

ORIGINAL ARTICLE

Abnormal liver enzymes two years after haematopoietic stem cell transplantation in children: prevalence and risk factors

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To establish the prevalence of elevated liver enzymes in children transplanted in a Dutch haematopoietic stem cell transplantation (HSCT) centre, we retrospectively assessed AST and ALT values at 2 years after HSCT. Age, sex, diagnosis, type of transplant, conditioning regimen and early post-transplant complications involving the liver (veno-occlusive disease, acute GVHD, viral reactivation) were analysed as risk factors. AST and ALT values were available at 2 years after HSCT in 216 of 290 patients (75%) alive at that time and were above normal in 53 (25%) and at least twice normal in 17 (8%) patients. Older age at HSCT and a diagnosis of benign haematological disease are risk factors for abnormal liver enzymes late after HSCT. In half of the patients with benign haematological disease, iron overload is the most likely aetiological factor. Chronic hepatitis B or C is uncommon in our centre. In conclusion, the prevalence of abnormal liver enzymes late after HSCT in our centre is lower than reported in previous studies. Abnormal liver enzymes occur more often in children who are older at HSCT and transplanted for benign haematological disease. Long-term follow-up is crucial to establish if elevated liver enzymes precede clinical liver disease.

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Introduction

In the first months after allogeneic haematopoietic stem cell transplantation (HSCT), most patients show elevated liver

enzymes at some point, but only few develop frank liver failure. Abnormal liver enzymes in the early phase after transplant may be due to drug toxicity, bacterial or fungal infection, viral infection or reactivation, acute GVHD and veno-occlusive disease (VOD).^{1–5} In children, only one study has been published on this subject, showing that 28% of children undergoing HSCT had transient hyperbilirubinaemia and 88% had transient elevation of aminotransferases during the early phase after transplant.⁶

In some patients, elevated liver enzymes may persist or recur late after transplantation. Only a few studies have investigated the prevalence of abnormal liver enzymes late after HSCT.^{7–11} And, to our knowledge, only three studies have been published that included paediatric patients.^{9–11} These studies have shown that long-term abnormal liver enzymes after HSCT are a common occurrence. The prevalence of persistently or recurrently elevated aminotransferases (ALT, AST) varied from 25 to 57% in these studies. However, few of these patients had abnormal liver function or clinical signs of liver disease.

In the majority of patients with elevated liver enzymes late after HSCT, a probable cause is found. Aetiology includes iron overload,^{7,12,13} chronic viral hepatitis B and/or C,^{8,9,14} and chronic GVHD.¹¹ A few patients have autoimmune hepatitis and in some patients the cause of elevated liver enzymes is unknown.¹⁰ In one paediatric study, the aetiology of persistently elevated liver enzymes was chronic viral hepatitis, mostly hepatitis C, in half of the patients.⁹ The prevalence, risk factors and aetiology of elevated liver enzymes late after HSCT in a Dutch Paediatric transplant centre may be different from what has been described in the above-mentioned studies. The prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection among voluntary blood donors in the Netherlands is low (less than 0.5%)¹⁵ and the risk of transfusion-transmitted hepatitis has been less than in some Mediterranean countries. Also, thalassaemia is less prevalent within our transplant population and iron overload may thus be less common. And lastly, the prevalence of chronic GVHD is low and less than 10% in our Paediatric transplant unit (personal communication).

The aim of our study was to establish the prevalence of elevated liver enzymes late after allogeneic HSCT in a

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Dutch paediatric population and to analyse risk factors in this group.

Patients and methods

Patients

All children who underwent an allogeneic HSCT in the Department of Paediatrics of the LUMC between 1 January 1980 and 1 January 2002 and were alive at least 2 years after transplant were eligible for the study. We also studied the cause of death in all patients who died within 2 years of transplant to assess if this was (at least in part) attributable to liver disease.

Methods

Patients eligible for the study were identified from the local database of the European Group for Blood and Marrow Transplantation. The following data were extracted from this database: sex, date of birth, alive or deceased, diagnosis (haematological malignancy, benign haematological disease, immunological disease, other inborn errors), donor type and stem cell source, type of conditioning regimen (CY and TBI/thoraco-abdominal irradiation (TAI), CY and BU, other including TBI/TAI, other without TBI/TAI, none) and the occurrence of VOD and of acute GVHD of the liver.

In our centre, cyclosporine was given for approximately 6 months and antibiotic prophylaxis after (spleen) irradiation for 1 year, and thus at 2 years after HSCT most patients were off drug therapy.

To establish if death was related to liver disease in the deceased patients, the occurrence of the following BMT related complications was collected from either the database or the case notes: VOD, acute GVHD of the liver, viral reactivation/infection involving the liver, hepato-renal syndrome and/or multi-organ failure including liver failure.

In the patients alive at least 2 years after transplant, serum AST and ALT were collected, retrospectively, from the hospital information system at 2 years (plus or minus 6 months) after HSCT. Abnormal liver enzymes at 2 years after transplant were defined as AST and/or ALT above the upper limit of normal of our laboratory (mean plus two standard deviations as determined in a normal Dutch population). To study the course of elevated liver enzymes in time, we also collected the liver enzyme values at 1 and 3 years from transplant in those patients who had abnormal values at 2 years.

Viral reactivation/infection was defined as the presence of viraemia as tested by nucleic acid detection with PCR for adenovirus, CMV and EBV. Since quantitative PCR for viral detection was introduced in our centre in 2001, results are available in a subgroup only. Before 2001, CMV viraemia was detected by the presence of pp65 in the leucocytes. Also, viral serology for EBV, CMV, hepatitis A virus (HAV), HBV and HCV (the latter from 1991 onwards) had been performed before HSCT.

VOD was defined according to the modified Baltimore criteria:¹⁶ hyperbilirubinaemia (bilirubin $\geq 34 \mu\text{mol/l}$) and

at least two of three symptoms (hepatomegaly, ascites or unexplained weight gain of $\geq 5\%$ from baseline) present before day 21 after HSCT when other possible causes of these clinical manifestations have been excluded. Hepatic acute GVHD was defined as the presence of clinical and histological evidence of other target organ involvement with GVHD in conjunction with the development of abnormal findings of serum liver biochemistry analysis.

In patients with liver enzymes elevated at least twice the upper limit of normal, aetiology was also investigated by testing of serum ferritin and MRI or biopsy of the liver (to establish iron overload), repeated viral serology for HAV, HBV, HCV, CMV and EBV and testing for auto-antibodies associated with auto-immune hepatitis. Ultrasound of the liver was also performed. We examined these children for clinical signs of liver disease and of chronic GVHD and tested synthetic liver function by measurement of clotting times and serum albumin level.

Statistical analysis

The frequency of elevated liver enzymes at 2 years after HSCT according to clinical characteristics was assessed. Potential risk factors for elevated liver enzymes were analysed in patients with liver enzyme values above the upper limit of normal (53 patients) and in the subgroup (17 patients) with liver enzyme values of at least twice the upper limit of normal, separately, using Pearson's χ^2 -test (discrete values) or Student's *t*-test (continuous values). Risk factors analysed included sex, age, diagnosis of malignant or benign haematological disease versus the other diagnosis groups, donor type (matched sibling donor or other), conditioning regimen with CY/TBI or with CY/BU, each versus all other types of conditioning, viral infection or reactivation in the acute phase after transplant, VOD and acute GVHD.

Results

Of the 456 patients who underwent allogeneic HSCT, 290 patients were alive at least 2 years after transplant. Of the 166 patients who died within 2 years from transplant, 15 (9%) died, at least in part, due to liver disease. The most frequent cause of death in the other 151 patients was relapse of disease, followed by transplant related mortality.

Of 22 patients who died more than 2 years after transplant, one died of chronic GVHD including the liver. In the other patients, cause of death was not associated with liver disease.

In 216 of 290 (74.5%) patients alive 2 years after HSCT, AST and ALT have been tested at 2 years after transplant. The clinical characteristics of these 216 patients are shown in Table 1. Their median age at HSCT was 7.6 (0.1–18.4) years. The majority of these patients were transplanted for a haematological malignancy and with bone marrow of a matched sibling donor. The majority of patients underwent conditioning with TBI. Only three patients had chronic hepatitis C, which in all three was present pre-HSCT and proven with HCV PCR. Viral reactivation of EBV, CMV and/or adenovirus after HSCT occurred in 35 patients

Table 1 Clinical characteristics of 216 children with liver enzyme values tested at 2 years after haematopoietic stem cell transplantation

Age at HSCT, median (range)	7.6 (0.1–18.4) years
Sex (male/female)	130/86
<i>Diagnosis^a</i>	
Haematological malignancy	129 (60%)
Benign haematological disease	54 (25%)
Immunological disease	22 (10%)
Other inborn errors	11 (5%)
<i>Donor type</i>	
MSD	141 (65%)
(M)MUD	55 (26%)
ORD	20 (9%)
<i>Haematopoietic stem cell source</i>	
BM	205 (95%)
PBSC	7 (3%)
CB	4 (2%)
<i>Conditioning regimen</i>	
CY + TBI/TAI	121 (56%)
CY + BU	69 (32%)
Other (+ TBI/TAI)	11 (5%)
Other (no TBI/TAI)	10 (5%)
None	5 (2%)
<i>Viral infection/reactivation</i>	
	<i>Patients pos/patients tested</i>
HBsAg positive at SCT	0/183
HCV seropositive at SCT	3/139 (2%)
CMV reactivation	25/117 (21%)
EBV reactivation	13/44 (30%)
Adenovirus reactivation	1/17 (6%)
<i>Liver disease <3 months after HSCT</i>	
VOD	14 (7%)
Acute GVHD	5 (2%)

Abbreviations: CB = cord blood; HCV = hepatitis C virus; HSCT = haematopoietic stem cell transplantation; MSD = matched sibling donor; (M)MUD = (mis)matched unrelated donor; ORD = other related donor; TAI = thoraco-abdominal irradiation; VOD = veno-occlusive disease.

^aHaematological malignancy: ALL, AML, CML, JMML, MDS and lymphoma; benign haematological disease: Fanconi anaemia, Blackfan–Diamond anaemia, Shwachman–Diamond syndrome, severe aplastic anaemia; immunological disease: immunodeficiency syndromes and haemophagocytic lymphohistiocytosis; other inborn errors: osteopetrosis, X-adrenoleucodystrophy.

(results not shown). VOD and acute GVHD involving the liver were uncommon. None of these patients was receiving immunosuppressive therapy.

A total of 53 of 216 patients (24.5%) had abnormal liver enzymes 2 years after transplant. In 17 patients (7.9%), AST and/or ALT were elevated at least twice the upper limit of normal.

In 23 of 36 patients with liver enzymes above normal but less than twice normal, liver enzyme values also have been tested at 1 and 3 years post-transplant. In 14 patients, liver enzymes normalised in time, whereas in 8 patients abnormal values (less than twice normal) persisted. In 13 of 17 patients with liver enzymes at least twice normal, liver enzymes had been tested at 1 and 3 years. In 12 abnormal values persisted in time and were at least twice normal in half of them.

In Table 2, the frequency of normal and elevated liver enzymes (above normal and at least twice normal) is shown

for sex, diagnosis, donor type, stem cell source and conditioning regimen. Children with elevated liver enzymes 2 years after HSCT ($n = 53$) were older at the time of transplant than those children with normal liver enzymes ($P = 0.027$). A diagnosis of benign haematological disease was the only other significant risk factor for liver enzymes above the upper limit of normal and for values at least twice the upper limit of normal with an OR, 2.59 (1.32–5.05) ($P = 0.005$) and OR, 2.76 (0.98–7.79) ($P = 0.048$), respectively. Patients with benign haematological disease appeared to be older at transplant than those patients with other diagnoses with a mean age of 9.23 years ($n = 54$) and 7.74 years ($n = 162$), respectively, and this is nearing significance and might be a factor if a larger cohort were studied ($P = 0.053$).

None of the other potential risk factors including sex, donor type, stem cell source, conditioning regimen, viral reactivation after HSCT, VOD and acute GVHD was found to be associated with a higher risk for elevated liver enzymes 2 years after transplant. Females and patients who had an EBV reactivation or VOD at the time of transplant were more likely to have liver enzymes at least twice normal (OR, 2.39 (0.86–6.60), $P = 0.09$, OR, 3.28 (0.81–13.3), $P = 0.08$, and OR 3.28 (0.81–13.3), $P = 0.08$ respectively) and although these risk factors were not significant, they might be in a larger cohort.

Aetiology was assessed only in patients with AST and/or ALT values at least twice the upper limit of normal. Four patients were lost during follow-up. One patient died of chronic GVHD and one patient has severe portal hypertension due to haemochromatosis of the liver. The other 11 patients are well with no clinical signs of liver disease and all have normal synthetic and excretory liver function. In 5 of 11 patients, iron overload was found to be the most likely cause of liver damage, confirmed by ferritin (5/5), liver biopsy (3/5) and/or MRI (1/5). In 3 of 11 patients chronic GVHD was the most likely cause for elevated liver enzymes. Two patients, one also with iron overload, had chronic hepatitis C, which was present pre-HSCT. In two patients aetiology was not clear and liver biopsy was not performed.

Discussion

The prevalence of abnormal liver enzymes in children 2 years after HSCT in our centre is 25%. Of these patients, 32% have liver enzyme values more than twice the upper limit of normal. The prevalence of abnormal liver enzymes late after transplantation is in agreement with the prevalence found by Frisk *et al.*,¹¹ but lower than that found by Locasciulli *et al.* and Tomás *et al.*^{9,10} Both studies found a prevalence of chronically elevated liver enzymes of more than 50%. But, like all three other paediatric studies on elevated liver enzymes late after SCT, we find that the majority of these children have neither clinical signs of liver disease nor any abnormal synthetic liver function. Only two children in our study had severe liver disease, one died of chronic GVHD and the other patient has liver cirrhosis and oesophageal varices due to iron overload.

Table 2 Liver enzyme values 2 years after HSCT in 216 patients according to clinical characteristics

	Normal liver enzymes (n = 163)	Above normal (n = 53)	> 2 times upper limit of normal (n = 17) ^a
Age at HSCT, median (range)	7.2 (0.1–18.1) years	9.9 (0.7–18.4) years	9.7 (0.2–18.1) years
Sex (male/female)	102 (78%)/61 (71%)	28 (22%)/25 (29%)	7 (5%)/10 (12%)
<i>Diagnosis^b</i>			
Haematological malignancy	101 (78%)	28 (22%)	7 (5%)
Benign haematological disease	33 (61%)	21 (39%)	7 (13%)
Immunological disease	18 (82%)	4 (18%)	3 (14%)
Other inborn errors	11 (100%)	0	0
<i>Donor type</i>			
MSD	107 (76%)	34 (24%)	8 (6%)
(M)MUD	42 (76%)	13 (24%)	6 (11%)
ORD	14 (70%)	6 (30%)	3 (15%)
<i>Haematopoietic stem cell source</i>			
BM	154 (75%)	51 (25%)	16 (8%)
PBSC	6 (86%)	1 (14%)	1 (14%)
CB	3 (75%)	1 (25%)	0
<i>Conditioning regimen</i>			
CY + TBI/TAI	86 (71%)	35 (29%)	7 (6%)
CY + BU	56 (81%)	13 (19%)	6 (9%)
Other (+ TBI/TAI)	9 (82%)	2 (18%)	1 (9%)
Other (no TBI/TAI)	8 (80%)	2 (20%)	2 (20%)
None	4 (80%)	1 (20%)	1 (20%)
<i>Viral infection/reactivation</i>			
HBsAg positive at SCT	0		
HCV seropositive at SCT	0	3 (100%)	2 (67%)
CMV reactivation	18 (72%)	7 (28%)	3 (12%)
EBV reactivation	10 (77%)	3 (23%)	3 (23%)
Adenovirus reactivation	0	1 (100%)	0
<i>Liver disease <3 months after HSCT</i>			
VOD	10 (71%)	4 (29%)	3 (21%)
Acute GVHD	3 (60%)	2 (40%)	1 (20%)

Abbreviations: CB=cord blood; HCV=hepatitis C virus; HSCT=haematopoietic stem cell transplantation; MSD,=matched sibling donor; (M)MUD=(mis)matched unrelated donor; ORD=other related donor; TAI=thoraco-abdominal irradiation; VOD=veno-occlusive disease.

^aThese 17 patients are a subgroup of the 53 patients with elevated liver enzymes.

^bHaematological malignancy: ALL, AML, CML, JMML, MDS and lymphoma; benign haematological disease: Fanconi anaemia, Blackfan–Diamond anaemia, Shwachman–Diamond syndrome, severe aplastic anaemia; immunological disease: immunodeficiency syndromes and haemophagocytic lymphohistiocytosis; other inborn errors: osteopetrosis, X-adrenoleucodystrophy.

From the case notes and laboratory tests, we tried to establish the aetiology of elevated liver enzymes only in the subgroup with values at least twice the upper limit of normal. In this group, iron overload was present in half of the patients. In only 2 of 13 patients aetiology was unclear. The percentage of patients with unknown aetiology of elevated liver enzymes varies substantially between studies, with Tomás *et al.* finding only 3% with unknown aetiology, whereas Locasciulli *et al.* describe unexplained aetiology in 42%. In the first study, more than half of the patients had a liver biopsy and in half of the patients iron overload was found, but the aetiology was multifactorial in 47%.¹⁰ In the study by Locasciulli *et al.*, half of the patients had chronic hepatitis C and it is unclear whether iron overload or chronic GVHD was present in any of the patients with unknown aetiology.⁹ As expected, the prevalence of chronic hepatitis C in our patients was low (three patients). These children had transfusion-transmitted HCV infection acquired pre-HSCT either abroad (two patients) or at a time before introduction of HCV screening of blood

products. Liver biopsy can help to assess fibrosis/cirrhosis and the level of iron overload that might be of help in the differential diagnosis of chronically elevated liver enzymes. However, in children with no clinical signs of liver disease, paediatricians tend to be reluctant in performing an invasive procedure such as liver biopsy. In only three of our patients with elevated liver enzymes, liver biopsy was performed.

We analysed if the clinical characteristics at and shortly after transplant were predictive for the occurrence of elevated liver enzymes late after HSCT. Only older age at HSCT and a diagnosis of benign haematological disease were shown to be significant risk factors for the presence of elevated liver enzymes 2 years after transplant. Half of the patients with benign haematological disease were shown to have iron overload, but five children with Fanconi anaemia (of 11 Fanconi patients in our study) also had liver enzymes above normal (but less than twice normal), with no signs of iron overload and here aetiology is unclear. We hypothesize that Fanconi patients are more at risk for drug-induced

liver injury by CY, even with the low doses given, due to the well known increased sensitivity for alkylating drugs in these children. Why older age would be a risk factor is unclear. Our hypothesis that children with a benign haematological disease were also older at transplant could not be confirmed. None of the other clinical characteristics or early transplant related complications was shown to be a risk factor for elevated liver enzymes 2 years after transplant. However, analysis of VOD and acute GVHD as risk factors might well have been hampered by the low patient numbers. In our study, only three patients had chronic hepatitis C, and none had hepatitis B, and all three showed elevated liver enzymes.

Although elevated liver enzymes are considered to be consistent with liver cell inflammation and could eventually lead to liver cell destruction with impaired liver function and even liver failure, studies so far, including our own, have shown that most patients are asymptomatic. Interestingly, in two thirds of patients with a minor elevation of liver enzymes, these values normalise in time, whereas values at least twice normal tend to persist and are thus more likely to be associated with liver disease. Since liver biopsies have been done in only a minority of these patients, the histopathological correlates are often unknown. The clinical relevance of abnormal liver enzymes and the risk for late liver dysfunction or even liver failure is currently unknown. However, it could be well concluded that persistence of elevated liver enzymes is a sign of ongoing liver cell inflammation possibly leading to liver cell destruction and impaired (metabolic) liver function later in life. Long-term follow-up is crucial in these patients to establish, if elevated liver enzymes precede liver disease with impaired liver function and should at some point prompt us to perform a liver biopsy.

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