Hepatic veno-occlusive disease in pediatric stem cell transplantation: impact of pre-emptive antithrombin III replacement and combined antithrombin III/defibrotide therapy

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Background and Objectives. Hepatic veno-occlusive disease (VOD) remains a serious complication after hematopoietic stem cell transplantation (HSCT). Based on a protective effect of antithrombin III (ATIII) on endothelial cells, we assessed the incidence of VOD after pre-emptive ATIII replacement and the outcome of VOD after combined high dose defibrotide and ATIII therapy.

Design and Methods. This prospective case series comprised two phases. In the first phase 71 children did not receive any specific VOD prophylaxis or therapy (controls). In the second phase 91 children were given pre-emptive ATIII replacement in case of decreased ATIII activity (≤70%). If VOD was diagnosed clinically (according to modified Seattle criteria), high dose defibrotide (60 mg/day) and ATIII replacement therapy were combined. The severity of VOD was determined according to the degree of multiple organ dysfunction.

Results. The incidence of VOD was similar in both groups (13/71, 18% vs. 14/91, 15%). All 14 patients in the second group who developed VOD showed decreased ATIII activity not more than 1 day prior to the clinical diagnosis of VOD. The resulting short duration of pre-emptive ATIII therapy failed to prevent VOD (OR 0.96). None of the patients (n=72) maintaining normal ATIII levels developed VOD. All 14 patients with VOD who received combined therapy achieved complete remission and 93 % (13/14) survived until day +100, compared to six survivors (46%) in the first group.

Interpretation and Conclusions. Pre-emptive ATIII administration did not alter the incidence of VOD. Combination treatment with ATIII and defibrotide was safe and yielded excellent remission and survival rates.

Key words: veno-occlusive disease, antithrombin III, defibrotide, hematopoietic stem cell transplantation, children.

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Veno-occlusive disease (VOD) of the liver is a major complication of allogeneic or autologous stem cell transplantation (HSCT), particularly after busulfan-based conditioning regimens.[1] Clinically, VOD is characterized by fluid retention, weight gain, painful hepatomegaly and hyperbilirubinemia. About half of the patients with VOD develop multiple organ dysfunction. Treatment with defibrotide has considerably improved the survival rates of patients with VOD. However, defibrotide treatment[2-6] alone has not completely eradicated VOD-associated mortality, and about one in three patients with VOD die from multiple organ dysfunction.[2,3,7,8] Common guidelines for VOD prophylaxis are still lacking, since the efficacy of various drugs used for prophylaxis, e.g. heparin preparations,[9-12] prostaglandin E,[13-14] ursodesoxy cholic acid,[15] or low dose defibrotide,[16,17] has not been adequately confirmed or the results are still preliminary. Some of these drugs are associated with an increased risk of hemorrhage, which must be weighed against the potential benefits. Successful strategies improving the prevention of VOD or the survival in patients with VOD would therefore represent a major progress in adjuvant therapy of HSCT.

Experimentally, antithrombin III (ATIII) has been shown to exert anti-inflammatory properties by binding to endothelial membranes and by increasing prostacyclin synthesis. ATIII appears to reduce the interaction between endothelial cells and neutrophils, limits platelet aggregation and decreases pro-inflammatory cytokine production.[18-21] Despite these favorable *in vitro* features and the negligible danger of hemorrhage, studies investigating the efficacy of ATIII administration as VOD prophylaxis or as a therapeutic agent have yielded contradictory results.[22-28] Reviewing the existing literature, we reasoned that the potential of regular monitoring of ATIII plasma levels, the pre-emptive administration of ATIII prior to the clinical diagnosis of VOD and plasma-level directed ATIII replacement therapy had not been sufficiently explored. Given the low incidence rate of VOD in a single institution, we designed a prospective cohort study to assess the possible impact of pre-emptive ATIII administration on the inci-

dence and outcome of VOD in a pure pediatric group of HSCT patients receiving busulfan-based conditioning regimens. The second aim was to investigate the safety and efficacy of a combined VOD therapy consisting of high dose defibrotide supplemented by plasma-level directed ATIII replacement.

Design and Methods

Study type and patients

This was a prospective cohort study on all 162 consecutive HSCT procedures conducted at the BMT Centre of the University Children's Hospital Zürich between 1997 and 2004. A major change in the approach to VOD prevention and therapy was introduced after the first three years of observation. From 1997 to May 2000, 71 HSCT patients did not receive any prophylaxis or specific anti-VOD therapy. These patients served as *controls*. From June 2000 to 2004, the study comprised 91 HSCT procedures in patients who received a) regular measurement of plasma ATIII levels (3×/week), b) pre-emptive ATIII replacement in the case of decreased ATIII activity (≤70 %) and c) administration of high dose defibrotide combined with plasma-level directed ATIII replacement in patients fulfilling modified Seattle criteria for the diagnosis of VOD. The latter children are termed the *treatment* group. The final study comprised 162 HSCT procedures in 147 infants, children and adolescents aged between 0.2 and 19.6 years (Table 1); 135 patients were treated with one HSCT, 9 underwent two procedures and 3 patients underwent three transplants. The protocol was approved by the Institutional Review Board. All patients and their families or other legal guardians gave informed consent.

Table 1. Patients' characteristics.

Characteristics patients	*Control patients*	*Treatment patients*	*p value**
Number of HSCT	71 (1997-2000)	91 (2000-2004)	
Median age, y (range)	8.2 (0.2 -19.6)	7.9 (0.5-19)	ns
Sex (male)	46 (64%)	52 (57%)	ns
Malignant disease	34 (48%)	48 (53%)	ns
ALL CR 1 and CR >1	12 (17%)	17 (19%)	ns
AML CR 1 and CR >1	7 (10%)	11 (10%)	ns
CML (CP) and MDS	4 (6%)	8 (8%)	ns
Solid tumor/lymphoma	11 (15%)	12 (13%)	ns
Genetic disorder	37 (50%)	43 (47%)	ns
Immunodeficiency/ metabolic disease	25 (35%)	31 (34%)	ns
Refractory anemias	12 (17%)	17 (19%)	ns
Autograft	16 (23%)	17 (19%)	ns
Allograft	55 (77%)	74 (81%)	ns
Median CD34 cell dose/kg recipient	4.0	4.1	ns
Donor (female)/recipient (male)	15 (21%)	18 (20%)	ns
Donor CMV (-)/recipient CMV (-)	58 (80%)	70 (77%)	ns
Busulfan (14-20 mg/kg)	53 (73%)	65 (71%)	ns
Cyclophosphamide (120-200 mg/kg)	42 (58%)	50 (55%)	ns
TBI (6-14 Gy)	9 (13%)	14 (15%)	ns
Thiotepa (300-900 mg/m^2)	6 (8%)	5 (5%)	ns
Melphalan (140-180 mg/m^2)	11 (15%)	13 (14%)	ns
Mylotarg® (7.5 mg/kg)	0 (0%)	2 (2%)	ns
Elevated liver enzymes at HSCT	12 (17 %)	17 (19 %)	ns
Multiple HSCT	13 (18 %)	14 (15 %)	ns

ALL: acute lymphoblastic leukemia: AML: acute myeloid leukemia; CML: chronic myeloid leukemia; MDS: myelodysplastic syndrome; CR1: first complete remission; CP: chronic phase; TBI: total body irradiation.
**χ^2 test for significance; ns: not significant.*

Preparation, conditioning and treatment prior to and during HSCT

Table 1 provides an overview of the diagnoses leading to HSCT and the treatment procedures employed. Patients with busulfan-based conditioning regimens (n=118; 73%), received the drug divided into four doses per day for four consecutive days. For prophylaxis of graft-versus-host-disease (GVHD) and rejection, rabbit anti-T-cell globulin (ATG), campath 1H (in haploidentical and matched unrelated donors), cyclosporine A and methotrexate were administered. Cytomegalovirus (CMV) serology prior to HSCT was documented for all donors and recipients and CMV-pp65 antigen or CMV-PCR were regularly determined. Antiviral treatment of CMV consisted of the administration of ganciclovir or foscavir. Platelets were transfused when the patients' levels fell below 20×10^9/L and red cell transfusions were given if hemoglobin levels declined below 90 µg/L. Intestinal decontamination consisted of vancomycin, colistine, gentamycin and amphotericin B. Intravenous vitamin K was given once weekly. Further data are provided in Table 1.

Diagnostic criteria for VOD and grading of severity

VOD was suspected clinically according to the modified Seattle criteria (extended until day +30) which require the presence of at least two of the following three clinical findings before day +30 after HSCT: (i) jaundice with bilirubin >34 µmol/L, (ii) painful hepatomegaly and (iii) fluid retention >5 % of the body weight at the time of HSCT. These features were evaluated on a daily basis. The first day of satisfying the clinical criteria was defined as day 0 of VOD. In all patients, the diagnosis of VOD was confirmed either by (i) demonstration of ascites and inversion of portal venous flow using Doppler sonography,[29] or (ii) by liver biopsy or autopsy. By combining clinical, radiological and biopsy criteria, patients with viral or toxic hepatitis and patients with hepatic GHVD were distinguished from patients with VOD. The severity of VOD was initially classified according to former McDonald criteria.[30,31] Since 2002, the VOD severity was reassessed according to Richardson's criteria:[2,3] patients with clinical signs of progressive VOD disease and considerable ascites and/or pleural effusion were classified as having moderate severity severity. All VOD patients requiring oxygen or artificial respiration and/or who had renal failure (doubling of baseline creatinine) and/or encephalopathy were classified as having severe VOD. Complete remission of VOD was defined as the resolution of VOD- and multiorgan dysfunction-related symptoms together with a bilirubin decrease to less than 34 µL with or without specific therapy for VOD. Survival was defined as being alive without signs of VOD beyond day +100.[2,3]

Analysis and measurement of VOD-relevant clinical and blood parameters

Total and conjugated bilirubin, aspartame, alanine and γ-glutamyl transferase, prothrombin time, activated partial thromboplastin, fibrinogen, and creatinine were determined at baseline and three times per week thereafter until at least day +30 after HSCT. Weight and abdominal circumference were measured daily. From 2000 to 2004, ATIII activity (normal range 70-120%) was measured at least three times per week beginning prior to conditioning (baseline) and ending at day +30. In case of VOD, all laboratory and clinical measures were continued until clinical remission of VOD.

Study design for pre-emptive ATIII replacement

Pre-emptive ATIII replacement consisted of the administration of virus-inactivated ATIII concentrate infusions (Kybernin®; Aventis Behring) at a dose of 50-100 units/kg in HSCT patients in whom plasma ATIII activity declined to ≤70%. Replacement was continued until the measured ATIII activity reached or exceeded 100%.

Study design for VOD treatment

The treatment of VOD in patients of the treatment group consisted of high dose defibrotide 60 mg/kg/day (divided into four doses, administered in 5% glucose over 2 hours) combined with plasma-level directed ATIII replacement aimed at maintaining the ATIII activity at 100% or above. Combined VOD therapy was continued until all clinical and laboratory signs of VOD resolved completely or until death. After resolution of VOD signs, therapy was tapered. No concurrent therapy with heparin, tissue plasminogen activator, warfarin, or non-steroidal inflammatory drugs was given. Patients in the control group did not receive any specific anti-VOD therapy.

Statistical methods

The χ^2 test was used to compare the clinical, laboratory and treatment features of the study and control patients. Continuous data were compared between groups using non-parametric tests (Wilcoxon's test and the Kruskal-Wallis test). Prediction of outcome was evaluated employing multivariable logistic regression analyses controlling for age. For these analyses, group membership was coded as a dummy variable. The small number of fatal VOD cases as well as the overall number of patients with VOD restricted the number of variables that could be simultaneously included in the multivariable logistic regression models. Therefore, several two and three variable models were tested. The fit of the models was compared according to minimisation of Akaiki's information criterion and c-statistics. Akaiki's information criterion introduces a penalty for inclusion of additional predictor variables. The c-statistics corresponds to the receiver operating characteristic curve in traditional test accuracy studies. Measures of association are expressed as odds ratio (OR) with 95% confidence intervals (CI). All tests were two-tailed with a probability of a type I error of less than 0.05 considered to indicate statistical significance. All computations were carried out using SAS (version 8.2, SAS Inc, Cary, NC, USA).

Results

Patients

The control and treatment groups were statistically comparable regarding age, gender, underlying disease (malignant or genetic), mode of HSCT (autologous or allogeneic), CD34-doses, donor-recipient gender ratio, constellation of CMV serology between donor and recipient, the use of chemotherapeutic agents (e.g. routes of busulfan intake and doses), total body irradiation, and VOD-specific risk factors (use of busulfan, thiotepa, melphalan, underlying liver disease and multiple HSCT) (Table 1).

VOD incidence, severity and mortality

Control group

Thirteen of 71 control patients developed VOD. The onset of VOD occurred on a median of day +12.5 (1-28) after HSCT. The overall incidence of VOD was 18 % (13/71 HSCT). Seven of 13 control patients with VOD, who had no specific VOD therapy, progressed to develop severe VOD. In the remaining six cases, VOD was of moderate severity. All seven patients with severe VOD died from VOD-related multiorgan dysfunction, which was confirmed by autopsy. Consequently, the complete remission and the day +100 survival rates were identical (46%). Further data are summarized in Table 2.

Treatment group

The overall incidence of VOD in the treatment group was 15% (14/91 HSCT). Twelve of 14 patients who developed VOD were shown to have inversion of portal flow by Doppler ultrasound; in the remainder VOD was proven by biopsy. The onset of VOD occurred on a median of day +12 (4-29) after HSCT. Further data are provided in Table 2. The mean baseline ATIII activity prior to conditioning was normal (95 %, range 80-110 %) (Table 2). After HSCT, ATIII levels declined to ≤70% in 19 of 91 procedures (21%). These patients received at least one pre-emptive ATIII infusion. The maximum time span from the first decline of ATIII ≤70% and clinical diagnosis of VOD was 1 day. Hence, none of the patients with subnormal ATIII levels received pre-emptive ATIII replacement for longer than 24 hours prior to fulfillment of the modified Seattle criteria. Hence, pre-emptive ATIII replacement failed to prevent the development of VOD (OR 0.96, 95% CI 0.41-2.3, p=0.93).

Under therapy with ATIII replacement and high dose defibrotide, three of the 14 patients with VOD progressed to have severe VOD, whereas the disease remained moderate in 11. The median duration of combined therapy was 16 days (5-65 days). VOD resolved in all patients. One patient with T-non-Hodgkin's lymphoma who underwent autologous HSCT after busulfan and melphalan conditioning developed VOD on day +29 and was successfully treated with ATIII and defibrotide for 9 days. This patient died on day +58 due to idiopathic pneumonitis, but with resolved VOD (normalized portal venous flow, no ascites, no liver VOD at autopsy). Further data are provided in Table 2.

Table 2. VOD incidence, severity and outcome in study patients.

Patients	*Control patients**	*Treatment patients†*
Number (n)	71	91
Patients with ATIII infusion	0	19 (21%)
VOD incidence		
Number of VOD in autologous HSCT (n, percent)	1 of 16 (6%)	3 of 17 (18%)
Number of VOD in allogeneic HSCT (n, percent)	12 of 55 (22%)	11 of 74 (15%)
Total VOD incidence (n, percent)	13 (18%)	14 (15%)
VOD incidence in patients with no decreased ATIII activity	N/A	0
Median age at HSCT (years)	7.9	8.2
Median day of VOD onset	12.5	12.0
Mean baseline ATIII activity (normal 70-120%)	not done	95 % (range 80-110%)
VOD grading		
Severe/moderate/none	7/6/58	3/11/77
Outcome		
Complete remission (n, percent)	6 of 13 (46 %)	14 of 14 (100%)
Survival (at day +100; n, percent)	6 of 13 (46 %)	13 of 14 (93%)††

*Patients receiving pre-emptive ATIII replacement plus combined therapy with ATIII and high dose defibrotide are compared with controls. *Control patients had no VOD prophylaxis and were not specifically treated in the case of proven VOD. †All 14 patients who developed VOD were treated with ATIII replacement and high dose defibrotide (60 mg/kg). ††Patient died of idiopathic pneumonitis in complete remission on day +58.*

Table 3. Predictors of VOD and survival.

Predictor variable	*Outcome*	*Odds ratio (95% CI)*	*Controlled for*	*p value*
All patients				
Pre-emptive ATIII administration	Any VOD	0.96 (0.41-2.3)	Age	.93
Decreased ATIII activity	Any VOD	13.8 (5.0-38)		< 0.001
Age	Any VOD	0.59 (0.42-0.83)	Pre-emptive ATIII administration	0.002
Busulfan 14 mg/kg	Any VOD	4.9 (0.98-26)	Age	0.053
Busulfan 16 mg/kg	Any VOD	7.2 (1.9-28)	Age	0 .004
Busulfan 20 mg/kg	Any VOD	17.1 (1.8-159)	Age	0.01
Busulfan and cyclophosphamide	Any VOD	3.5 (1.4-9.0)	Age	0.009
VOD patients only				
Combined therapy vs. no therapy	Severe vs.moderate	0.29 (.055-1.6)		0.14
Combined therapy vs. no therapy	CR	3.0 (1.56-5.8)		0.003
Combined therapy vs. no therapy	Survival d +100	16.4 (1.64-164)		0.006
<Nessuno(a)>				

CR: complete remission.

Drug-related toxicity and complications of the prophylaxis and therapy group

Patients receiving pre-emptive or therapeutic ATIII replacement therapy had no detectable toxicity or adverse effects. Three of the 14 VOD study patients receiving combined ATIII and high dose defibrotide therapy experienced non life-threatening bleeding complications: two had bladder hemorrhage and one had hemorrhage after drainage of a pleural effusion.

These complications were successfully managed by repeated platelet and red cell infusions and did not require interruption of VOD therapy. However, hemorrhage only ceased after termination of high dose defibrotide. All surviving patients receiving ATIII concentrates (n=18) remained HIV1-, HCV-, HBV-PCR negative 6 months after HSCT.

ATIII levels as a diagnostic criterion for VOD

Fourteen of the 19 HSCT patients with ATIII levels ≤70% developed VOD. None of the patients (n=72) maintaining unchanged ATIII activity developed VOD. The sensitivity of ATIII levels ≤70% as a diagnostic criterion for VOD was 100% (95% CI, 82-100%), the specificity 94% (95% CI, 87-97%), and the likelihood ratio was 15.4 (95% CI 7.8-30.4). Likelihood ratios above 10 are considered to provide clinically relevant information.

The negative predictive value of normal ATIII levels was 100%, hence normal ATIII levels practically ruled out VOD. A retrospective analysis of the five patients with low ATIII levels suggested that the ATIII decline was attributable to subclinical disseminated intravascular coagulation during ATG infusion, which induced transient non-VOD-associated ATIII consumption.[32,33] After cessation of ATG and after transient ATIII replacement all subsequently measured ATIII plasma activities remained above 70%.

Risk factors for VOD

Table 3 presents the results from multivariable logistic regression analysis to elucidate risk factors for VOD or severe VOD. As expected, the administration of busulfan increased the risk of VOD in a dose-response fashion (OR 17.1, 7.2 and 4.9, respectively; controlled for age, Table 3). Combined anti-VOD therapy with ATIII and defibrotide had a significant impact on complete remission and day +100 survival (OR 3.0 and 16.4; p=0.003 and 0.006). The lack of a group receiving defibrotide alone prevented analysis of the additional effect of ATIII beyond the effect of defibrotide.

Discussion

The present cohort study compared 71 HSCT procedures with neither prophylaxis nor therapy to 91 subsequent HSCT procedures managed with a pre-emptive ATIII replacement regimen for prophylaxis and combined defibrotide/ATIII administration for therapy of emerging VOD. To our knowledge, this is the first report of regular ATIII measurements and plasma-level directed ATIII replacement regimen in pediatric HSCT patients. Three main findings emerged from the study. First, normal ATIII levels practically ruled out VOD (predictive value 100%). Second, in patients with VOD, ATIII activity declined to ≤70% not more than 24 hours prior to the clinical diagnosis of VOD according to modified Seattle criteria. Hence the window of opportunity for pre-emptive ATIII replacement was brief. Compared to the incidence in historical

controls, pre-emptive ATIII replacement failed to reduce the incidence rate of VOD (odds ratio 0.96). Third, VOD resolved in all 14 patients who received the combined defibrotide/ATIII therapy, whereas there was a VOD-associated mortality rate of 54% among the 13 VOD cases not receiving any specific treatment. While the observed VOD mortality from the combined therapy is lower than rates reported in the literature for defibrotide alone,[2,3,7,8] the lack of a group of patients with VOD receiving monotherapy with defibrotide prevents any enumeration of the possible additional benefit of combined defibrotide/ATIII therapy over defibrotide therapy alone. Our study rationale was based on reports demonstrating significantly lowered ATIII activities (measured once weekly) in patients with moderate and severe VOD.[34] Adult HSCT patients with ATIII levels between day +14 and +28 after HSCT declining to less than 70% had an increased rate of VOD and multiorgan dysfunction.[28,32] While other data suggested a therapeutic benefit of ATIII treatment in single and multiple organ dysfunction in adults[23,24] as well as in proven VOD of pediatric HSCT patients,[25-27] a study by Budinger and colleagues[22] failed to demonstrate a preventive effect of prophylactic high dose ATIII concentrate infusions (target range 150-200%) in 35 adult HSCT patients. We showed that plasma-level directed pre-emptive ATIII replacement failed to prevent VOD. Our hypothesis that a lowered ATIII activity might allow anticipation of VOD, opening a window of opportunity for well-timed pre-emptive treatment was not confirmed. While normal ATIII levels practically ruled out VOD, the clinical diagnosis of VOD was usually established only briefly after detecting declining ATIII levels. Probably, once ATIII levels declined in patients who subsequently progressed to develop clinically evident VOD, hepatic endothelial damage had already occurred. It should, however, be noted that VOD is not the only cause of reduced ATIII levels in HSCT patients (specificity of 94%). About one out of four patients with reduced ATIII levels in our study did not progress to develop VOD (positive predictive value 74%). The pathophysiological rationale for combining both ATIII and defibrotide was based on the expectation of increasing the anti-inflammatory activity of both drugs[4,5,18-21] while preventing further secondary thrombosis of the hepatic veins and the portal vein via the natural anticoagulatory activity of ATIII.[35] Herewith, we intended to reduce the incidence of multiorgan dysfunction.[23-28] The incidence of VOD in both groups (15% versus 18%; Table 2) was similar to that reported for recent pediatric cohorts[36,37] receiving no specific VOD prophylaxis. Regarding outcome, the complete remission and the +100 day survival rates of 100% (14 of 14) and 93% (13 of 14), respectively, after combined therapy in patients with proven VOD were higher than the previously published results with defibrotide alone.[2,3,7,8] The only pure pediatric study amongst the latter reports published by Corbacioglu and colleagues reported a complete remission rate of 76% and a day +100 survival of 64% after defibrotide treatment.[8] Our patient population was comparable to that of other reports with respect to conditioning regimens, adjuvant treatments and underlying diagnoses. Hence, our observation of complete VOD remission in all 14 affected patients suggests a possible synergistic effect of combined ATIII and defibrotide treatment beyond treatment with defibrotide alone.

The major limitations of the present study are the lack of randomization and the lack of a pure defibrotide treatment group. Definite evidence can only be generated from randomized, controlled trials. However, given the low frequency of pediatric HSCT procedures, meticulously documented cohort studies or case series involving new treatment modalities may provide first hunches towards possible improvements of therapeutic modalities. Possible causes for the observed results that are not attributable to a superior efficacy of the combined treatment include random outliers (referral of patients inherently prone to less severe VOD). A further limitation is the application of the less stringent Seattle criteria for the diagnosis of VOD as compared to the Baltimore criteria. However, the incidence rates in the control and treatment group were comparable. Moreover, all cases of VOD were confirmed by biopsy and/or demonstration of inverted portal vein flow. Thus, it is unlikely that a classification bias distorted the results between the control and the treatment group. Admittedly, no biopsies were routinely performed in patients without a clinical diagnosis of VOD. Therefore, VOD-like subclinical endothelial damage not leading to reduced ATIII levels or clinical symptoms cannot be ruled out. Because of the historical nature of our control group, we cannot entirely rule out that advances in other supportive measures may have contributed to the better outcomes in the treated patients.

With these limitations, our data suggest that treatment with combined ATIII and high dose defibrotide is safe and may yield excellent results even in patients with proven inversion of portal venous flow and an unfavorable prognosis.[6] Regarding adverse effects, no hemorrhage was observed under the pre-emptive ATIII infusion, while three VOD patients experienced non-life threatening bleedings under combined ATIII and high dose defibrotide treatment. As no clear dose-dependent effect of defibrotide on hemorrhage has been reported in the literature, we cannot rule out a moderately increased risk of hemorrhage if ATIII and high dose defibrotide are combined, as for the combined administration of heparin and ATIII.[38]

We conclude that the strategy of pre-emptive ATIII replacement in the case of decreased ATIII activity failed to prevent VOD. In light of the favorable remission and outcome rates after combined treatment presented in this study, a randomized controlled trial comparing the efficacy of combined high dose defibrotide/ATIII replacement therapy with the efficacy of defibrotide therapy alone is suggested.

UH, TG: contributed equally to the work reported in this paper; TG, RS: conceived and designed the study; UH, SE, FS and TG: responsible for data collection; UH, TG and JJF: interpreted the data; UH, JF and TG wrote the manuscript; JF: performed the statistical analysis.

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