Coexisting or Underlying Risk Factors of Hepatic Veno-Occlusive Disease in Pediatric Hematopoietic Stem Cell Transplant Recipients Receiving Prophylaxis

Müge Gökce, Baris Kuskonmaz, Mualla Cetin, Duygu Uckan Cetinkaya, Murat Tuncer

Abstract

Objectives: **To evaluate the characteristics of veno-occlusive disease in pediatric hematopoietic stem cell transplant recipients, and their effect as a prophylactic regimen on severity and outcome.**

Materials and Methods: **This study included 204 allogeneic hematopoietic stem cell transplants performed on 187 children whose data retrospectively described the risk factors, prophylaxis, and treatment modalities of veno-occlusive disease. A prophylactic regimen composed of enoxaparin versus ursodeoxycholic acid and vitamin E was given to 167 of 204 patients.**

Results: **Veno-occlusive disease developed in 22 patients (10.8%). Nineteen patients experienced veno-occlusive disease despite this prophylactic regimen. The prophylaxis seemed ineffective in preventing veno-occlusive disease (*P* = .657). Regarding risk factors, oral busulphan use, liposomal amphotericin B vancomycin treatment, and total parenteral nutrition were associated with an increased risk of veno-occlusive disease. Conversely, renal impairment also was associated with increased mortality in patients with veno-occlusive disease. The mortality rate in the first 100 days after a hematopoietic stem cell transplant was higher in the patients with veno-occlusive disease than it was in those without the disease.**

Conclusions: **Our prophylactic regimen may have played a role in the fairly low incidence of veno-occlusive disease in a pediatric population with high-risk features.**

Key words: *Hematopoietic stem cell transplant, Low–molecular-weight heparin, Pediatric, Veno-occlusive disease*

Introduction

Despite the recent advances in hematopoietic stem cell transplant (HSCT), hepatic veno-occlusive disease (VOD) remains a significant cause of morbidity and mortality. In pediatric population, its incidence shows a wide range between 5% to 39% because of differences in the underlying risk factors.[1,2]

Pre-existing liver disease, younger age, gemtuzumab-ozogamicin, myeloablative conditioning regimens with busulphan (BU), total parenteral nutrition (TPN), previous abdominal radiotherapy and pediatric diseases such as hemophagocytic lymphohistiocytosis and osteopetrosis are well-established risk factors for VOD.[2-5]

Several agents, such as classic heparin, low–molecular-weight heparin, prostaglandin E1, have been tried to prevent of VOD but unfortunately, the results of these studies are inconclusive .[6,7] Prognosis depends on the extent of the hepatic injury and the development of the multiorgan failure.[8]

The objective of this study was to retrospectively evaluate the characteristic features of VOD in pediatric HSCT recipients with various underlying diseases and to analyze the effect of a prophylactic regimen on incidence, severity, and outcome of VOD.

Materials and Methods

Between July 1996 and April 2012, two hundred four allogeneic HSCTs were performed on 187 children at

From the Unit of Pediatric Hematopoietic Stem Cell Transplantation, Ihsan Dogramaci Children's Hospital, Hacettepe Medical Faculty, Ankara, Turkey

Acknowledgements: *The authors have no conflicts of interest to declare.*

Corresponding author: *Müge Gökce, MD, Gokturk Mah., Doga Sok. No:2/16, 34070 Kemerburgaz, Eyup, Istanbul, Turkey*
Phone: *+90 533 292 1387* ***Fax:*** *+90 312 305 1172* ***E-mail:*** *drgundogdu@yahoo.com*

Experimental and Clinical Transplantation (2013) 5: 440-446

DOI: 10.6002/ect.2012.0265

the Pediatric Hematopoietic Stem Cell Transplantation Unit of Ihsan Dogramaci Children's Hospital, Hacettepe University, Ankara, Turkey. Patients' data were retrospectively reviewed from hospital charts that describe the demographic features of patients, risk factors, prophylaxis, and treatment modalities of VOD within 100 days after HSCT. The study was approved by Hacettepe University Ethical Committee, and all protocols were performed according to the 1975 Declaration of Helsinki.

All patients were hospitalized in HEPA-filtered single rooms until discharge. Acyclovir and fluconazole were given for prophylaxis of herpes simplex virus and fungal infection. Trimethoprim-sulfamethoxazole was used for prophylaxis of *Pneumocystis carinii*.

A VOD prophylactic regimen composed of low–molecular-weight heparin, ursodeoxycholic acid (UDCA), and vitamin E was given from the beginning of the conditioning regimen until late discharge or day twenty-eight in 167 of 204 HSCT recipients (81.8%). The remaining patients did not receive VOD prophylaxis either because they did not use nonmyeloablative or reduced-intensity conditioning regimens (not expected to increase risk of VOD) or because they had a medical condition that might be associated with an increased risk of bleeding. Enoxaparin and ursodeoxycholic acid were given at a dosage of 0.8 mg/kg sc daily and 20 mg/kg in 2 doses daily. Vitamin E was given daily at 100 IU above 12 years of age, 50 IU for ages 2 to 12 years, and 25 IU for those patients younger than 2 years of age. Cyclosporine and a short course of methotrexate or methylprednisolone were given for prophylaxis of acute graft-versus-host disease.

Definitions

The diagnosis of VOD was established according to Baltimore criteria.[9] In addition to elevated serum bilirubin level above 34.2 μmol/L, presence of 2 of the following events in the first 21 days after HSCT confirmed the diagnosis: tender hepatomegaly and a ≥ 5% weight gain because of fluid accumulation and ascites. No other explanation for these clinical findings was present at the time of the diagnosis of VOD. The first day of diagnosis and duration of VOD, the presence of renal involvement, treatment approach, maximum serum bilirubin level, liver transaminase levels, and clinical outcomes were noted.

Conversely, the severity of VOD was based on the criteria of McDonald and associates. Veno-occlusive disease that resolved spontaneously without intervention, and VOD that resolved completely with supportive treatment, were classified as mild and moderate. Veno-occlusive disease leading to multiorgan failure or death despite treatment was considered severe. Defibrotide is not widely available in our country, so it was used only in a few patients (n=13). When available, defibrotide was given as 2-hour infusion, 4 times daily, at a dosage of 25 mg kg^{-1}.

Neutrophil engraftment was defined as *the first day of neutrophil count reaching to* $\geq 0.5 \times 10^9\ L^{-1}$ *for 3 consecutive days*. Platelet engraftment was accepted as platelet count $\geq 20 \times 10^9\ L^{-1}$ for 1 week without thrombocyte transfusion.

Acute graft-versus-host disease was diagnosed and classified according to Glucksberg's criteria.[10] Renal impairment was evaluated according to serum creatinine levels based on RIFLE criteria.[11] Hemorrhagic cystitis was defined as *painful hematuria with a negative urine culture for bacteria and fungus, without any other explanations such as general bleeding diathesis, urinary tract catheterization for reasons other than hemorrhagic cystitis, urinary calculi, or bladder neoplasms.*[12]

Risk factors for veno-occlusive disease

The risk factors for VOD were classified in 2 groups: (1) Pretransplant risk factors: age, sex, baseline body mass index, underlying diagnosis, number of transplants, prior chemotherapy or radiotherapy, pre-existing liver disease, serum ferritin levels, and hepatitis serology; or (2) HSCT-related risk factors: donor type, stem cell source, conditioning regimen, the route of BU administration, antibiotherapy, renal involvement, and neutrophil/platelet engraftment days.

The ages were grouped below 2 years of age, 2 to 8 years, and > 8 years of age. Body mass index was evaluated according to Center for Disease Control.[13] Serum ferritin levels also were classified as < 500 ng mL^{-1}, 500 to 1000 ng mL^{-1}, and > 1000 ng mL^{-1}.

The underlying disorders were analyzed with 9 different titles: (1) Thalassemia major (n=27), (2) Fanconi Anemia (n=35), (3) acute lymphoblastic leukemia (n=10), (4) myeloid malignancies (n=48) [myelodysplastic syndromes (n=11), acute myeloid leukemia (n=20), juvenile myelomonocytic

leukemia (n=1), and chronic myeloid leukemia (n=16)], (5) immunologic disorders (n=18) (Wiskott-Aldrich syndrome, Griscelli syndrome, Chédiak–Higashi syndrome, MHC class II deficiency, and chronic granulomatous disease), (6) acquired aplastic anemia (n=20), (7) hemophagocytic lymphohistiocytosis (n=8), (8) metabolic disorders (n=10) (adrenoleukodystrophy, metachromatic leukodystrophy, and I-cell disease), and (9) osteopetrosis (n=10). One patient had hypereosinophilic syndrome. The underlying disorders also were grouped as malignant (n=58) or nonmalignant diseases (n=129).

Preparative regimens were classified as myeloablative, reduced intensity, and BU-containing or not. The dosage of BU was based on both the age group and the actual body weight as follows: 1 mg/kg for patients ≤ 4 years of age, and 0.8 mg/kg for patients > 4 years of age, every 6 hours, for 16 dosages.[14] The route of the administration (IV or oral) of BU also was noted.

Statistical analyses

Risk factors were evaluated initially by univariate analysis using chi-square and Fisher exact test. Then multivariate analysis was performed using logistic regression model and the odds ratios were determined with 95% CIs. The mortality predictors of VOD were calculated by Cox proportional hazards regression model. Valves for $P < .05$ were accepted as statistically significant. Survival analysis was estimated by the Kaplan-Meier test. Survival in patients with or without VOD was compared with the log-rank test. Statistical analyses were performed with SPSS software (SPSS: An IBM Company, version 15.0, IBM Corporation, Armonk, NY, USA).

Results

We performed a total of 204 HSCTs in 187 patients. Seventy-one patients were female (37.6%) and 116 patients were males (62.4%). The median age at transplant was 7 years (range, 0.25-21 years). The features of the patients and HSCTs are shown in Table 1.

Features of veno-occlusive disease

Veno-occlusive disease developed in 22 patients with a ratio of 10.8%. The median age of the patients with VOD was 7 years (range, 0.33-17 y). Thirteen were boys (59.1%). Nine patients (40.9%) had severe and 6 (27.2%) had moderate disease. The remaining 7 were diagnosed as mild VOD (Table 2).

Table 1. General Features and Complications of Hematopoietic Stem Cell Transplants

Number of patients	187
Number of HSCT	204
Sex (f/m)	71/116
Median age (y)	7 (min, 0.25-max, 21)
Underlying disorders	
Thalassemia	30
FA	39
ALL	10
Myeloid malignities	53
Immunologic disorders	19
FA	21
HLH	9
Inborn errors of metabolism	10
Osteopetrosis	11
HES	2
HLA disparity (No unrelated donor)	
10/10	186
9/10	17
≤ 8/10	1
Stem cell source	
BM	151
PBSC	50
Cord blood + BM	3
Median nucleated cell count	5.6 × 108 kg-1 (max, 0.75 × 108kg-1-max, 38 × 108/kg-1)
Median neutrophil engraftment (d)	Day + 14
Median platelet engraftment (d)	Day + 21
GVHD prophylaxis	
Mtx + CsA	164
Others (MPZ, MMF)	40
Acute GVHD (n, %)	41 (20.1)
Chronic GVHD (n,%)	21 (10.3)
Hemorrhagic cystitis (n,%)	33 (16.1)
Hepatic VOD (n,%)	22 (10.8)
Mortality in +100 days (%)	28 (13.7)

Abbreviations: AAA, acquired aplastic anemia; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BM, bone marrow; CsA, cyclosporine; FA, Fanconi anemia; GVHD, graft-versus-host disease; HES, hypereosinophilic syndrome; HLA, human leukocyte antigen; HLH, hemophagocytic lymphohistiocytosis; HSCT, hematopoietic stem cell transplant; MDS, myelodysplastic syndrome; MMF, mycophenolate mofetil; MPZ, methylprednisolone; Mtx, methotrexate; PBSC, peripheral blood stem cell; VOD: veno-occlusive disease

Nineteen patients (86.3%) experienced VOD despite the prophylactic regimen. The prophylaxis seemed ineffective for preventing VOD ($P = .657$).

Median onset of VOD was day 11 (range, 0-19 d). Median duration in survivors was 4 days (range, 3-26 d). All patients had hepatomegaly and weight gain above 5%. Ascites was noted in 4 patients (18.1%). Doppler ultrasonography could be performed in 5 patients only (25%) and prominent increase in echogenicity of periportal areas, increase in portal vein diameter, and reverse blood flow in portal vein were demonstrated. The remaining 15 patients were

Table 2. Features of Patients With Veno-Occlusive Disease

No.	Disease	Conditioning Regimen	HLA	Stem Cell Source	Day of VOD Diagnosis	Duration Prophylaxis	Defibrotide	Mortality in 100 Days
1	FA	Fludarabine/cyclophosphamide	10/10	PBSC	Day 11	8 d +	+	-
2	AML	BU-melphalan-cyclophosphamide	10/10	PBSC	Day 8	16 d +	+	+
3	FHL	BU-VP-16-cyclophosphamide	10/10	PBSC	Day 12	8 d +	+	+
4	DOCK8 def	BU-cyclophosphamide	10/10	BM	Day 7	9 d +	+	-
5	MHC Class2	BU-cyclophosphamide	10/10	BM	Day 9	14 d +	+	-
6	AAA	BU-fludarabine	10/10	BM	Day 11	35 d +	+	+
7	Osteopetrosis	BU-cyclophosphamide	10/10	BM	Day 6	9 d +	+	+
8	CML	BU-cyclophosphamide	10/10	PBSC	Day 12	3 d +	+	-
9	AML	BU-cyclophosphamide	10/10	BM	Day 9	12 d +	+	+
10	Thalassemia	BU-cyclophosphamide	10/10	BM	Day 12	3 d +	+	-
11	MDS	BU-melph-cyclophosphamide	10/10	BM	Day 0	3 d +	+	-
12	AML	BU-cyclophosphamide -TBI	10/10	BM	Day 16	26 d +	-	-
13	FA	BU-cyclophosphamide	10/10	PBSC	Day 15	24 d +	-	+
14	Thalassemia	BU-cyclophosphamide	10/10	BM	Day 19	3 d +	-	-
15	FA	Flu-cyclophosphamide	10/10	BM	Day 13	18 d -	-	+
16	Griscelli syn	BU-VP16-cyclophosphamide	10/10	BM	Day 15	12 d +	+	+
17	FA	Flu-cyclophosphamide	10/10	BM	Day 12	7 d +	+	-
18	MDS	BU-cyclophosphamide	10/10	BM	Day 7	4 d -	-	-
19	CML	BU-cyclophosphamide	10/10	BM	Day 11	3 d +	-	-
20	CML	BU-cyclophosphamide	10/10	BM	Day 7	3 d +	-	-
21	FA	BU-cyclophosphamide	10/10	BM	Day 5	14 d -	-	+
22	Thalassemia	BU-cyclophosphamide	10/10	BM	Day 14	14 d -	-	-

Abbreviations: AAA, acquired aplastic anemia; AML, acute myeloid leukemia; BU, busulphan; CML, chronic myeloid leukemia; DOCK8: Dedicator of cytokinesis 8; FA, Fanconi anemia; FHL, familial hemophagocytic lymphohistiocytosis; HLA, human leukocyte antigen; MDS, myelodysplastic syndrome; MHC, major histocompatibility complex; PBSC, peripheral blood stem cell; VOD, veno-occlusive disease

immobile and Doppler ultrasonography could not be performed.

Renal impairment was seen in 13 patients (59.1%). Renal injury (doubling of the basal serum creatinine) and renal failure (increase above 3 times basal creatinine level) developed in 8 patients (36.3%) and 5 patients (22.8%) with VOD.[12]

Analysis of risk factors for veno-occlusive disease

Sex and body mass index were not found as risk factors for VOD (P = .927 and P = .913). Five of the patients with VOD were under 2 years of age, whereas 9 were older than 8 years of age. The age at which the patient had the HSCT was not a risk factor (P = .30).

As the largest group, myeloid malignities constituted 31.8% of the patients with VOD and 25.3% of them without VOD. The diagnosis of the underlying disease did not affect development of VOD (P = .861). In addition, the malignant or nonmalignant nature of the disease did not affect the incidence of VOD (P = .561).

The presence of abnormal liver function tests, abnormal ultrasonographic findings, and serum ferritin levels in the pre-HSCT period were not found to be significant risk factors for developing VOD in our cohort (P = .769, P = .288, and P = .869).

There also was no difference according to donor type and hematopoietic stem cell source. Use of BU in the preparative regimen was not associated with increased risk (P = .40). However, the oral route of BU was a risk factor for developing VOD (P = .05).

The day of neutrophil and platelet engraftment did not differ in patients with or without VOD. The presence of proven bacterial infection and cytomegalovirus polymerase chain reaction positivity were not risk factors (P = 1.00 and P = .72). Antibiotics were analyzed separately. Use of liposomal amphotericin B (P = .06) and vancomycin treatment (P = .04) longer than 10 days were associated with an increased risk of developing VOD. Total parenteral nutrition use for longer than 1 week also was associated with an increased risk of VOD (P = .00).

In the multivariate logistic regression analysis, only TPN was found to be associated with an increased risk of VOD (P = .03, OR=3.99, 95% CI: 1.3-4.3). Risk factors for the development of VOD are shown in Table 3.

Table 3. Significant Risk Factors for Veno-Occlusive Disease

Risk Factors	*P* Values
Oral vs IV busulphan	.05
Total parenteral use longer than a week	.00
Vancomycin use longer than 10 days	.04
Amphotericin B use longer than 10 days*	.06

*Not statistically significant but important

Mortality predictors of hepatic veno-occlusive disease

Nine patients (40.9%) with VOD died within 100 days after HSCT. One died because of a relapse of the disease 19 months after HSCT. The median day of the death was day 34 (range, 26-98 d). No chronic liver

disease developed on follow-up of a median of 52.5 months in survivors (range, 4.3-146 mo). Thirteen patients (59%) also were given defibrotide. Of them, VOD resolved in only 6 patients (46.2%).

Renal impairment (especially above the doubling of basal serum creatinine) and TPN were related to mortality in VOD (P = .00 and P = .02). The median maximum serum total bilirubin level was 61.6 μmol/L (range, 35.9-632.7 μmol/L). Serum bilirubin concentrations above 85.5 μmol/L were significant for mortality (P = .04). On the other hand, median peak alanine amino transferase and aspartate aminotransferase levels greater than 150 IU L-1 were not found to be predictors of mortality.

The mortality rate in 100 days after HSCT was 40.9% of patients with VOD and 9.8% of patients without VOD (P = .001). The overall survival with a median follow-up of 24.2 months was 54.5% for patients with VOD and 83.5% for patients without VOD (P = .00).

Discussion

Hepatic VOD is believed to result from oxidative injury to the vascular endothelium of the hepatic sinusoids, triggered by high-dose chemotherapy and radiotherapy.[9,15,16] The endothelial injury is usually followed by superimposed thrombosis and progressive fibrosis.[17] Its incidence varies significantly among HSCT centers, explained by the presence of miscellaneous risk factors, patient selection, and different definition criteria. In previous studies, the incidence of hepatic VOD showed a range between 5% and 39%.[1,2,4] In our cohort, VOD developed in 22 of 204 patients who received a HSCT, with a rate of 10.8%. The relatively low incidence of VOD in the present study is attributed to the use of HLA fully-matched donors in 91% of transplants and improvements in supportive therapies. Although this was not confirmed by this study, our prophylactic regimen may have played role.

Several risk factors were established for developing hepatic VOD. One factor was a young age. Owing to narrow sinusoid lumens leading to easy occlusion, it was defined as a risk factor for VOD in previous studies but not in our cohort.[2] Meanwhile, several diseases—such as thalassemia major, osteopetrosis, and hemophagocytic lymphohistiocytosis—especially during childhood, have been proposed to increase risk of VOD.[2,5] In our study, the patients were divided in 9 disease groups. Myeloid malignancies (n=53, 25.9%) were the largest group, followed by Fanconi anemia (n=39, 19.1%), and thalassemia major (n=30, 14.7%). However, none of the underlying disease diagnoses was associated with an increased risk of VOD. In thalassemic patients, pre-existing liver iron burden and associated hepatic inflammation/fibrosis traditionally have been thought of as the leading cause of VOD. The median age of 30 patients with thalassemia who underwent first their first HSCT was 5 years in our cohort (range, 1-16 y). In terms of minimizing the risk, optimal chelation before HSCT, younger transplant age, and the use of a prophylactic regimen, might have been useful in present study.

The type and intensity of the conditioning regimen were major factors that influence the incidence and severity of hepatic VOD.[2,18] Veno-occlusive disease increases with increasing TBI and BU dosages and the route of administration of BU.[19] Busulphan is well-known to be associated with hepatic VOD. Although intravenous BU seems to be associated with a lower risk in adults, this difference is not impressive in children.[2] Busulphan, particularly when given orally via enterohepatic circulation, reaches to, and crystallizes in, hepatocytes, and causes depletion of glutathione, leading to oxidative injury that triggers chemical hepatitis and subsequently, VOD. Approximately 70% of our patients and 80% of patients with VOD were prepared for HSCT with BU-containing regimen. Unfortunately, we could not perform the pharmacokinetics studies for BU, and we did not obtain its targeted levels. A BU regimen was not found a risk factor (P = .4), whereas oral BU was associated with high risk of VOD (P = .05). This observation underscores the importance of enterohepatic circulation in the metabolism of BU.

Another well-known and significant risk factor was TPN administration for longer than 1 week. Total parenteral nutrition was administered to patients with intolerability to enteral feeding because of severe mucositis which is unwanted side effect of preparative regimen. Severe mucositis could possibly be associated with severe hepatic endothelial injury. Conversely, it is well-known that hepatobiliary complications may be encountered in pediatric patients on TPN because of different reasons, and its incidence changes between 7.4% and 84%.[20]

Diminished bile acid synthesis or secretion, decreased gall-bladder contraction, bile stasis, decreased gastrointestinal hormones, and motility leading to bacterial overgrowth, fat deposition in the liver, and carnitine deficiency in TPN solutions may be responsible for hepatobiliary complications of TPN.[21] Total parenteral nutrition use has been demonstrated as risk factor for VOD in previous studies.[2,4] Despite cyclic administration of TPN between 8:00 PM and 6:00 AM, it was shown as a risk factor and a mortality predictor for VOD in present study.

Progressive sinusoidal obstruction, reverse blood flow in portal system, and subsequent volume and sodium leakage to the extracellular space underlie the pathophysiology of VOD. The renin-angiotensin-aldosterone system activates, and edema and weight gain develop. Decreased blood flow also leads to deterioration in renal perfusion and tubulointerstitial injury.[22] Renal involvement usually points to moderate-to-severe forms of VOD.[23] Serum creatinine levels are reliable predictors of renal injury.[11] Doubling of the basal creatinine level was associated with increased mortality in the present study.

Considering the pathophysiology of VOD, it has been thought that anticoagulants such as unfractionated or low–molecular-weight heparin may play a role in VOD prophylaxis, but the results of clinical trials has been variable and inconclusive.[7,24-26] Bearman and associates reported that they had to stop continuous heparin infusion given for the purpose of prophylaxis because of a high ratio of moderate-to-severe bleeding (21 of 28 patients).[27] In our center, enoxaparin (0.8 mg/kg/d, once daily, sc), which was thought to be safer than classic heparin, was used as prophylaxis for VOD. Prophylaxis was used in 167 HSCT recipients (81.8%) in the present cohort.

Another aim for using enoxaparin was to prevent catheter-related thrombotic events. Neither severe bleeding nor increased rate of hemorrhagic cystitis was found to be related to enoxaparin. Furthermore, none of the patients experienced thrombosis, including catheter-related thrombosis. Ursodeoxycholic acid and vitamin E also have been used as prophylaxis. Ursodeoxycholic acid, an antioxidant and an antiapoptotic agent, that is a hydrophilic bile acid, previously had been used for hepatic VOD prophylaxis. It has been shown that ursodeoxycholic acid at a dosage of 600 mg/day may be useful in preventing VOD.[28,29] It is not feasible to compare the incidence of VOD in those who received prophylaxis or not, because prophylaxis was not given to those with lower risk of VOD, or to those with increased risk of bleeding because of their medical history. The incidence of VOD in those receiving prophylaxis was 11.3% (19/167), whereas it was 8.1% in the other group (3/37). Regarding the low general incidence of VOD (10.8%) in the whole cohort, we believe that the prophylactic regimen may have been a contributory factor, particularly when a high-risk population is considered.

Defibrotide, which is a profibrinolytic, anti-inflammatory, and antithrombotic agent, in previous studies, has been shown effective in treating VOD.[5,30] Unfortunately, until recently, it has not been widely available in many countries including Turkey. Only 13 patients were treated with defibrotide, and favorable response were obtained in 6 patients (46.2%). Nonetheless, it has been reported that management with defibrotide or its prophylactic use might be promising.

There are some limitations to our study. The major one in this retrospective evaluation is the lack of patient randomization to VOD prophylaxis. Second, the cross-sectional nature allows us to reveal only associations rather than causations. Further studies are required to confirm our results.

In conclusion, hepatic VOD has been troublesome after HSCT, particularly in the presence of multiple risk factors. Decreasing the incidence of VOD by reducing its risk factors could improve transplant outcomes. Careful and close monitoring is important, especially in high-risk patients, so that early suspicion and aggressive treatment of VOD can be instituted promptly.

Our study suggests that using a VOD prophylactic regimen consisting of enoxaparin, ursodeoxycholic acid, and vitamin E may have played a role in the fairly low incidence of VOD in a pediatric population with high-risk features. New prophylactic agents that prevent oxidative damage, the initial step of VOD before thrombotic occlusion, and the use of defibrotide as prophylaxis are being studied.

References

1. Carreras E. Veno-occlusive disease of the liver after hemopoietic cell transplantation. *Eur J Haematol*. 2000;64(5):281-291.

2. Cheuk DK, Wang P, Lee TL, et al. Risk factors and mortality predictors of hepatic veno-occlusive disease after pediatric hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2007;40(10):935-944.
3. McKoy JM, Angelotta C, Bennett CL, et al. Gemtuzumab ozogamicin-associated sinusoidal obstructive syndrome (SOS): an overview from the research on adverse drug events and reports (RADAR) project. *Leuk Res*. 2007;31(5):599-604.
4. Reiss U, Cowan M, McMillan A, Horn B. Hepatic venoocclusive disease in blood and bone marrow transplantation in children and young adults: incidence, risk factors, and outcome in a cohort of 241 patients. *J Pediatr Hematol Oncol*. 2002;24(9):746-750.
5. Corbacioglu S, Hönig M, Lahr G, et al. Stem cell transplantation in children with infantile osteopetrosis is associated with a high incidence of VOD, which could be prevented with defibrotide. *Bone Marrow Transplant*. 2006;38(8):547-553.
6. Carreras E, Bertz H, Arcese W, et al. Incidence and outcome of hepatic veno-occlusive disease after blood or marrow transplantation: a prospective cohort study of the European Group for Blood and Marrow Transplantation. European Group for Blood and Marrow Transplantation Chronic Leukemia Working Party. *Blood*. 1998;92(10):3599-3604.
7. Attal M, Huguet F, Rubie H, et al. Prevention of hepatic veno-occlusive disease after bone marrow transplantation by continuous infusion of low-dose heparin: a prospective, randomized trial. *Blood*. 1992;79(11):2834-2840.
8. McDonald GB, Hinds MS, Fisher LD, et al. Veno-occlusive disease of the liver and multiorgan failure after bone marrow transplantation: a cohort study of 355 patients. *Ann Intern Med*. 1993;118(4):255-267.
9. Jones RJ, Lee KS, Beschorner WE, et al. Venoocclusive disease of the liver following bone marrow transplantation. *Transplantation*. 1987;44(6):778-783.
10. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation*. 1974;18(4):295-304.
11. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P; Acute Dialysis Quality Initiative workgroup. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care*. 2004;8(4):R204-R212.
12. Hassan Z, Remberger M, Svenberg P, et al. Hemorrhagic cystitis: a retrospective single-center survey. *Clin Transplant*. 2007;21(5):659-667.
13. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, et al. CDC growth charts: United States. *Adv Data*. 2000;314:1-27.
14. Nguyen L, Fuller D, Lennon S, Leger F, Puozzo C. I.V. busulfan in pediatrics: a novel dosing to improve safety/efficacy for hematopoietic progenitor cell transplantation recipients. *Bone Marrow Transplant*. 2004;33(10):979-987.
15. Shulman HM, McDonald GB, Matthews D, et al. An analysis of hepatic venocclusive disease and centrilobular hepatic degeneration following bone marrow transplantation. *Gastroenterology*. 1980;79(6):1178-1191.
16. Sabuncuoğlu S, Kuşkonmaz B, Uckun Çetinkaya D, Ozgüneş H. Evaluation of oxidative and antioxidative parameters in pediatric hematopoietic SCT patients. *Bone Marrow Transplant*. 2012;47(5):651-656. doi: 10.1038/bmt.2011.145.
17. Shulman HM, Fisher LB, Schoch HG, Henne KW, McDonald GB. Veno-occlusive disease of the liver after marrow transplantation: histological correlates of clinical signs and symptoms. *Hepatology*. 1994;19(5):1171-1181.
18. Styler MJ, Crilley P, Biggs J, et al. Hepatic dysfunction following busulfan and cyclophosphamide myeloablation: a retrospective, multicenter analysis. *Bone Marrow Transplant*. 1996;18(1):171-176.
19. Girinsky T, Benhamou E, Bourhis JH, et al. Prospective randomized comparison of single-dose versus hyperfractionated total-body irradiation in patients with hematologic malignancies. *J Clin Oncol*. 2000;18(5):981-986.
20. Kelly DA. Liver complications of pediatric parenteral nutrition--epidemiology. *Nutrition*. 1998;14(1):153-157.
21. Kumpf VJ. Parenteral nutrition-associated liver disease in adult and pediatric patients. *Nutr Clin Pract*. 2006;21(3):279-290.
22. Hingorani SR, Guthrie K, Batchelder A, et al. Acute renal failure after myeloablative hematopoietic cell transplant: incidence and risk factors. *Kidney Int*. 2005;67(1):272-277.
23. Hasper D, Jörres A. New insights into the management of hepato-renal syndrome. *Liver Int*. 2011;31(suppl 3):27-30. doi: 10.1111/j.1478-3231.2011.02586.x.
24. Or R, Nagler A, Shpilberg O, et al. Low molecular weight heparin for the prevention of veno-occlusive disease of the liver in bone marrow transplantation patients. *Transplantation*. 1996;61(7):1067-1071.
25. Forrest DL, Thompson K, Dorcas VG, Couban SH, Pierce R. Low molecular weight heparin for the prevention of hepatic veno-occlusive disease (VOD) after hematopoietic stem cell transplantation: a prospective phase II study. *Bone Marrow Transplant*. 2003;31(12):1143-1149.
26. Simon M, Hahn T, Ford LA, et al. Retrospective multivariate analysis of hepatic veno-occlusive disease after blood or marrow transplantation: possible beneficial use of low molecular weight heparin. *Bone Marrow Transplant*. 2001;27(6):627-633.
27. Bearman SI, Hinds MS, Wolford JL, et al. A pilot study of continuous infusion heparin for the prevention of hepatic veno-occlusive disease after bone marrow transplantation. *Bone Marrow Transplant*. 1990;5(6):407-411.
28. Ohashi K, Tanabe J, Watanabe R, et al. The Japanese multicenter open randomized trial of ursodeoxycholic acid prophylaxis for hepatic veno-occlusive disease after stem cell transplantation. *Am J Hematol*. 2000;64(1):32-38.
29. Essell JH, Schroeder MT, Harman GS, et al. Ursodiol prophylaxis against hepatic complications of allogeneic bone marrow transplantation. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med*. 1998;128(12 Pt 1):975-981.
30. Cappelli B, Chiesa R, Evangelio C, et al. Absence of VOD in paediatric thalassaemic HSCT recipients using defibrotide prophylaxis and intravenous Busulphan. *Br J Haematol*. 2009;147(4):554-560. doi: 10.1111/j.1365-2141.2009.07871.x.