositivity in HSCT recipients and affecting factors in an intermediate endemic region, Turkey.

MATERIALS AND METHODS

Patients

HSCT patients in Hacettepe University Faculty of Medicine, Adult and Paediatric HSCT units from 2000 to 2007 were evaluated. Patients who were aged <1 year, and cases with severe combined immunodeficiency (SCID) receiving intravenous immunoglobulin (IVIG) regularly prior to HSCT were excluded from the study. In total, 80 patients whose pre- and post-transplant hepatitis A serology was known and who were not vaccinated with HAV vaccine and who had a disease-free survival of at least 1 year after HSCT were included. The prevalence of HAV seropositivity was 85% ($n=68$) before HSCT. Seropositivity was 76·9% in transplant cases aged <18 years and 100% in transplant cases aged ⩾18 years. Of these 68 seropositive patients, six had autologous HSCT and these patients were seropositive during the study period; median follow-up of the six autologous patients was 34·5 months (range 28–56 months). Therefore, the remaining 62 patients with allogeneic-related HSCT were included in this study. Stem cell source was bone marrow in 61 patients and cord blood in one patient. From these patients, a total of 189 samples had been tested for HAV seropositivity and median number of samples was three (range 1–6) per patient after HSCT.

Patients' files were evaluated retrospectively in terms of age at transplantation, gender, underlying diseases, type of HSCT, donor age, conditioning regimen, history or presence of GVHD, and duration of IVIG, immunosuppressive medications and HAV seropositivity.

Laboratory method

HAV IgG antibodies were analysed using a qualitative commercially available chemiluminescent microparticle immunoassay (CMIA) (Architect® HAVAb-IgG, Abbott Diagnostic Division, Germany) and expressed as positive or negative at the hospital of Hacettepe University Laboratory of Biochemistry.

Statistical analysis

All analyses were performed with SPSS for Windows (SPSS Inc., USA). The cumulative duration of HAV seropositivity was calculated by the Kaplan–Meier method and the differences on the duration of HAV seropositivity were analysed by a log-rank test. Estimated means and standard errors were calculated. Cox regression models [backward-stepwise (likelihood ratio)] were performed to assess the impact of factors including age at the time of transplantation (<18 *vs*. ⩾18 years), gender, donor age (<18 *vs*. ⩾18 years), conditioning regimen (myeloablative, reduced),

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Table 1. *Patient characteristics* ($n=62$)

Variable	*n* (%)
Male	42 (67·7)
Age, years, median (range)	17·0 (2·5–56·0)
Age at the time of transplantation, years, median (range)	12·5 (1·3–53·2)
Donor age, years, median (range)	19·0 (0·0–52·0)
Underlying diseases	
Acute myeloid leukaemia	17 (27·4)
Aplastic anaemia	11 (17·7)
Chronic myeloid leukaemia	9 (14·5)
Thalassaemia major	6 (9·7)
Acute lymphoblastic leukaemia	5 (8·1)
Fanconi aplastic anaemia	4 (6·5)
Adrenoleukodystrophy	2 (3·2)
Myelodysplastic syndrome	2 (3·2)
Non-Hodgkin's lymphoma	2 (3·2)
Haemophagocytic lymphohistiocytosis	1 (1·6)
Juvenile myelomonocytic leukaemia	1 (1·6)
Congenital neutropenia	1 (1·6)
Osteopetrosis	1 (1·6)
Conditioning regimen	
Myeloablative	32 (51·6)
Reduced	30 (48·4)
Graft *vs*. host disease	
Absence	50 (80·6)
Acute	4 (6·5)
Chronic	8 (12·9)

history or presence of GVHD, duration of IVIG (<4 *vs*. $\geqslant 4$ months) and administration of immunosuppressive medications $\geqslant 12$ months after HSCT on the duration of HAV seropositivity. Hazard ratios with 95% confidence intervals (CI) were used to quantify the strength of these associations. P values $<0{\cdot}05$ were considered significant [14].

RESULTS

The median age of the seropositive patients with allogeneic HSCT ($n=62$) was 17 years (range 2·5–56·0) and 67·7% of the patients were male. The most common underlying diseases were haematological disorders (Table 1). After Kaplan–Meier analysis, estimated mean time to persistence of HAV antibodies was 48·6 months after transplantation (Table 2). The percentage of seropositive and seronegative patients during follow-up is shown in Figure 1. Four (6·5%) patients were still seropositive 54 months after HSCT. The factors associated with persistence of HAV antibodies were older age ($\geqslant 18$ years) at transplantation and older ($\geqslant 18$ years) donor. Gender, conditioning regimen, duration of IVIG and immunosuppressive medications, and presence of GVHD did not affect the duration of HAV seropositivity after HSCT in univariate analysis (Table 2). Cox backward-stepwise regression confirmed older age of recipient at transplantation was the only significant parameter for HAV seropositivity (hazard ratio 3·70, 95% CI 1·08–12·64, $P=0{\cdot}037$).

DISCUSSION

In our study HAV seroprevalence was 85% before HSCT. Seropositivity increased with age at transplantation: it was 76·9% for patients aged <18 years and 100% for patients aged $\geqslant 18$ years. Seroprevalence rates were similar with previous epidemiological studies from Turkey [4–9]. Unal Ince *et al*. have recently reported that seroprevalence was 75·5% in paediatric allogeneic HSCT recipients in Turkey [15]. A study in Brazil reported the prevalence of HAV antibodies as 92·2% before HSCT with the patients' average age 26 years (range 3–58) [16]. In Argentina, Dignani *et al*. reported that 68% of peripheral stem cell transplant recipients aged 14–66 years were seropositive before transplantation [17]. There was no history of HAV vaccine application in the above-mentioned studies before transplantation, therefore all seropositive patients reflected naturally acquired HAV immunity.

In the present study, estimated mean time to loss of HAV seropositivity was 48·6 months with 30 months of median follow-up period (range 12–65 months) in allogeneic HSCT patients; 6·5% of the patients were still seropositive at 54 months. Median time to loss of HAV antibodies was reported in two studies as 358 days [17] and 12 months [15] and median follow-up was 12 months (range 1–51) and 56 months (range 21–123), respectively. An advantage of our study was that we used the Kaplan–Meier method which took into account 'censored' data – analysis of the case before the final outcome is observed [14].

Older age ($\geqslant 18$ years) at transplantation was found to be the only risk factor for persistence of HAV antibodies in our study. In accord, Godoi *et al*. reported that loss of HAV antibodies was significantly associated with younger age [16]. Their patient population was also similar to our study. However, we showed that donor age ($\geqslant 18$ years) was a significant risk factor in univariate analysis and this is the first study showing the relevance of donor age in the persistence of HAV antibodies after HSCT. A limitation

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Table 2. *Risk factors associated with the loss of hepatitis A antibodies after HSCT*

				Log rank	
	n	Mean	S.E.M.	χ^2	*P*
Overall	62	48·6	3·0		
Age at time of transplantation (years)					
<18	40	43·4	3·9	5·125	**0·024**
⩾18	22	53·2	3·0		
Gender					
Female	20	47·8	4·3	0·414	0·520
Male	42	47·2	3·6		
Donor age (years)					
<18	28	37·0	3·8	5·458	**0·019**
⩾18	34	54·6	3·4		
Regimen					
Myeloablative	32	44·9	4·5	1·589	0·207
Reduced	30	48·1	3·3		
Duration of IVIG after HSCT (months)					
<4	11	36·0	4·9	0·129	0·720
⩾4	51	48·9	3·3		
Any immunosuppressive medications ⩾12 months after HSCT (months)					
No	41	50·1	3·6	0·542	0·462
Yes	21	35·7	3·3		
Graft *vs*. host disease					
No	50	49·2	3·3	0·854	0·652
Acute	4	47·3	10·2		
Chronic	8	33·4	4·1		

HSCT, Haematopoietic stem cell transplant.

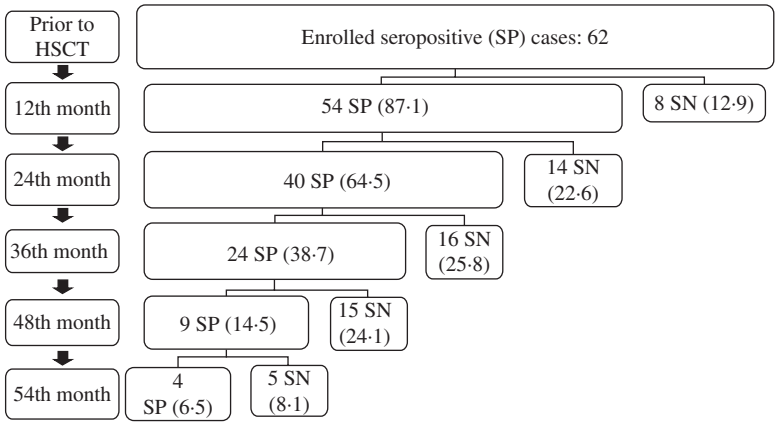

Fig. 1. The percentage of seropositive (SP) and seronegative (SN) patients during follow-up [*n* (%)].

of our study was that we did not know the serology of HAV in donors but it was assumed that older age reflects natural infection because of high seroprevalence rates with increasing age in Turkey [4–9]. In a previous study, Ljungman *et al*. showed that a young donor is a significant risk factor in univariate analysis for becoming seronegative against measles [18]. These authors also reported that patients having previously been vaccinated against measles were much more likely to become seronegative during

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follow-up than patients who had experienced a natural measles infection [18]. In our study the persistence of HAV antibodies in older age at transplantation and older donors could be explained by naturally acquired infection.

Godoi *et al*. also reported that the loss of HAV antibodies was significantly associated with longer follow-up and acute GVHD [16]. In our study the possible impact of other factors such as gender, donor age, conditioning regimen (myeloablative, reduced), GVHD (acute, chronic), duration of IVIG and application of immunosuppressive medications 12 months after transplantation were not found to be statistically significant on HAV serology. Unal Ince *et al*. also showed that the loss of HAV antibodies was high (43%) in paediatric HSCT recipients but they did not detect any significant risk factor [15]. Dignani *et al*. also failed to report any predictor factor for loss of HAV antibodies in peripheral stem cell transplant recipients [17]. These results are related to the limited number of patients in this field.

Hepatitis A infection is a widespread vaccine-preventable disease that can cause liver failure and some haematological complications [1–3, 10, 11]. These potential problems of hepatitis A infection might make follow-up of HSCT recipients difficult and can increase morbidity and mortality. Therefore, HSCT recipients should be vaccinated when they become seronegative. In our study, child and adult HSCT recipients in an intermediate endemic country were evaluated. We showed that older age ($\geqslant$18 years) at transplantation affected the persistence of HAV antibodies. It is obvious that children need to be vaccinated earlier than adult HSCT recipients. However, further studies with larger numbers of HSCT recipients can reveal the predictive factors for loss of HAV antibodies and the role of donor immunity.

DECLARATION OF INTEREST

None.

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