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Gastrointestinal Toxicity, Systemic Inflammation, and Liver Biochemistry in Allogeneic Hematopoietic Stem Cell Transplantation

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A B S T R A C T

Liver toxicity is frequently seen in relation to allogeneic hematopoietic stem cell transplantation (HSCT), but pathogenesis and the risk factors are poorly understood. The purpose of this study was to investigate associations between liver toxicity, gastrointestinal toxicity, and levels of immune-regulating cytokines during the early post-transplantation period. We prospectively included 81 children and adults undergoing HSCT after myeloablative conditioning. Alanine aminotransferase (ALT), total bilirubin levels, and international normalized ratio were measured longitudinally until 3 months after the transplantation and related to levels of inflammatory markers (C-reactive protein [CRP], IL-6, and IL-10) and to plasma citrulline as a marker of intestinal toxicity during the first 3 weeks after HSCT. The majority of patients experienced ALT levels above the normal range (45 U/L) with significant increases at 3 months after HSCT. Increased levels of total bilirubin were observed in 26% during the 3-month period. Citrulline levels decreased significantly to a nadir at day 7 (B = .23; 95% confidence interval [CI], .12 to .35; $P < .0001$), but citrulline levels at nadir were not associated with parameters of liver toxicity. However, a faster reconstitution of mucosa with higher citrulline levels at day +21 correlated with lower bilirubin levels 3 months after HSCT (r = -.26, $P = .034$) and increased overall survival (hazard ratio, .88; 95% CI, .79 to .97; $P = .008$) . Increased levels of CRP and IL-6 at day 7 after HSCT correlated positively with ALT and bilirubin, and in the multivariate analysis, IL-6 at day 7 appeared to be the only predicting risk factor for increased mean bilirubin during the early post-transplantation phase (B = .01; 95% CI, .01 to .02; $P = .001$) as well as maximum levels of bilirubin (B = .3; 95% CI, .12 to .48; $P = .001$) and occurrence of sinusoidal obstruction syndrome during the first 3 months after HSCT (odds ratio, 1.003; 95% CI, 1.001 to 1.005; $P = .002$). The results of this study indicate that liver toxicity after HSCT is associated with an increased inflammatory response mounted during the phase of maximal gastrointestinal toxicity in the early phase after transplantation.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a treatment of high-risk leukemia and a number of nonmalignant hematological diseases [1]. Although mortality and morbidity have improved over the last decade, organ toxicity is still of vast concern in HSCT, with a nonrelapse mortality of 9% to 12% at 10 years after HSCT [2].

Liver involvement in the early toxic phase is frequently seen in relation to HSCT [3,4]. Elevated liver enzymes have been reported in up to 72% of adult patients within the first year after HSCT [4-6], while a pediatric study reported elevated liver enzymes in about one-quarter of the patients at

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2-year follow-up [7]. Previous studies from our group have suggested that even mild liver affection during the early post-HSCT phase and at 1-year follow-up are predictive of poor long-term survival [8].

Despite this, our insight into causes of liver involvement in HSCT is still limited; however, it is thought to be multifactorial and include infections and side effects of the pretransplantation conditioning regimen and supportive treatments, as well as acute and chronic graft-versus-host disease (GVHD).

Cytotoxic effects of the chemotherapy and irradiation on the gastrointestinal (GI) tract are thought to play a key role in the pathogenesis of treatment-related complications in HSCT because of translocation of endotoxins originating from the intestinal microbiota [9,10]. This influx of microbiologically derived products may lead to an increased concentration of cytokines in the portal blood, thereby exposing the liver to a high load of pro-inflammatory signals, possibly predisposing to further tissue damage and enhanced alloreactivity. However, possible associations between measures of toxic damage to the GI tract and liver involvement, including sinusoidal obstruction syndrome (SOS), have not previously been investigated in detail.

The purpose of this prospective study was to investigate the hypothesis that liver affection after HSCT is associated with increased GI toxicity and increased systemic levels of inflammatory mediators during the early post-transplantation period. We studied liver toxicity and markers of systemic inflammation within the first 6 months after HSCT in a cohort of children and adults. We used plasma citrulline as a measure of GI toxicity, as citrulline reflects the loss of functioning intestinal epithelial cells and correlates inversely with clinical and functional scores of GI mucositis [11,12].

PATIENTS AND METHODS

We consecutively recruited HSCT patients in a prospective study at the national HSCT center at the Copenhagen University Hospital Rigshospitalet, Denmark, from June 2010 to January 2013. Inclusion criteria were first myeloablative allogeneic stem cell transplantation and age >1 year at the time of transplantation. All patients received full-intensity conditioning and identical antibiotic prophylaxis consisting of ceftriaxone and fluconazole. Fifty-four patients received cyclophosphamide as part of the conditioning.

The cohort has previously been described and included 81 of a total of 154 patients who underwent transplantation in the study period [13]. Participating patients had a lower recipient age than patients who were not included (mean age, 24.3 versus 32.2 years; $P < .0001$). Fewer included patients received total body irradiation as part of the conditioning (57% versus 70%; $P = .021$) and more participating patients were treated with antithymocyte globulin (ATG) (46% versus 23%; $P = .007$). None of the patients had any pre-HSCT liver comorbidities.

Sixteen patients did not fulfill a complete set of assessments because of relapse of leukemia (n = 5), graft failure (n = 1), treatment-related death (n = 6), or transfer to other hospital for clinical follow-up (n = 4). Clinical characteristics of included patients and transplantations are listed in Table 1.

Laboratory Tests

Plasma samples used for cytokines and citrulline analyses were collected at fixed time intervals, as follows: before the start of conditioning regimen, at the day of transplantation before graft infusion, at day +7, and at day +21. EDTA-anticoagulated blood and heparinized blood were centrifuged within 2 hours after collection, and plasma was isolated and cryo-preserved in .5-mL aliquots at –80°C until analyses.

Table 1
Baseline Patient and Transplantation-Related Characteristics

Characteristic	Toxicity	Patients n (% calculated separately for each period)	
		Three-Month Follow-up	Six-Months Follow-Up
Total	81 (100)	72 (100)	65 (100)
Sex			
Male	49 (60)	42 (58)	39 (60)
Female	32 (40)	30 (42)	26 (40)
Age, mean (range), yr	24.3 (6.3-52.1)	25.1 (6.4-50.7)	25.5 (6.4-51.9)
Underlying disease			
Malignant diseases	63 (78)	55 (76)	50 (77)
Nonmalignant diseases	18 (22)	17 (24)	15 (23)
Karnofsky score ≤ 90	40 (49)	34 (47)	31 (48)
Karnofsky score >90	36 (44)	33 (46)	29 (45)
Donor type			
Matched sibling donor	18 (22)	15 (21)	13 (20)
Matched unrelated donor	46 (57)	41 (57)	38 (58)
Mismatched unrelated donor	17 (21)	16 (22)	14 (22)
Stem cell source			
Bone marrow	57 (70)	51 (71)	48 (74)
Peripheral blood stem	16 (20)	13 (18)	11 (17)
Umbilical cord blood	8 (10)	8 (11)	6 (9)
Conditioning regimen			
Cyclophosphamide based	28 (35)	24 (33)	21 (32)
Total body irradiation based	26 (32)	25 (35)	23 (35)
Other TBI based	18 (22)	14 (19)	14 (22)
Other chemotherapy based	9 (11)	9 (13)	7 (11)
GVHD prophylaxis			
CsA	7 (9)	6 (8)	4 (6)
CsA + methotrexate	70 (86)	62 (86)	57 (88)
CsA + corticosteroids	3 (4)	3 (4)	3 (5)
aGVHD grade 0-1	48 (59)	44 (61)	42 (65)
aGVHD grade 2-4	33 (41)	28 (39)	23 (35)
aGVHD: no	33 (41)	30 (42)	28 (43)
aGVHD: yes	48 (59)	42 (58)	37 (57)
SOS	18 (22)		

TBI indicates total body irradiation; aGVHD, acute graft-versus-host-disease.