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Liver Stiffness Measurement Allows Early Diagnosis of Veno-Occlusive Disease/Sinusoidal Obstruction Syndrome in Adult Patients Who Undergo Hematopoietic Stem Cell Transplantation: Results from a Monocentric Prospective Study

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A B S T R A C T

Veno-occlusive disease (VOD), also known as sinusoidal obstruction syndrome (SOS), is a life-threatening complication affecting patients undergoing hematopoietic stem cell transplantation (HSCT). The survival rate is higher when specific therapy is initiated early; thus, improving early, noninvasive diagnosis of VOD/SOS is an important need. In an adult population undergoing HSCT, we aimed to assess the role of liver stiffness measurement (LSM), evaluated by transient elastography (TE), for diagnosing VOD/SOS. Between April 2016 and March 2018, 78 consecutive adult patients with indications for allogeneic HSCT were prospectively included. LSM was performed before HSCT and at days +9/10, +15/17, and +22/24 post-HSCT. New European Society for Blood and Marrow Transplantation criteria were used to establish VOD/SOS diagnosis. Four patients developed VOD/SOS (5.1%) during the study period, with a median time of +17 days post-HSCT. A sudden increase in LSM compared with previously assessed values and pre-HSCT values, was seen in all patients who developed VOD/SOS. LSM increases occurred from 2 to 12 days before clinical SOS/VOD appearance. The VOD/SOS diagnostic performance of increased LSM over pre-HSCT assessment showed an area under the receiver operating characteristic curve of 0.997 (sensitivity 75%; specificity 98.7%). LSM gradually decreased following successful VOD/SOS-specific treatment. Interestingly, LSM values did not increase significantly in patients experiencing hepatobiliary complications (according to the Common Terminology Criteria) other than VOD/SOS. LSM by TE can be considered a promising method to perform an early, preclinical diagnosis and follow-up of VOD/SOS.

INTRODUCTION

Veno-occlusive disease (VOD), also known as sinusoidal obstruction syndrome (SOS), is a relatively rare but potentially life-threatening complication that can affect patients after hematopoietic stem cell transplantation (HSCT) [1]. It occurs in roughly 14% (5% to 60%) of patients after HSCT [2], depending on several known factors [1,3–5]. From a pathophysiological standpoint, conditioning regimens can cause a toxic injury in sinusoidal endothelial cells, with consequent damage of hepatic acini and blood outflow blockage, causing sinusoidal portal hypertension (PH) [3,6,7]. Clinical signs such as ascites, weight gain, painful hepatomegaly, and jaundice, which are the clinical diagnostic criteria for VOD/SOS [8–11], are directly connected to PH. These clinical criteria are crucial to establishing a diagnosis of VOD/SOS. Several conditions can mimic or overlap VOD/SOS, (eg, cholangitis, bacterial sepsis, acute graft-versus-host disease [GVHD], biliary sludge), and histological confirmation is often needed to accurately discriminate among multiple differential diagnoses [12]. Moreover, severe forms of VOD/SOS frequently lead to severe multiorgan dysfunction/failure (MOD/MOF) [3], with reported mortality of up to 80% rate in a historical cohort [13]. However, it has been demonstrated that an early and prompt diagnosis, leading to earlier

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initiation of treatment [14], is associated with significantly improved day +100 survival in patients with MOF and in those without MOD [15,16]. For this reason, the recognition of VOD/SOS in its milder forms or at earlier stages [17] is highly important. New ultrasonography scores and technological ultrasound improvement may help to confirm the diagnosis in suspected VOD/SOS [18–20]; however, early recognition of VOD/SOS remains a challenge. Invasive methods, such as the measurement of hepatic vein pressure gradient (HVPG) and transjugular liver biopsy, can lead to a conclusive diagnosis of VOD/SOS with high specificity, although their use may be limited in selected cases and in well-trained centers [12].

Recently, ultrasound elastography techniques to perform liver stiffness measurement (LSM) have been investigated as a possible surrogate for PH and its complications [21–24]. Studies and meta-analyses have confirmed the close relationship between LSM and HVPG, the gold standard for evaluating PH, in patients with advanced chronic liver disease (ACLD) [25–27].

To date, among these techniques, only transient elastography (TE) [28–30] and point-shear wave elastography by acoustic radiation force impulse imaging [29,31] have been evaluated for assessment of liver complications in pediatric patients undergoing HSCT. No study has yet been reported on the role of LSM in VOD/SOS assessment in the setting of adult HSCT. Here we report the results of a monocentric prospective study evaluating the role of LSM as assessed by TE in the development of VOD/SOS in adult patients undergoing allogeneic HSCT for hematologic malignancies.

METHODS

Patients and Study Design

This monocentric prospective study included consecutive adult patients undergoing allogeneic HSCT at the Institute of Hematology L. and A. Seràgnoli, Sant'Orsola-Malpighi University Hospital, Bologna, Italy between April 2016 and March 2018. The inclusion criteria were allogeneic HSCT from any type of donor and hematopoietic stem cell source with either myeloablative and reduced-intensity conditioning, hematologic indication for allogeneic HSCT, and age 18 to 70 years. The exclusion criteria were body mass index (BMI) >40 kg/m^2 with ascites and the presence of ACLD at pre-HSCT assessment, to avoid possible technical LSM bias.

The primary objective was to investigate the clinical role of LSM in the development of VOD/SOS in the setting of adult allogeneic HSCT as assessed by TE. Secondary objectives included assessing the predictive factors associated with VOD/SOS diagnosis, evaluating the role of LSM in follow-up management of VOD/SOS, comparing the application of LSM and ultrasound in assessment of VOD/SOS, and defining the role of LSM in the differential diagnosis among other liver-related complications of HSCT.

The study protocol included clinical evaluation, biochemical assays, gray-scale and color Doppler ultrasound, and LSM evaluation of all patients included at enrollment (within 1 month before HSCT). Subsequent LSM evaluations were done on a dense assessment schedule on days +9/10 (T1), +15/17 (T2), and +22/24 (T3) after HSCT, 3 days/week assessment of biochemical laboratory tests, and a daily clinical assessment of the presence of VOD/SOS criteria from the initiation of the conditioning regimen until 30 days after HSCT. In cases of clinical suspicion of VOD/SOS according to the European Society for Blood and Marrow Transplantation (EBMT) criteria [8], in the interval between 2 subsequent scheduled time points (T0-T1, T1-T2, T2-T3, or >T3), in addition to the foregoing study protocol, color Doppler ultrasound, LSM, and intensive clinical monitoring were carried out based on the seriousness of the clinical features. If increased LSM values were found at any scheduled time point, color Doppler ultrasound, biochemical testing, and intensive clinical monitoring were further assessed to confirm/exclude the diagnosis, according to the EBMT recommendations. When VOD/SOS was diagnosed, weekly LSM evaluations were performed until clinical VOD/SOS was resolved.

This trial was conducted in compliance with the Declaration of Helsinki and was approved by the Institutional Ethics Committee of Sant'Orsola-Malpighi University Hospital (protocol 125/2015/O/Sper). Written informed consent was obtained from each patient.

VOD/SOD Diagnosis

VOD/SOS of the liver was defined by EBMT clinical diagnostic criteria [8]. Accordingly, painful hepatomegaly, weight gain, and serum bilirubin ≥2.0 mg/dL with or without ultrasound signs (only in late VOD) were considered typical clinical signs for VOD/SOS diagnosis; in doubtful cases, further invasive diagnostic analyses (liver biopsy and HVPG) were performed to confirm the clinical diagnosis. VOD/SOS grading was defined in accordance with EBMT criteria for severity grading [8]. VOD with MOF was defined as the presence of renal and/or pulmonary dysfunction in addition to VOD.

Other Hepatobiliary Complications

The occurrence of other hepatobiliary complications after HSCT, such as hepatic GVHD, infective hepatobiliary diseases, and other drug-induced liver injury (DILI) in accordance with clinical definitions [32–35], were also evaluated. Patients with liver biochemical alterations were stratified based on the Common Terminology Criteria for Adverse Events version 4 (CTC) [36].

Liver Stiffness Measurement (LSM)

LSM values were assessed at the patients' bedside by TE using the FibroScan apparatus with an "M" probe (Echosens, Paris, France) after an overnight fast and after a complete abdominal ultrasound examination. LSM values were obtained as described previously [37]. The reliability criteria were considered in accordance with the latest European Federation of Societies for Ultrasound in Medicine and Biology's guidelines and recommendations on the clinical use of ultrasound elastography [38]; the reliability criterion was at least 10 measurements with a ratio of the interquartile range (IQR) of liver stiffness to the median (IQR/M) ≤30%.

Abdominal Ultrasound

All patients underwent baseline gray-scale and color Doppler ultrasound examinations before HSCT, when LSM increased and in case of the presence of one among the VOD/SOS clinical signs criteria [8] in the interval between scheduled time points. Ultrasound findings were evaluated according to the Lassau criteria [18]. During hospitalization, ultrasound examinations were performed at the bedside with a Noblus with a UST-9147 convex ultrasound transducer (Hitachi Medical, Tokyo, Japan).

Supportive Care

Patients were allocated in single positive-pressure rooms with HEPA-filtered air. Anti-infectious prophylaxis was administered according to the local practice, with levofloxacin and antifungal drugs (fluconazole for HLA-identical sibling transplant and voriconazole for all other transplants) during the neutropenic phase. All patients received acyclovir and cotrimoxazole on a standard schedule until 9 months after HSCT or throughout the duration of immunosuppression.

CMV and EBV monitoring was performed once or twice weekly, depending on the risk of the pairs during the first 100 days after transplantation, and once a month thereafter. All patients received filtered and irradiated blood products.

Data Management and Statistical Analysis

Data were collected and managed using REDCap electronic data capture tools hosted at Department of Medical and Surgical Sciences, University of Bologna [39]. Categorical data are expressed as number (percentage) and continuous variables are expressed as median and IQR. For group comparisons, the Wilcoxon signed-rank test was used for continuous variables, and the Fisher exact test was used for categorical variables. Univariate logistic regression analyses were performed to evaluate predictors of VOD/SOS development after HSCT. A receiver operating characteristic curve was created to calculate the area under the curve (AUC) for LSM changes over pre-HSCT assessment. All P values are 2-tailed. Statistical analyses were performed using Stata/SE version 14.2 for Windows (StataCorp, College Station, TX).

RESULTS

Over the study period, of the 89 patients referred to the transplantation center, 2 were excluded because of BMI >40 and ACLD at enrollment, and 9 did not undergo HSCT (due to donor unavailability or disease progression) after being enrolled initially. As shown in Figure 1, 78 patients met all the inclusion criteria and underwent HSCT. During the study period, 2 patients were transferred to an intensive care unit, and 4 patients died from non-liver-related causes. One patient died from severe VOD/SOS.

The clinical and transplantation characteristics of the enrolled patients are summarized in Table 1 and Table 2, respectively. Before HSCT, almost all patients (92.3%) received blood transfusions (median serum ferritin level, 738 ng/mL [IQR, 68 to 1333 ng/mL]), and 32 patients (41.1%) received

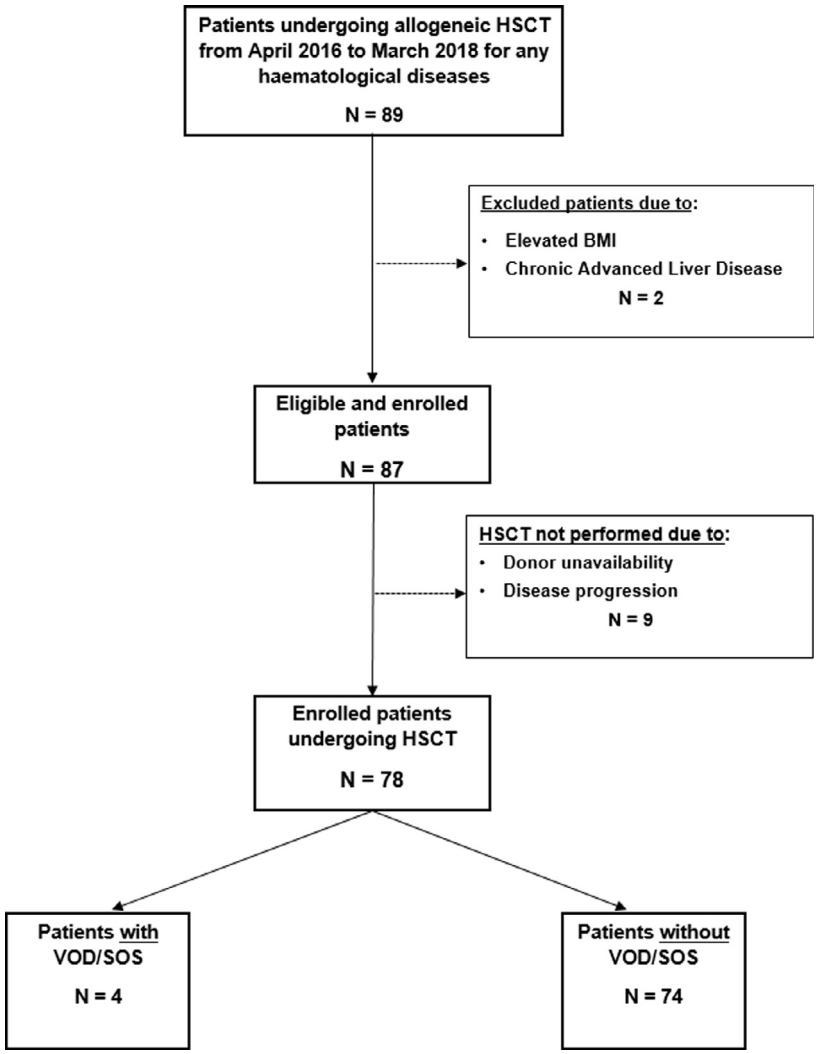

Figure 1. Flow chart of the enrolled patients.

Table 1
Baseline Characteristics of Study Population and by VOD/SOS Diagnosis

Characteristic	Study population (N = 78)	Without VOD/SOS (n = 74; 94.9%)	With VOD/SOS (n = 4; 5.1%)	*P* Value*
Male sex, n (%)	39 (50)	37 (50)	2 (50)	1
Age, yr, median (IQR)	54 (40-60)	54 (41-60)	46 (31-60)	.696
BMI, kg/m^2, median (IQR)	25 (22.6-27.5)	25.1 (23-27.5)	21.5 (21.1- 23.8)	.358
Ethnicity				.106
Caucasian	73 (93.6)	70 (94.5)	3 (75)	
African/African American	3 (3.8)	3 (4.1)	0 (0)	
Asiatic	2 (2.6)	1 (1.4)	1 (25)	
Diagnosis, n (%)				.133
Acute myelogenous leukemia	35 (44.9)	34 (45.9)	1 (25)	
Acute lymphoblastic leukemia	14 (17.9)	12 (16.2)	2 (50)	
Myelodysplastic syndrome	11 (14.1)	11 (14.9)	0 (0)	
Hodgkin lymphoma	5 (6.4)	5 (6.8)	0 (0)	
Non-Hodgkin lymphoma	4 (5.1)	4 (5.4)	0 (0)	
Multiple myeloma	2 (2.6)	2 (2.7)	0 (0)	
Myelofibrosis	3 (3.8)	3 (4.1)	0 (0)	
Chronic myelogenous leukemia	3 (3.8)	3 (4.1)	0 (0)	
Severe aplastic anemia	1 (1.3)	0 (0)	1 (25)	
Blood transfusion history, n (%)				**.029**
≤20 transfusions	46 (58.9)	46 (62.2)	0 (0)	
>20 transfusions	32 (41.1)	28 (37.8)	4(100)	
HCT-CI Sorror total score, median (IQR)	0 (0-2)	0 (0-1.5)	2 (.75-3)	.247
LSM at baseline, median (IQR)	4.2 (3.8- 5.3)	4.2 (3.8-5.2)	6.9 (5.7-7.8)	.079

Significant *P* values are in bold type.
HCT-CI indicates hematopoietic cell transplantation comorbidity index.
* Wilcoxon rank-sum test/Fisher exact test.

Table 2
Transplantation Characteristics According to VOD/SOS Occurrence

Characteristic	Study Population (N = 78)	Without VOD/SOS (n = 74; 94.9%)	With VOD/SOS (n = 4; 5.1%)	*P* Value*
Conditioning regimen, n (%)				.190
Myeloablative conditioning	52 (66.7)	48 (92.3)	4 (7.7)	
TBI-Cy	2 (3.8)	2 (4.2)	0 (0.0)	.899
TBF	47 (90.5)	45 (93.7)	2 (50)	.523
Bu- Flu	2 (3.8)	1 (2.1)	1 (25)	**.004**
Cy200	1 (1.9)	0 (0.0)	1 (25)	**.05**
Reduced-intensity conditioning	26 (33.3)	26 (100)	0 (0)	.190
TBF	13 (50)	13 (50)	0 (0.0)	.475
TCF	13 (50)	13 (50)	0 (0.0)	.475
GVHD prophylaxis, n (%)				
ATLG-CNI-MTX	73 (93.6)	69 (93.2)	4 (100)	.537
CNI-MTX	2 (2.6)	2 (2.7)	0 (0)	.899
CNI-MMF-PT-Cy	3 (3.8)	3 (4.1)	0 (0)	.852
Stem cell donor, n (%)				
Matched related donor	12 (15.4)	11 (14.9)	1 (25)	.495
Unrelated donor†	53 (67.9)	50 (67.6)	3 (75)	.616
Haploidentical donor	3 (3.8)	3 (4.1)	0 (0.0)	.852
Cord blood	5 (6.4)	5 (6.7)	0 (0.0)	.721
Stem cell source, n (%)				.676
Peripheral blood	58 (74.4)	54 (73)	4 (100)	
Bone marrow	15 (19.2)	15 (20.3)	0 (0.0)	
Umbilical cord blood	5 (6.4)	5 (6.8)	0 (0.0)	

Significant *P* values are in bold type.
TBI indicates total body irradiation; Cy, cyclophosphamide; TBF, thiotepa-busulfan-fludarabine; Bu-Flu, busulfan-fludarabine; TCF, thiotepa-cyclophosphamide-fludarabine; ATLG, anti-T lymphocyte globulin; CNI, calcineurin inhibitor; MTX, methotrexate; MMF, mofetil mycophenolate; PT-Cy, post-transplantation cyclophosphamide.
* Fisher exact test.
† Thirty-four patients (64.1%) were 10/10 HLA-matched, and 19 (35.9%) were <10/10 HLA-matched.

more than 20 RBC transfusions. The baseline median LSM was 4.2 kPa (IQR, 3.8 to 5.3 kPa).

During the study period, 4 of 78 patients (5.1%) met the clinical diagnostic criteria for VOD/SOS. The median day of clinical VOD/SOS diagnosis was day +17 (IQR, day +4 to day +28) post-HSCT. Characteristics of the patients who developed VOD/SOS are reported in Table 3. Three of the 4 cases of VOD/SOS were severe/very severe, and 1 case led to MOF and death several days after onset (patient 3). These patients received a myeloablative conditioning regimen based on busulfan or cyclophosphamide and a greater number of pretransplantation blood transfusions (>20) compared with the patients who did not develop VOD/SOS (Tables 1 and 2).

Table 4 shows that among the EBMT-defined pre-HSCT risk factors for VOD/SOS [8], these 4 patients underwent previous therapy with inotuzumab ozogamicin (n = 3) or other hepatotoxic drugs (n = 1), and 2 of them had pre-HSCT iron overload.

Regarding the ultrasound findings at VOD/SOS diagnosis (Table 5), according to the Lassau criteria [18] (increased diagnostic likelihood when ≥3 criteria were met), 3 of the 4 patients met ≥3 gray-scale ultrasound morphological criteria and no patients met ≥3 color Doppler criteria. Only portal vein diameter >12 mm was seen in all 4 patients diagnosed with VOD/SOS.

LSM values at each time point are reported in Figure 2. LSM values were increased in all patients who developed VOD/SOS (red continuous lines). The median pre-HSCT (T0) LSM values did not differ between patients with VOD/SOS and those without VOD/SOS (6.9 kPa versus 4.2 kPa; $P = .079$). LSM values were increased at the assessment performed before the clinical diagnosis of VOD/SOS, and these values were significantly different from those measured at T0 and at previous assessments. In general, the LSM values showed a sudden increase from days 2 to 12 before the clinical VOD/SOS diagnosis (Table 3). After the initiation of treatment for VOD/SOS (defibrotide and diuretics), LSM values decreased, reaching pretransplantation values within 2 to 4 weeks after VOD/SOS diagnosis. One patient, who died 20 days after being diagnosed with severe VOD/SOS, did not exhibit decreased LSM values (Figure 3).

Logistic regression analysis demonstrated that an increase in LSM values after HSCT over pre-HSCT assessment was significantly associated with the likelihood of diagnosis of VOD/SOS (odds ratio, 1.837; 95% confidence interval, 1.1107 to 3.0384; $P < .001$). Furthermore, receiver operating characteristic curve analysis of LSM increases over pre-HSCT assessment revealed an area under the curve of .997 with a sensitivity of 75% and specificity of 98.7%. For instance, an increase of ≥+10 kPa over pre-LSM-HSCT assessment showed a sensitivity of 100% and specificity of 98.7% for diagnosing VOD/SOS.

During the study period, among the 74 patients who did not develop VOD/SOS, 62 experienced no or mild-to-moderate therapy-associated complications without severe liver involvement after HSCT (CTC grade 0 to 3). Twelve patients experienced severe liver complications (CTC grade 4 to 5), including 2 with acute cholecystitis, 1 with cholangitis, 2 with DILI related to antimycotic drug use, 1 with hepatic GVHD, 5 with isolated liver biochemical alterations, and 1 with fulminant Epstein-Barr virus-related hepatitis reactivation.

The median LSM values after HSCT were not significantly different between the patients with CTC grade 0 to 3 and those with CTC grade 4 to 5 (6.1 kPa [IQR, 5 to 6.8 kPa] versus 6.6 kPa [IQR, 4.8 to 8.6 kPa]; $P = .724$); however, these values were significantly different ($P < .001$) between patients with CTC grade 4 to 5 and patients with VOD/SOS (Figure 4). Regardless, only 1 patient, a 67-year-old patient with Philadelphia chromosome-positive acute lymphoblastic leukemia, showed a sudden increase in LSM values (13.5 kPa) in the absence of any clinical signs of VOD/SOS (Figure 2). Over the next month, the patient developed mildly painful hepatomegaly and a tender abdomen with ascites. HVPG was 21 mmHg, and transjugular liver

Table 3
Characteristics of the Patients with VOD/SOS

Patient	Sex, Age, yr	Disease	Baseline LSM, kPa	Number of Risk Factors*	Conditioning Regimen	Clinical VOD/SOS Diagnosis*	Serum Bilirubin >2 mg/d	Hepatomegaly	Weight Increase >5%	Ascites on ultrasound	Decreased Mean PV Flow Velocity	Increased LSM, kPa	VOD/SOS Severity Grading*	Management
1	Male, 26	Aplasia	6.4	6	MAC	+7	Yes, +7	Yes, +7	Yes, +7	Yes, +7	No	10.4 at +5; 26.3 at +7	Severe	Defibrotide, diuretics
2	Female, 60	AML	3.7	7	MAC	+1	Yes, +1	Yes, -1	Yes, -1	Yes, +3	Yes, +7	14.4 at -1	Severe	Defibrotide, diuretics
3	Male, 32	ALL	7.4	6	MAC	+27†	Yes, +7	Yes, +27	Yes, +27	Yes, +27	Yes, +46	9.2 at +7; 12.6 at +15; 16.6 at +21; 22.8 at +46	Very severe (MOF)	Defibrotide, diuretics
4	Female, 60	ALL	8.8	6	MAC	+29	Yes, +29	Yes, +29	Yes, +27	No	Yes, +27	11.4 at +18; 34.3 at +26	Moderate	Diuretics

PV indicates paraumbilical vein; MAC, myeloablative conditioning; AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia.
* According to Mohty et al [8].
† Diagnosis also made with HVPG assessment (+21 mmHg).

biopsy revealed widespread dilatation of hepatic sinusoids and the presence of atypical elements such as B lymphoblastic leukemia infiltration. At this time, the patient's LSM value was 20 kPa. We believe that this case described above is an uncommon clinical situation in which the infiltration of liver sinusoids by leukemia mimics the presence of sinusoidal obstruction typical of the pathogenesis of VOD/SOS after HSCT.

DISCUSSION

The aim of this study was to evaluate the clinical role of LSM values measured by TE on VOD/SOS development in a prospective cohort of adult patients undergoing allogeneic HSCT. The rationale for using LSM as an early detector of VOD/SOS is related to its pathogenesis, with congestion from blood outflow block and inflammation from loss of sinusoidal wall integrity by toxic injury leading to sinusoidal PH. These conditions were previously identified as determinants of LSM increase in PH [38].

Our data show significantly increased LSM values compared with previous measurements and pre-HSCT measurements only in patients who developed VOD/SOS. This allows for a preclinical diagnosis because the sudden increase in stiffness anticipated the standard EBMT-based clinical diagnosis by 2 to 12 days (Figure 2).

In our study population, the incidence of VOD/SOS was 5.1%, with a 3.8% rate of severe disease, in line with the current reports in adult cohorts [40,41]. As expected, VOD/SOS occurred mostly in patients with a higher number of VOD/SOS risk factors according to the latest EBMT classification scheme [8]. Indeed, in the present study, patients who developed VOD/SOS received a significantly more intensive busulfan-based chemotherapy (myeloablative conditioning regimen), and received more than 20 blood transfusion pre-HSCT (leading to a liver iron overload) and hepatotoxic drugs (mainly inotuzumab ozogamicin) (Table 4). These findings support previous reports [42] and the findings of a recent expert panel review [43].

The importance of having, along with clinical VOD/SOS criteria, a radiologic technique able to better identify and differentiate VOD/SOS from other clinical complications of HSCT is well recognized. Thus, our protocol included gray-scale and color Doppler ultrasound examinations when LSM values were increased or a diagnosis of VOD/SOS was established. Indeed, we confirmed that the majority of patients who developed VOD/SOS met ≥3 Lassau criteria [18] (Table 5). Our results support the EBMT and British Committee for Standards in Haematology/British Society for Blood and Marrow Transplantation guidelines [8,12] suggesting that ultrasound examination could have a pivotal role as the first bedside technique for use in the differential diagnosis among several abdominal post-HSCT complications and to confirm the VOD/SOS diagnosis in uncertain cases. However, the positivity of ultrasound signs is consistent with the clinical occurrence of VOD/SOS criteria, precluding its use in predicting diagnosis.

The main finding of this study is that for the first time in a prospective study of adult HSCT recipients, the increase in LSM values after HSCT as assessed by TE has been identified as an accurate predictor of VOD/SOS development. Of note, an LSM increase after HSCT over the pre-HSCT assessment precedes the clinical VOD/SOS diagnosis by 2 to 12 days. This increase in LSM values compared with previous measurements was significantly associated with the diagnosis of VOD/SOS. Our results obtained in an adult cohort are consistent with the findings of our previous study in a pediatric setting [28] using the same

Table 4
VOD/SOS Risk Factors According to EBMT and by VOD/SOS Diagnosis

VOD/SOS Risk Factors	Study Population (n = 78)	Without VOD/SOS (n = 74; 94.9%)	With VOD/SOS (n = 4; 5.1%)	*P* Value*
Transplantation-related factors				
Unrelated donor	63 (80.8)	60 (81.1)	3 (75)	.582
HLA-mismatched donor	32 (41)	31 (41.9)	1 (25)	.640
Non-T cell-depleted transplant	78 (100)	74 (100)	4 (100)	1
Myeloablative conditioning regimen	49 (62.8)	47 (63.5)	2 (50)	.625
Oral or high-dose busulfan-based regimen	62 (79.5)	59 (79.7)	3 (75)	.609
High-dose TBI-based regimen	3 (3.8)	3 (4.1)	0 (0)	.852
Second HSCT	7 (9)	6 (8.1)	1 (25)	.319
Patient and disease-related factors				
Older age	21 (26.9)	21 (28.4)	0 (0)	.569
Karnofsky Performance Status score <90%	0 (0)	0 (0)	0 (0)	—
Metabolic syndrome	10 (12.8)	9 (12.2)	1 (25)	.429
Female receiving norethisterone	3 (3.8)	3 (4.1)	0 (0)	.852
Advanced disease (beyond CR2 or relapsed/ refractory)	23 (29.5)	21 (28.4)	2 (50)	.577
Thalassemia	0 (0)	0 (0)	0 (0)	—
Genetic factors (eg, GSTM1 polymorphism, C282Y allele, MTHFR 677CC/1298CC haplotype)	1 (1.3)	1 (1.4)	0 (0)	.949
Hepatic-related factors				
Transaminases 2.5× ULN	0 (0)	0 (0)	0 (0)	—
Serum bilirubin 1.5× ULN	1 (1.3)	1 (1.4)	0 (0)	.949
Active viral hepatitis	3 (3.8)	3 (4.1)	0 (0)	.852
Cirrhosis	0 (0)	0 (0)	0 (0)	—
Abdominal or hepatic irradiation	1 (1.3)	1 (1.4)	0 (0)	.949
Previous use of inotuzumab ozogamicin	4 (5.1)	1 (1.4)	3 (75)	**<.00001**
Other hepatotoxic drugs	2 (2.6)	1 (1.4)	1 (25)	**.004**
Iron overload	3 (3.8)	1 (1.4)	2 (50)	**.006**

Significant *P* values are in bold type.
ULN indicates upper limit of normal.
* Wilcoxon rank-sum test/Fisher exact test.

Table 5
US Criteria According to Lassau et al [18] in Patients with VOD/SOS

Criteria	Patient 1	Patient 2	Patient 3	Patient 4
Clinical VOD/SOS diagnosis	+7	+1	+27	+29
Ultrasound and color Doppler criteria	4	3	8	3
Gray-scale ultrasound morphological criteria, n	4	3	6	2
Hepatomegaly	Yes, +7	Yes, +3	Yes, +27	Yes, +26
Splenomegaly	None	None	Yes, +21	None
Gallbladder wall thickening >6 mm	Yes, +7	None	Yes, +27	None
Portal vein diameter >12 mm	Yes, +7	Yes, +7	Yes, +12	Yes, +26
Hepatic vein diameter <3 mm	None	None	None	None
Ascites	Yes, +7	Yes, +3	Yes, +27	None
Visualization of paraumbilical vein	None	None	Yes, +27	None
Ultrasound color Doppler criteria, n	0	0	2	1
Mean portal flow velocity <10 cm/s or hepatofugal flow	None	None	None	None
Flow recorded in paraumbilical vein	None	None	Yes, +27	Yes +26
Monophasic flow or flow recorded in hepatic veins	None	None	None	None
Hepatic Artery Resistive Index >.75	—	—	Yes, +27	None

elastographic method and assessment schedule and with results reported by Reddivalla et al [31] using a different ultrasound elastography method (ie, acoustic radiation force impulse), again in a pediatric population.

In addition, in patients who developed VOD/SOS and were specifically treated, LSM values were reduced within 2 to 4 weeks after diagnosis, reaching pretransplantation values. It could be argued that these findings, if confirmed in future studies, could drive the duration of treatment, an issue that has not yet been addressed in clinical trials.

Finally, LSM was able to discriminate between VOD/SOS and other post-HSCT complications, enabling a definitive differential diagnosis. We have shown that the median LSM values of patients with VOD/SOS were significantly higher than those of patients with other therapy-associated liver complications (eg, acute cholecystitis, cholangitis, DILI related to antimycotic drugs, hepatic GVHD, isolated liver biochemical alterations, fulminant Epstein-Barr virus-related hepatitis reactivation) regardless of CTC grade (Figure 4). This result is apparently in contrast with the findings of Karlas et al [29], who reported significantly higher LSM values measured by TE in 5 of 59 HSCT recipients who developed severe hepatic involvement after HSCT (CTC grade 4 to 5) compared with the others who did not (mean, 6.2 ± 1.5 kPa versus 4.7 ± 1.7 kPa). A possible explanation for this discrepancy is the fact that VOD/SOS was not analyzed separately but was included in the severe complication group without any specifications.

However, the predictive role and meaning of pre-HSCT LSM values in liver complications remains unclear. Even if,

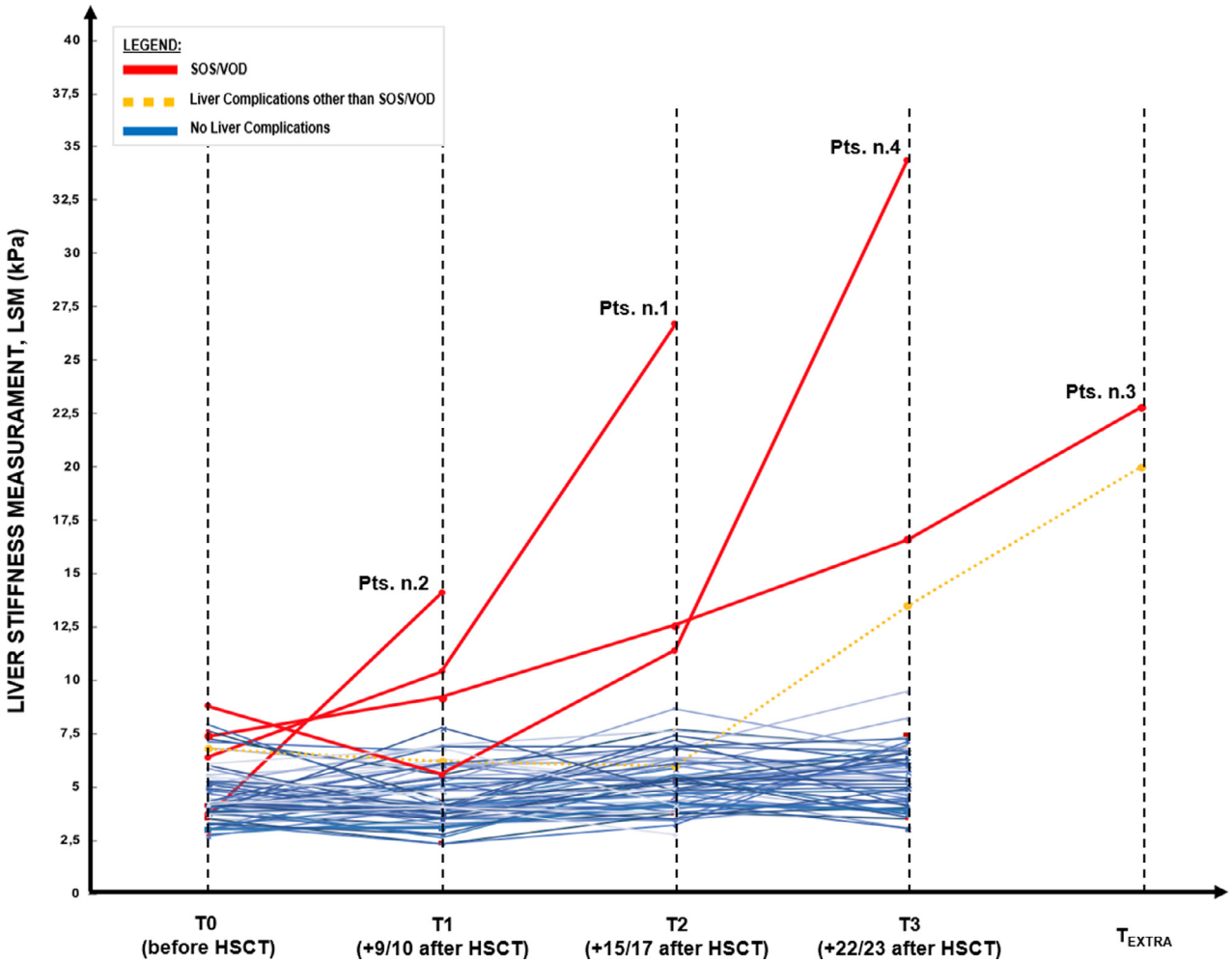

Figure 2. Variation of LSM values at each determination for all patients. The red solid line represents patients who developed VOD/SOS after HSCT; blue solid line, patients who did not develop VOD/SOS after HSCT; yellow dotted line, patients who developed liver complications other than SOS/VOD.

the initial observation by Auberger et al [30], who showed that a pre-HSCT LSM cutoff of 8 kPa measured by TE was able to identify patients developing liver toxicity (defined as bilirubin >2 mg/dL) after HSCT. Our present findings and data by Reddivalla et al [31] do not support the foregoing findings. Indeed, we observed no significant differences in pre-HSCT LSM values between patients who developed VOD/SOS and those who did not.

The present study has several limitations. A relatively small number of patients with VOD/SOS were included, although the number was in line with the prevalence of this disease in adults reported in the literature [1]. The LSM assessments were scheduled arbitrarily on 3 consecutive visits (days +9/10, +15/17, and +22/24). Evidence for a standard LSM assessment schedule is not yet available, and our schedule was arbitrarily applied owing to the experimental role of LSM in this setting. A daily LSM assessment, which is hardly practicable in the actual clinical setting, could identify the real advantages of TE for assessing LSM. Another limitation is that in this study, we assessed LSM only by TE, and thus it is difficult to compare our findings with results obtained with other ultrasound-based elastography techniques, as demonstrated recently [44]. Nevertheless, even though a dedicated device is needed, TE is still considered the best validated and readily available elastography technique, and most centers use TE as a standard technique to assess LSM even in the consolidated hepatologic indications [38,45,46]. Finally, we excluded patients with pathological obesity (BMI >40 kg/m^2) owing to the technical limitations of the TE device' s "M" probe. The use of an "XL" probe [47] (a dedicated probe for obese patients) or other ultrasound-based elastography techniques could bypass this technical limitation.

In conclusion, this prospective study in a large number of patients resembles a real-life HSCT practice and suggests that LSM assessment by TE may be a promising technique for obtaining an early, preclinical diagnosis and predicts VOD/SOS. In addition, it also may be useful for assessing treatment response in adult HSCT recipients who develop VOD/SOS. Moreover, this noninvasive bedside method is reproducible and well tolerated by patients. In addition, it provides a standardized quantitative measurement, avoiding the use of radiation and i.v. contrast agents and precluding the risk of bleeding or infection. LSM as assessed by TE appears to accurately differentiate between VOD/SOS and other liver-related HSCT complications. Further multicenter prospective studies in adult and pediatric settings are needed to confirm the role of TE assessment of LSM in the

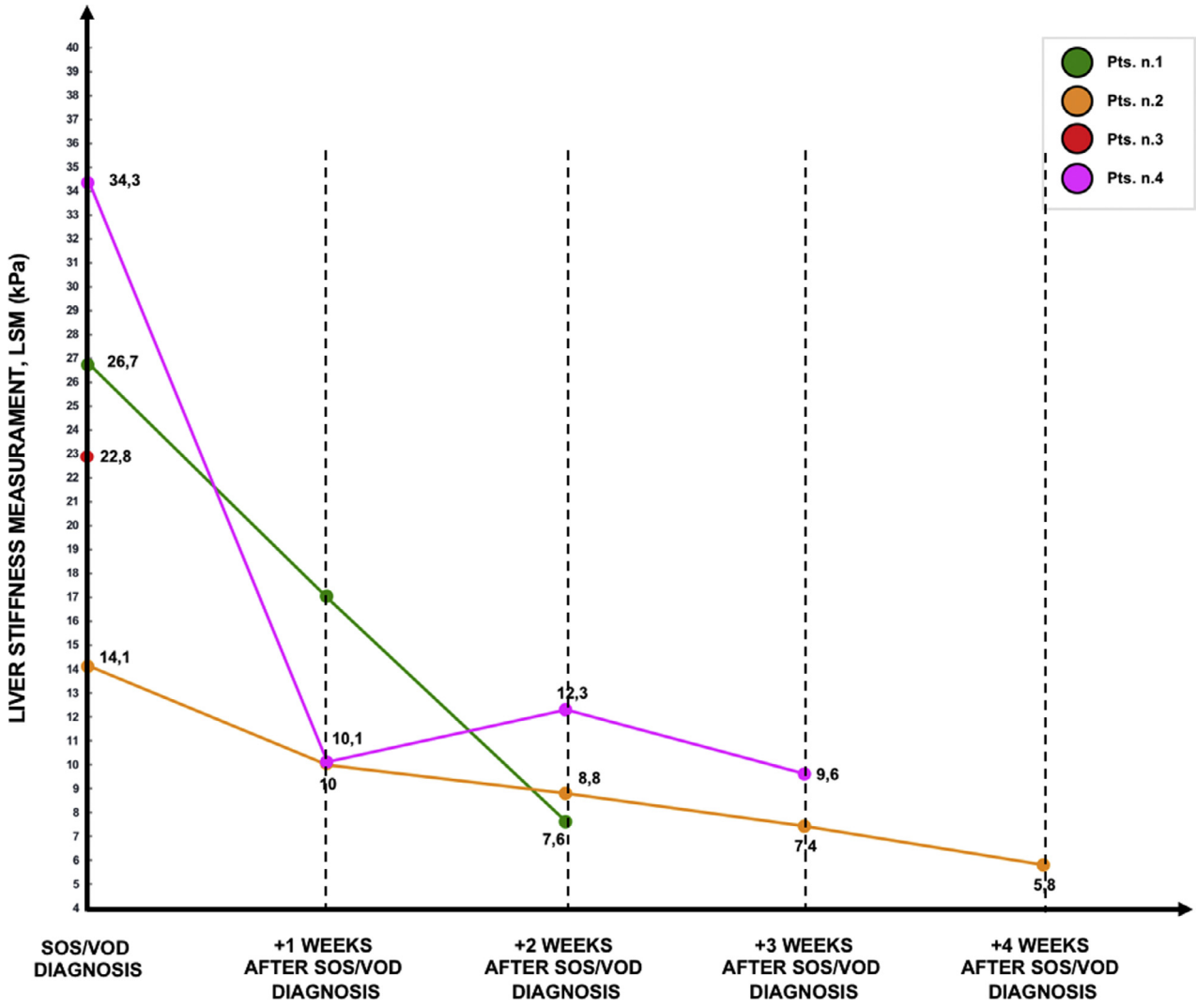

Figure 3. Variation in LSM values after VOD/SOS-specific treatment of patients with VOD/SOS diagnosis.

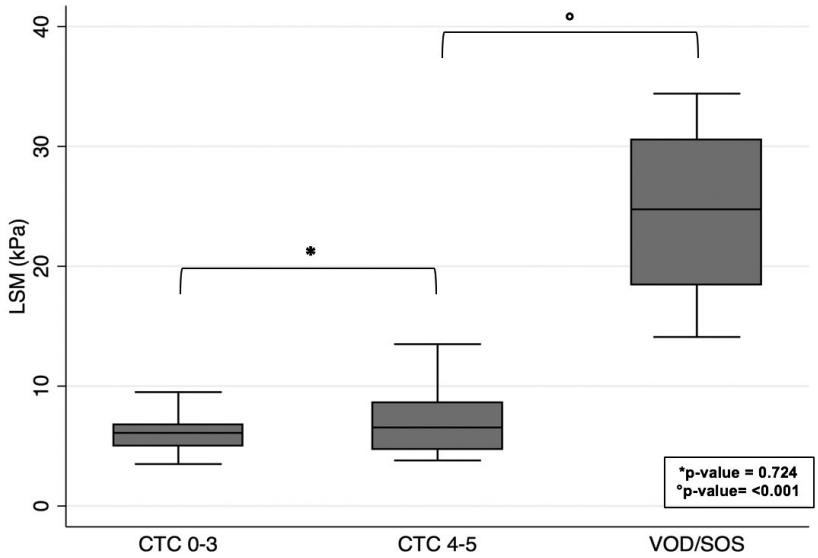

Figure 4. Boxplot of maximum LSM values after HSCT in patients with VOD/SOS diagnosis by CTC degree.

preclinical diagnosis of VOD/SOS, as assessed by ultrasound elastography techniques.

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