



A prospective survey on incidence, risk factors and therapy of hepatic veno-occlusive disease in children after hematopoietic stem cell transplantation

Simone Cesaro
Marta Pillon
Enrico Talenti
Tiziana Toffolutti
Elisabetta Calore
Gloria Tridello
Liliana Strugo
Roberta Destro
Maria Vittoria Gazzola
Stefania Varotto
Gabriella Errigo
Modesto Carli
Luigi Zanesco
Chiara Messina

Background and Objectives. Veno-occlusive disease (VOD) is one of the most frequent complications after stem cell transplantation. We conducted a prospective survey of 244 hematopoietic stem cell transplants in children to determine the incidence of VOD, its main risk factors, treatment and effect on the transplant.

Design and Methods. Two hundred and forty-four hematopoietic stem cell transplants (HSCT) performed in 220 pediatric patients from 1993 to 2003 were evaluated. The series included 127 males and 93 females with a median age of 6.7 years at the time of transplantation.

Results. VOD was diagnosed following 26 of the 244 transplants (cumulative incidence 11%), but a higher incidence was found in patients with at least one known risk factor for VOD (cumulative incidence 20%). In multivariate analysis, risk factors for VOD were age < 6.7 years; type of VOD prophylaxis, and busulphan-containing conditioning regimens. Routine treatment of VOD was based on supportive care and, starting from 1999, defibrotide was used. All patients were monitored with daily Doppler ultrasound (US) for early diagnosis of inversion of portal blood flow. Twelve patients developed inversion of portal flow (9 had severe VOD; 3 had moderate VOD) and were promptly started on fibrinolytic and anticoagulant therapy with heparin and recombinant tissue plasminogen activator (rt-PA). Hepatic flow reverted to normal in all 12 patients; only 4 patients ultimately developed multiorgan failure and died. The transplant-related-mortality (TRM) rate in patients with or without inversion of portal flow was 33% vs 7%, ($p=0.1$). The TRM in patients with or without VOD was 19% vs 8% ($p=0.001$).

Interpretations and Conclusions. This study showed that younger age, type of VOD prophylaxis, and busulphan-based conditioning regimens are independent risk factors for VOD. Inversion of portal flow was found in 9 of 10 patients with severe VOD. Doppler US monitoring may be helpful in early identification of the patients with VOD-induced inversion of portal flow who might benefit from therapy with heparin and rt-PA.

Key words: veno-occlusive disease, pediatric hematopoietic stem cell transplantation fibrinolytic therapy, ultrasound Doppler.

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From the Clinic of Pediatric Hematology Oncology, Department of Pediatrics, University of Padova, Italy (SC, MP, EC, GT, LS, RD, MVG, SV, GE, MC, LZ, CM); Unit of Pediatric Radiology, Institute of Radiology, University of Padova, Italy (ET, TT).

Correspondence:
Simone Cesaro, M.D., Clinic of Pediatric Hematology Oncology Department of Pediatrics, University of Padova, Via Giustiniani 3, 35128 Padova, Italy.
E-mail: simone.cesaro @unipd.it

Veno-occlusive disease (VOD) usually occurs in the first 3-4 weeks after hematopoietic stem cell transplantation (HSCT) as a result of endothelial and hepatocyte damage caused by the chemo-radiotherapy of the conditioning regimen.¹⁻⁴ Recently, it has been proposed to rename this form of liver disease as sinusoidal obstruction syndrome because the sinusoidal cells are the primary target of the toxic injury.⁵

The diagnosis of VOD relies on a combination of clinical signs such as hepatomegaly, right upper quadrant pain, ascites, weight gain and hyperbilirubinemia.^{4,6-9} The reported incidence of VOD is 5-40% in children^{6,10} and similar or higher rates have been reported in adults.^{6-8,11,12} Known risk factors for VOD are pre-existing liver disease such as viral hepatitis, hepatic fibrosis or cirrhosis,^{3,13,14} second myeloablative transplant or

myeloablative allogeneic transplantation beyond the second relapse,^{12,15,16} prior abdominal irradiation,^{3,13} previous therapy with gemtuzumab ozogamicin,¹⁷ busulphan- cyclophosphamide and/or melphalan-containing regimens,^{1,18,19} and some pediatric diseases such as osteopetrosis, hemophagocytic lymphohistiocytosis, adrenoleukodystrophy, and neuroblastoma.¹⁸⁻²¹ The mortality rate varies according to the severity of the clinical presentation, multi-organ failure being the cause of death in more than 90% of patients with severe VOD.^{4,15,17} Various drugs such as prostaglandin E1, ursodiol, pentoxifylline, heparin and low molecular weight heparin (LMWH), have been used in different trials for the prevention of VOD but their efficacy has not been adequately confirmed or the results are still preliminary.²²⁻³¹

The therapeutic approaches for estab-

lished VOD are based on supportive care and on the use of drugs with anticoagulant or fibrinolytic activity, i.e. recombinant tissue plasminogen activator (rt-PA), heparin or LMWH and, more recently, defibrinolytic.³²⁻³⁶ Their use is suggested by the fact that VOD is associated with the obliteration of hepatic venules or sinusoids by deposits of fibrin, fibrinogen, factor VIII and cellular debris which may lead to hepatocellular necrosis and widespread fibrotic replacement of normal liver.^{2,5} Moreover, several abnormalities of hemostasis, which give rise to a procoagulant and hypofibrinolytic state, have been described in VOD patients.^{37,38} A major drawback in the use of rt-PA and heparin is the high risk of life-threatening bleeding due to thrombocytopenia and refractiveness to platelet transfusions due to portal hypertension and splenic sequestration.^{17,32} Another major dilemma is the right time to start the therapy with rt-PA. The possible greater efficacy if used early, before multi-organ failure is established, needs to be balanced against the low mortality rate of mild or moderate VOD.^{15,31,32}

We report a prospective study on the incidence, risk factors and therapy of VOD in pediatric patients who underwent HSCT. The effect of VOD on the main transplant outcomes were also assessed. Given the pathogenesis of VOD and the association between the alteration of portal hemodynamic parameters and severity of VOD, daily monitoring of hepatic portal flow was used in this study as the criterion on which to select the patients eligible for the more risky therapy with heparin and rt-PA.^{2,5,39-42}

Design and Methods

Patients and transplant procedures

From January 1993 to January 2003, 244 consecutive HSCT were performed at our Center in 220 pediatric patients (127 males, 93 females; median age at HSCT 6.7 years, range 0.3–18). Of these 244 transplants, 119 were autologous and 125 were allogeneic. One hundred and ninety-seven patients underwent one HSCT, 22 patients two transplants and 1 patient three transplants. In 99 HSCT (41%), one or more known risk factors for VOD were present at HSCT and their distribution was as follows: chronic viral hepatitis C, 4 (2%); second myeloablative HSCT, 10 (4%); myeloablative allogeneic HSCT beyond the second relapse, 16 (7%); diagnosis of neuroblastoma, osteopetrosis, adrenoleucodystrophy, or hemophagocytic lymphohistiocytosis, 40 (16%); and a busulphan-based conditioning regimen associated with cyclophosphamide and/or melphalan, 57 (23%). All patients had a Lansky or Karnofsky performance score $\geq 90\%$ and liver function tests (transaminase, bilirubin, and serum albumin) within the normal

range for age. Table 1 shows the main demographic characteristics, origin of stem cells, and type of conditioning regimen used. The chemotherapy-based conditioning regimens were divided into four groups (busulphan, melphalan, cyclophosphamide and carboplatin) that were all considered myeloablative according to the dose used.

Two hundred and nine HSCT were performed for a neoplastic disease. According to pre-HSCT remission status and type of neoplastic disease, 101 (48%) of the transplants, including those for acute lymphoblastic leukemia (ALL) in first or second complete remission (CR), acute myeloid leukemia (AML) in first CR, and chronic myeloid leukemia (CML) in first chronic phase, were considered as standard-risk; the other 108 transplants (52%) were considered high-risk because of more advanced acute or chronic leukemia, myelodysplastic syndrome, solid tumor, non-Hodgkin's lymphoma, or hemophagocytic lymphohistiocytosis.

All the patients were nursed in a HEPA-filtered room and standard measures were adopted to prevent infectious complications. Phenytoin was given at doses adjusted to maintain therapeutic levels as prophylaxis of busulphan-related seizures.

Neutrophil and platelet engraftment was defined as the first of three consecutive days on which the neutrophil and platelet counts exceeded $0.5 \times 10^9/L$ and $50 \times 10^9/L$, respectively. Early post-HSCT toxicity was classified according to Bearman's criteria.⁴³

Diagnosis and classification of VOD

The diagnosis of VOD was based on Jones's criteria: jaundice (bilirubin $> 34.2 \mu M$ [2 mg/dL]) and at least two of the following signs, hepatomegaly and/or right upper quadrant pain, and $> 5\%$ weight gain with or without ascites.^{8,36} The severity of VOD was defined according to established criteria: mild for clinically manifested VOD that resolved without intervention; moderate for VOD that required treatment but resolved completely; and severe for VOD that caused death or progressed to multi-organ failure. Multi-organ failure was defined as either an oxygen requirement with an oxygen saturation of $< 90\%$ on room air and/or ventilator dependence; renal insufficiency (doubling of baseline creatinine level and/or dependence on dialysis); and/or encephalopathy.^{3,21,31,36} In order to be VOD-related, multiorgan failure had to be diagnosed within 28 days of the diagnosis of VOD.

Prophylaxis of graft-versus-host disease

In patients undergoing allogeneic HSCT prophylaxis of graft-versus-host disease (GVHD) was cyclosporine or cyclosporine, short-term methotrexate and rabbit antithymocyte serum according to whether the source of the stem cells was a matched sibling donor or a mismatched family or unrelated donor, respectively.

Standard criteria were used to define acute and chronic GVHD.^{44,45}

Prophylaxis and management of VOD

The prophylaxis of VOD changed over the years. From 1993 to 1994 prostaglandin E1 and pentoxifylline were used in 31 and 7 HSCT, respectively. Because of the inconsistency of data regarding their prophylactic effect, no prophylaxis at all was used from 1995 to 1996 during which time 35 HSCT were performed. From 1997, VOD prophylaxis with sodium heparin, 100 IU/kg, administered intravenously over 24 hours, was started for all the remaining 171 HSCT.

Once VOD was clinically diagnosed, standard supportive care measures were adopted, such as restriction of daily sodium and fluid intake, diuretics, and hematologic support. From 1999, all patients with a clinical diagnosis of VOD were promptly treated with defibrotide, 25 mg/kg/day, in 4 doses.³⁴⁻³⁶ All patients were monitored daily with portal Doppler US. Doppler US was performed using a mobile Hitachi EUB 450 or Toshiba Ecocee device equipped with a 3.5-MHz probe. Each examination lasted approximately 20-30 minutes and was performed by an expert radiologist (E.T. or T.T.) who assessed the following signs: hepatomegaly, splenomegaly, ascites, thickening of the gallbladder wall, and the decrease of portal flow velocity (normal value, > 10 cm/sec). The presence of all these signs was used to confirm the diagnosis of VOD, but only the detection of inversion of hepatic portal flow was used to start therapy with rt-PA, 0.2 mg/kg/day intravenous infusion over 4 hours, and heparin, 150 U/kg/day intravenous infusion over 24 hours. If the patient was already on defibrotide, this drug was withdrawn. Therapy with rt-PA and heparin continued until normal portal flow was restored and pretreatment bilirubin level decreased by at least 50% or death or life-threatening hemorrhage occurred.³²

Statistical analysis

The data were analyzed as at 31st January, 2004. The patients' characteristics were compared using the χ^2 or Fisher's exact test (as appropriate) in the case of discrete variables, and the *t* test or Mann-Whitney test, in the case of continuous variables. The end-points of the study were the cumulative incidence of VOD, transplant-related mortality; overall survival and event-free survival. Cumulative incidence curves were used in a competing-risk setting, with death from relapse treated as a competing event to calculate the probability of VOD.⁴⁶

The time to VOD was calculated from the date of HSCT to the date of clinical diagnosis. The duration of VOD was defined as the interval between the clinical diagnosis and the complete resolution of symptoms with reduction of bilirubin by at least 50% or death.

Table 1. Main demographic and transplant data of the patients.

Patients	220
Age (years)	
Median, range	6.7 (range 0.3-18)
Sex	M 127, F 93
Number of HSCT	244
Autologous HSCT	119
Allogeneic HSCT	125
FD	45
UD	59
CB	9
FM/haplo	12
Conditioning regimens	
Based on total body irradiation	95 (39%)
1200 cGy+TT+Cy	58
1200 cGy+L-PHAM	13
1200 cGy+VP16	6
1200 cGy+TT+Flu	4
1200 cGy+VCR+Cy	2
1200 cGy+other*	3
1440 cGy+Ara-C	9
Based on busulphan	63 (26%)
Bu+L-PHAM	18
Bu+L-PHAM+TT	4
Bu+Cy+TT	8
Bu+Cy+VP16	4
Bu+TT+VP16	5
Bu+Cy+Flu	1
Bu+TT+Flu	1
Bu+Cy	4
Bu+Cy+L-PHAM	18
Based on melphalan	51 (21%)
L-PHAM	7
L-PHAM+Cy	13
L-PHAM+Flu	5
L-PHAM+TT	9
L-PHAM+Carboplatin	4
L-PHAM+Flu+VP16	2
L-PHAM+TT+Flu	4
BEAM (L-PHAM+ Ara-C+ VP16+ BCNU)	7
Based on cyclophosphamide	13 (5%)
Cy	8
Cy+TT	1
Cy+TT+VP16	2
Cy+Flu	2
Based on carboplatin	20 (8%)
Carboplatin	2
Carboplatin+VP16	7
Carboplatin+VP16+lfo	11
Other (VP16 +TT + Flu)	2 (1%)

HSCT: hematopoietic stem cell transplantation; FD: family (sibling or matched parent) donor; UD: unrelated donor; CB: cord blood; FM/haplo: family mismatched or haploidentical donor; TBI: total body irradiation; cGy: centigray; Cy: cyclophosphamide, 120-200 mg/kg; L-PHAM: melphalan, 140-200 mg/m²; VP16: etoposide 1000-1800 mg/m²; TT: thiotepa, 10 mg/kg; Ara-C: cytarabine, 24 g/m²; Bu: busulphan, 16 mg/kg; Flu: fludarabine, 120-150 mg/m²; carboplatin 1500-1800 mg/m²; lfo: ifosfamide, 12 g/m²; BEAM: BCNU, 300 mg/m², etoposide, 800 mg/m², cytarabine, 1600 mg/m², melphalan, 140 mg/m².

Chronic GVHD was assessed only in patients who underwent allogeneic-HSCT and survived at least 100 days after the transplant. Overall survival was calculated from the date of HSCT to death from any cause or

to the date of the latest follow-up. Event-free survival was calculated from the date of HSCT to the date of the first event (death from any cause, relapse or second malignancy) or to the date of the latest follow-up. Transplant-related mortality for the patients with or without VOD was estimated by the cumulative incidence method, death from relapse being the competing event. The groups were compared with Gray's k -sample test.⁴⁷ Overall and event-free survival were estimated by the Kaplan-Meier method. The differences between patients with or without VOD were calculated by log-rank test. The following variables were included in the analysis of prognostic factors for VOD: gender (male vs female); median age at HSCT; type of HSCT (autologous vs allogeneic); source of stem cells (bone marrow vs other); type of allogeneic donor (related vs unrelated); underlying disease (malignant vs non-malignant); diagnosis (leukemia/lymphoma vs solid tumor vs other); pre-HSCT status of malignant disease (standard-risk vs high-risk); presence of at least one known risk factor for VOD; type of conditioning regimen (total body irradiation-based vs busulphan-based vs other; busulphan-based vs other; cyclophosphamide vs other); type of VOD prophylaxis (heparin vs other); and severity of acute GVHD (0-I vs II-IV). The variables proving significant at univariate analysis were included in a multivariate Cox regression analysis.^{48,49} Co-variables found to be significant at a p -value < 0.1 were subsequently introduced in the stepwise procedure. All reported p values are two-sided, and a significance level of $\alpha = 0.05$ was used. The statistical analysis was performed using the SAS statistical program (SAS Institute, Cary, NC, USA), NCSS (Number Cruncher Statistical Systems, Kaysville, Utah, USA) and R, ver1.9 (available at <http://www.r-project.org> provided by The R Foundation for Statistical Computing, Vienna, Austria).

Results

Incidence and characteristics of VOD

VOD was diagnosed following 26 of 244 transplants at a median time of 10.5 days (range, 3-54) after HSCT. The cumulative incidence of VOD was 11%, confidence interval (C.I.) 8-16, and 20%, C.I. 14-30, in the total series and in patients with at least one recognized risk factor, respectively. Considering the age at HSCT, the patients who developed VOD were younger than those who did not (3.3 years [range 0.3-18] vs 7.8 years [range 0.5-18], $p=0.002$). VOD occurred in 18 of 119 autologous transplants procedures (15%) and 8 of 125 allogeneic grafts (6%), ($p=0.03$). No difference was found in the median duration of VOD following autologous or allogeneic transplantation (13 days [range, 8-31] vs 18 days [range, 6-38], $p=0.2$). The incidence of VOD was 6% (11/171) in the group that received

Table 2. Main clinical and biochemical characteristics of the 26 patients who developed VOD.

Hepatomegaly and/or RUQ pain	26/26
Ascites and/or increased BW >5%	19/26
Median value of the bilirubin peak level, $\mu\text{mol/L}$, (range)	57 (38-277)
Median value of the ALT peak level, IU/L (range)	500 (338-2430)
Severity of VOD	Mild, 6; moderate, 10; severe, 10
Hepatic portal flow inversion (distribution according to VOD severity)	12/26 (mild, 0/6; moderate, 3/10; severe, 9/10)
Death related to MOF-VOD	4/26*
Death from any cause	12/26 (PD, 5; VOD-MOF, 4; PI, 2; 2 nd tumor, 1)

RUQ: right upper quadrant pain; BW: body weight; MOF: multi-organ failure; VOD: veno-occlusive disease; *: $p=0.02$ for the incidence of inversion of portal flow in severe vs moderate VOD; *all these four patients had severe VOD; PD: progression of disease; IP: interstitial pneumonia.

heparin prophylaxis versus 21% (15/73) in the group given other types of prophylaxis (pentoxifylline, prostaglandin E1) or no prophylaxis at all ($p=0.001$). No difference was found among patients with or without VOD as regards the total number of nucleated cells (bone marrow stem cell source) or CD34⁺ cells (peripheral blood stem cell source) infused, and neutrophil engraftment (*data not shown*). Platelet engraftment occurred later in patients with VOD (median 43 days; range 17-165), than in patients without VOD (median 30 days; range 11-405) ($p=0.01$).

Table 2 shows the main clinical characteristics of the VOD patients. All patients had painful hepatomegaly. Ascites and fluid retention were found in 19/26 (73%) patients with a median increase of body weight of 10% (range, 5-30). VOD resulted mild, moderate and severe in 6, 10 and 10 patients, respectively. Inversion of portal flow was found in 12/26 (46%) patients at a median time of 5 days (range 0-5) after clinical diagnosis of VOD; four of these patients progressed to develop multi-organ failure, irrespective of any treatment. The occurrence of inversion of portal flow was significantly associated with the severity of VOD, the incidence being 0, 30% and 90% in mild, moderate and severe VOD, respectively ($p=0.02$ for the incidence of inversion of portal flow in severe vs moderate VOD).

Treatment and outcome of VOD

All patients received appropriate supportive care while the six patients who were diagnosed after 1999

Table 3. Results of univariate and multivariate analyses of risk factors for VOD.

Prognostic factor	HSCT	VOD episodes	Univariate P value	Multivariate P value	Hazard Ratio (95% CI)
Gender					
Male	140	14/140 (10%)	n.s.		
Female	104	12/104 (12%)			
Age					
< 6.7 years	123	21/123 (17%)	0.001	0.001	9.5 (2.6-34.7)
≥ 6.7 years	121	5/121 (4%)			
Source					
Autologous HSCT	119	18/119 (15%)	0.027	n.s.	
Others	125	8/125 (6%)			
Stem cell source					
Bone marrow	153	19/153 (12%)	0.23		
Other	91	7/91 (8%)			
Type of donor					
Related	60	5/60 (8%)	n.s.		
Unrelated	65	3/65 (5%)			
Underlying disease					
Malignant	209	23/209 (11%)	0.7		
Non-malignant	35	3/35 (9%)			
Diagnosis					
Leukemia/Lymphoma	134	12/134 (9%)	0.4		
Solid tumor	75	11/75 (15%)			
Non-malignant	35	3/35 (9%)			
Presence of at least one known risk factor for VOD					
Yes	87	17/87 (20%)	0.001	n.s.	
No	157	9/157 (6%)			
Pre-HSCT status of malignant disease					
Standard risk	101	9/101 (9%)	n.s.		
High risk	108	14/108 (12%)			
Conditioning regimen					
Busulphan	63	16/63 (25%)	< 0.001	< 0.001	8.1 (2.8-23.7)
Others	181	10/181 (13%)			
Conditioning regimen					
Cyclophosphamide	122	12 (10%)	0.8		
Others	122	14 (11%)			
Conditioning regimen					
Total body irradiation	95	5/95 (5%)	<0.001		
Busulphan	63	16/63 (25%)			
Others	86	5/86 (6%)			
Prophylaxis					
Others	73	15/73 (21%)	0.001	< 0.001	7.6 (2.8-20.6)
Heparin	171	11/171(6%)			
Acute GVHD					
0 - I	58	3/58 (5%)	0.7		
II-IV	67	5/67 (7%)			

HSCT: hematopoietic stem cell transplantation; VOD veno-occlusive disease; GVHD: graft-versus-host disease.

were treated promptly with defibratide. VOD was mild (6 patients), moderate (7 patients) and severe (1 patient) in the 14 of 26 patients (54%) who did not develop inversion of portal flow, and resolved after a median of 14 days (range, 6-30). Two of these patients had been treated with defibratide. The other 12 patients (46%) were started on heparin and r-tPA after

the occurrence of inversion of portal flow; four of them were already receiving defibratide. Portal flow reverted in all patients in a median time of 4 days (range 2-25), but four patients eventually died of multi-organ failure. Two patients had moderate upper gastro-intestinal bleeding that occurred within one week after the withdrawal of heparin and r-TPA.

Risk factors for VOD

The role of several factors in the development of VOD is shown in Table 3. The factors that reached significance in univariate analysis were age <6.7 years-old ($p=0.001$); autologous-HSCT, ($p=0.027$); the presence of at least one known risk factor for VOD, ($p=0.001$); a busulphan-based conditioning regimen, ($p<0.001$); and type of VOD prophylaxis ($p=0.001$). According to age, the risk of VOD was significantly higher in children aged 0-4 years than in those aged 5-9 or older than 10 years (19% vs. 8%, vs. 3%, $p=0.002$). Patients with neuroblastoma had a significantly higher risk of VOD than did patients with other diseases (29% vs. 8%; $p=0.001$).

In a multivariate analysis the factors that were significantly associated with VOD were: age <6.7 years; VOD prophylaxis without heparin, and busulphan-containing regimens, the hazard ratios being 9.5, 7.6, and 8.1, respectively.

Transplant outcome

Five of 26 patients with VOD (19%, C.I. 9-42) died within 100 days after HSCT, the cause of death being VOD-related multi-organ failure in four patients, and interstitial pneumonia in one. The transplant-related mortality rates on day 100 of patients with or without inversion of portal flow were 33% (15-74) vs 7% (C.I.1-47) ($p=0.1$), whereas those for patients with or without VOD were 19% (C.I. 9-42) vs. 8% (C.I. 5-13) ($p=0.001$). Figure 1 shows transplant-related mortality, overall survival and event-free survival according to the presence or absence of VOD: the transplant-related mortality rates were 23% (C.I.-12-47) vs 13% (C.I. 9-19) ($p=0.1$); the overall survival rates were 50% (C.I. 29-70) vs 53% (44-62) ($p=0.2$); and event-free survival rates were 40% (21-60) vs 45% (36-54) ($p=0.3$).

Discussion

VOD is caused by hepatocyte and sinusoidal endothelial vessel damage that can occur early after HSCT, and, in the severe form, may lead to liver failure, hepato-renal syndrome, portal hypertension and, eventually, death from multi-organ failure. In this study, the cumulative incidence of VOD was 11%. This figure, though not negligible, is less than in other previous reports, and may be explained in part by the fact that all patients had a good performance status and normal liver function tests prior to transplantation.¹¹ Considering the patients with at least one known predisposing factor, the incidence of VOD almost doubled, being 20%. These data suggest that patients at high-risk for VOD can be identified prior to transplantation and this group could benefit from preventive strategies.

The multivariate analysis confirmed that busulphan

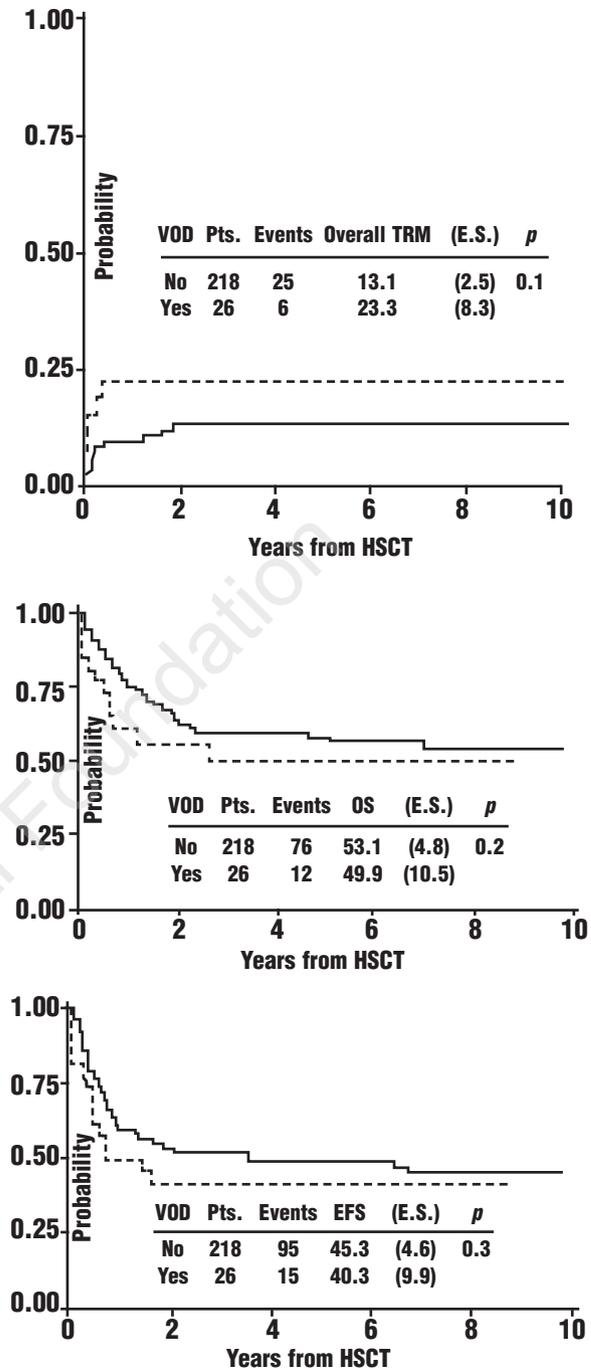


Figure 1. Overall transplant-related mortality (TRM), overall survival (OS) and event-free survival (EFS) are shown. No difference was found in the main outcomes of the patients with or without VOD.

is an independent risk factor for VOD^{6-8,11,12,15,50,51} but, interestingly, so is younger age. Although a higher dose of busulphan is sometimes recommended in patients less than 2 years old, because of rapid clearance, the standard dose of 16 mg/kg was used in all our patients treated with busulphan, i.e. 1 mg/kg every 6 hours for 4 days, irrespective of age. The higher incidence of

VOD which was observed in the younger patients is probably related to the occurrence of some VOD-predisposing pediatric diseases in the first years of life, especially neuroblastoma, rather than a greater age-specific variability of oral busulphan bio-availability or drug-to-drug interaction.⁵¹⁻⁵² The modulation of the total dose of busulphan by determining the area under the curve or the use of intravenous busulphan to eliminate the toxicity due to the *first pass* effect are possible options to overcome this undesired negative effect of busulphan.^{53,54} Indeed, a decreased incidence of VOD has been described with intravenous busulphan both in adults and in pediatric patients.^{55,56} Recently, the adoption of reduced intensity conditioning regimens and the use of drugs with less hepatic toxicity such as treosulphan and fludarabine, have been associated with a lower incidence of VOD.⁵⁷⁻⁵⁹

Given the high morbidity and mortality associated with VOD several prophylactic drugs have been adopted during the last two decades, though definite results on efficacy are still awaited. Our need for an effective prophylaxis was influenced by the different experiences published over the years. In our analysis, heparin prophylaxis had a protective effect on VOD incidence but these data, though consistent with other reports,^{29,30} need to be validated by a prospective controlled study. Recently, ursodiol, a hydrophilic, non-hepatotoxic bile salt, showed efficacy in reducing VOD in a randomized placebo-controlled trial but this finding was not confirmed in two more recent studies.^{23,24,28} Defibrotide is potentially one of the most valuable options available for prophylaxis of VOD but data on its efficacy in the prophylactic setting are still lacking.⁶¹

The development of VOD has been associated with abnormalities in the coagulation cascade such as the onset of a procoagulant and hypofibrinolytic status. In view of this, the use of fibrinolytic and anticoagulant therapy for VOD may be indicated but there is considerable risk of subsequent hemorrhage because of the patient's thrombocytopenia and high platelet transfusion requirement. Other authors have previously reported that rt-PA and heparin, though potentially useful, are associated with significant iatrogenic morbidity and mortality.³¹⁻³³ In this study, we observed an association between severe VOD and inversion of portal flow. The use of daily monitoring of hepatic portal flow by Doppler US allowed treatment with heparin and rt-PA to be administered only to those patients with portal hypertension, avoiding any hemorrhagic risk in patients who did not develop severe VOD or severe hemodynamic abnormalities of portal circulation. Portal flow reverted to normal in a median time of 4 days in all patients who received treatment with heparin and rt-PA. Only two episodes of non-fatal gastrointestinal bleeding, possibly-related to rt-PA, were reported. Four of 12 treated patients (33%) developed

severe renal and lung dysfunction and ultimately died. Interestingly, our data did not show any statistically significant difference in overall transplant-related mortality, overall survival and event-free survival between patients with or without VOD. We did find a statistically significant association between the inversion of portal flow and severity of VOD ($p=0.02$); nevertheless, the patients with inversion of portal flow did not show a higher 100-day transplant-related mortality. This result needs to be confirmed by a larger prospective study because it could have been influenced by the low number of patients with severe VOD or by the improvement of supportive care in recent years. Considering that only four of ten patients with severe VOD died of multi-organ failure, we may speculate that the early intervention with heparin and rt-PA in case of inversion of portal flow may have contributed to reducing mortality from VOD.

Although Doppler US studies were not found to be more sensitive than clinical criteria in the early diagnosis of VOD,³³ our data showed that this examination is useful in early detection of the patients with VOD-induced portal hypertension and that it might be helpful in selecting those patients eligible for a higher risk treatment.

In recent years, several authors have reported that defibrotide is an effective treatment for VOD with a good safety profile.^{34-36,61} Richardson *et al.* found response rates of 42% and 32% in patients with severe VOD and multi-organ failure, respectively. In an evaluation of compassionate use in Europe, Chopra *et al.* reported a higher efficacy of defibrotide in patients with moderate VOD, suggesting that early administration may be helpful.³⁵ According to the time of these reports, defibrotide was introduced as first-line therapy for VOD at our center from 1999; so only the six patients diagnosed after this date received it promptly. Unfortunately, the limited number of patients treated and our local policy of treating any case of inversion of portal flow with heparin and rt-PA did not allow us to make any conclusion on the efficacy of defibrotide as therapy. Interestingly, four of six patients treated initially with defibrotide developed inversion of portal flow which regressed completely with heparin and rt-PA. This finding suggests that a strategy based on two-step therapy, defibrotide and/or, for non-responders with inversion of portal flow, heparin and rt-PA could be explored in the future to reduce VOD mortality.

In conclusion, this study showed that both busulphan-based conditioning regimens and younger age are independent risk factors for VOD. The combination of adequate supportive measures and VOD therapy enabled us to reduce the high mortality rate. In this context, Doppler US may be helpful in identifying the patients with inversion of portal flow who might benefit from a higher risk therapy with heparin and rt-PA.

Further prospective controlled studies are needed to design the best strategy of prophylaxis and therapy for VOD and to clarify the optimal use of defibrotide.

CM, SC, and MP designed the study; MP, SV, LS, and GE collected the data; GT, SC and MP performed the statistical analysis; SC and CM wrote the paper; LZ, MC, SC and CM were responsible for the allocation of patient to stem cell transplantation; SC, MP, EC, and CM contributed equally to the routine clinical management of the patients; RD and MVG were respon-

sible of stem cell collection and processing; ET and TT performed all ultrasound examinations in patients who developed veno-occlusive disease. The authors declare that they have no potential conflict of interest.

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