Elastography improves accuracy of early hepato-biliary complications diagnosis after allogeneic stem cell transplantation

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Supplementary materials for:

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Online Methods

Patients

Between July 2017 and July 2019, 212 patients underwent an allo-HSCT in the adult hematology and transplantation unit at Saint Louis Hospital (Paris, France). As a standard care, all patients had ultrasonography, Doppler, TE and 2D-SWE elastography before transplantation and at day+7 and day+14 after allo-HSCT. A total of 161 patients were included. All patients received ursodeoxycholic acid (15 mg/kg/day) as SOS prophylaxis, from conditioning initiation until day+100. Prophylaxis for infections included valacyclovir, trimethoprim/sulfamethoxazole, and antifungal therapy (fluconazole or voriconazole). CMV and EBV monitoring was performed twice weekly during hospitalization and weekly until day+100. In patients with prior HBV infection, reactivation was prevented by entecavir treatment until withdrawal of all immunosuppressive drugs. Liver blood tests were routinely performed at least twice a week until day+100 and more frequently if needed. Clinical data were extracted from medical records and included gender, age, CMV serological status, underlying hematological disease, previous history of autologous or allo-HSCT or radiotherapy, HLA matching, stem cell source, T-cell depletion, GvHD prophylaxis, GvHD status and grade if any, date and medical status at the last follow-up. The intensity of conditioning regimen was based on the Bacigalupo classification[14]. Disease risk index (DRI) was used to risk-stratify patients[15]. This study has been conducted in compliance with the Declaration of Helsinki. All patients gave their written consent for the registration of clinical and biological data (CNIL number 2093819). Data were collected and processed anonymously in a dedicated study after authorization of the National Commission for Data Protection and Liberties (CNIL number 2211540) and of the IRB 00003888 (study number 20-697).

Ultrasonography and elastography

Ultrasonography, Doppler, and elastography were performed at baseline (before conditioning regimen), at day+7, and at day+14, by one experienced radiologist (PB, AMZ or MDB) after 4-hour fasting period. Radiologists were blinded for biological or clinical status of patients at the time of examination. Two methods were used for Elastography: transient elastography (TE) with Fibroscan® (Echosens, Paris, France) and 2D real time shear wave (2D-SWE; Aixplorer, SuperSonic Imaging SA, Aix-en-Provence, France) with a 3.5MHz convex ultrasound probe (SCX-6-1) for abdominal exam and a 7.5Mhz linear ultrasound probe (SL-10-2) for gallbladder exam. For TE, 10 measurements were obtained. TE measurements were considered unreliable when they showed a interquartile (IQR) / median (M) ratio > 30%, according to international consensus criteria[16]. We defined measure failure as the impossibility to obtain reliable value. For the 2D-SWE, 3 acquisitions were obtained and mean value was calculated using "multi Q box" function. The box was positioned within the liver parenchyma, placed at more than 2 cm beneath the Glisson capsule and avoiding the big vascular structures, with a region of interest (ROI) of 100 mm^2. Failure measure of 2D-SWE was defined by impossibility to obtain any value, with < 50% fill-in color in the elastogram box. For all ultrasonography and Doppler examination, the following criteria, were assessed by radiologists: liver (preaortic and midclavicular vertical axis) and splenic (3 orthogonal axis) measurements, measurement of the gallbladder wall, ascites (none, mild, moderate, profuse), portal vein diameter, portal vein direction flow and maximal flow velocity, spectral waveforms of the hepatic veins (triphasic, biphasic, monophasic). Based on Lassau et al[6], and EBMT classification, an ultrasound-Doppler score based on 7 criteria was performed: (1) Hepatomegaly (increase of 2 of 3 measures, greater than 2 cm relative to baseline measure), (2) Splenomegaly (increase greater than 1 cm relative to the baseline measure of greatest axis), (3) Gall bladder wall thickening (> 6 mm),

(4) Dilatation of main portal vein (> 12 mm), (5) Ascites, (6) Decrease mean velocity of portal vein (less than or equal to 10 cm/sec), (7) Hepatofugal flow or no flow in portal vein. In case of liver blood test abnormalities or when a liver involvement was suspected, additional ultrasonography and elastography could be performed at the discretion of the physician.

Liver test and definition of liver involvements

Before allo-HSCT, previous history of liver disease was explored by analyzing medical history and liver blood tests: serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase, alkaline phosphatase, bilirubin, albumin, prothrombin time, ferritinemia, nuclear antigen testing, viral load and serology for hepatitis B, C and E viruses.

During hospitalization, liver involvement was considered if increased serum AST or ALT level above twice the upper limit of normal values (ULN) in two consecutive measures, hyperbilirubinemia (above 17 µmol/L) in two consecutive measures, or both, occurred. Cholestasis without hyperbilirubinemia or elevated aminotransferase was not considered.

All medical records were retrospectively reviewed to determine the final liver diagnosis, based on clinical examination, laboratory results, medical imaging (ultrasonography, CT scan), hepatic venous portal gradient (HVPG) and pathological reports (if any) and clinical evolution under treatment.

GvHD was graded according to the modified Glucksberg's classification[17] and liver GvHD diagnosis was considered in patients without evidence of infectious disease (no bacterial, fungal or viral documentation, including A, B, C and E hepatitis and herpes virus plasma viral load), normal imaging, without clinical sign for SOS (i.e. increased weight, ascites, or painful

hepatomegaly) or drug-induced liver toxicity. When patients had no other organ involvement than suspected liver GvHD, a biopsy was performed to ascertain the diagnosis (n=3).

SOS diagnosis was suspected when EBMT, Baltimore or modified Seattle clinical criteria were present in patients[18–20]. Diagnosis was retained only if proven on liver biopsy (n=3), or using ultrasonography and Doppler criteria, as described in EBMT classification[20]. If not proven on biopsy or ultrasonography, SOS diagnosis was considered only in the absence of infectious disease, drug toxicity or GvHD (n=3), as recommended by EASL guidelines [8,21]. Retrospective review of medical history was used to adjudicate the final diagnosis according to clinical evolution and treatment efficiency, blinded about elastography measures.

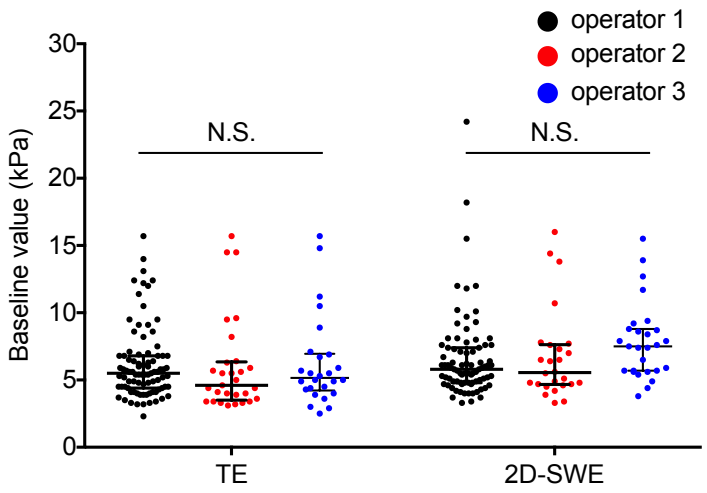
Drug-induced liver injury (DILI) was defined according to EASL guidelines[22] after exposure to a drug already known to be associated with hepatotoxicity, with normal imaging to exclude steatohepatitis or biliary tract disease, no infectious disease (including A, B, C, E hepatitis and herpes virus nucleic acid detection), and if liver blood tests improved after drug was withdrawn. Biopsy confirmed DILI diagnosis in one case in which a SOS was first suspected.

Statistical analysis

Categorical variables were expressed as numbers and percentages, and continuous variables as median and interquartile range (IQR). All statistical tests were performed using Prism v7.0a (GraphPad) or R v3.6.0. Two-group comparisons were performed with Mann-Whitney U test and multiple comparisons were performed with Kruskal-Wallis test followed by a Dunn's correction for multiple comparisons. Two-way ANOVA test followed by Dunnet correction was used for multiple comparisons of data with normal distribution and equal variance. ROC curves were built for continuous variable and area under the ROC curve (AUROC) was

calculated for SOS diagnosis using all ultrasound and doppler criteria, 2D-SWE and TE measurements at baseline, day+7 and day+14. Best cut-off value was determined using Youden index. Scores performance were calculated using an intention to diagnose approach using 3x2 table, as previously described to assess performance of diagnostic tests[21,23]. All statistical tests were two-tailed with a significance level of 0.05.

Supplementary Figure 1, Debureaux et al.



Supplementary figure 1: TE and 2D-SWE measures are not operator dependent

Baseline TE and 2D-SWE were performed by three experienced radiologists. The comparison of values between operators did not show any significant difference (two-way ANOVA with Tukey test for multiple comparison).

Supplementary table 1. Characteristics, diagnosis and outcomes of positive EBMT patients

N°	Gender, age	Date of onset	Clinic signs	Max Cytolysis (ULN)	Max Bilirubin (µmol/L)	Imaging	2D-SWE measures			Additional data	Liver biopsy	Treatment	Outcomes at day+100	**Final diagnosis**
							B	D+7	D+14					
1	Female, 62y	D+13	Bilirubin, weight gain, painful HMG	200	91	Not done	6.1	5.7	D	No GvHD sign	SOS (Post mortem)	None	Death at D+14 from multiorgan failure	**Very Severe SOS**
2	Male, 21y	D+6	Bilirubin, weight gain, ascites, painful HMG	2	157	HSMG, ascites, flow	9.3	18.5	13.9	No GvHD sign	Not done	Defibrotide	Improvement with defibrotide	**Very severe SOS**
3	Male, 48y	D+12	Bilirubin, weight gain, ascites, painful HMG	1,5	355	HMG, ascites, velocity, flow	6.1	13.7	F	No GvHD sign	Not done	None	Death from multiorgan failure	**Very severe SOS**
4	Female, 61y	D+21	Weight gain, ascites, painful HMG	2	23	HSMG, ascites, velocity	6	8	14.5	HVPG 10 mmHg	SOS	Defibrotide	Improvement with defibrotide then death from sepsis	**Severe SOS**
5	Male, 45y	D+15	Bilirubin, weight gain, ascites	20	50	Ascites	6.1	5.2	9.1	None	Not done	None	Resolved without treatment	**Moderate SOS**
6	Male, 61y	D+22	Weight gain, ascites, painful HMG	2	17	HSMG, ascites	12	8.7	12.8	HVPG 12 mmHg	SOS	Defibrotide	Improvement on defibrotide	**Mild SOS**
7	Male, 67y	D+23	Bilirubin, weight gain, ascites	12	155	HMG, ascites	9.4	9.6	10.5	HVPG 4 mmHg, Gut GvHD	liver GvHD	IS	Improvement on IS	**GvHD**
8	Male, 61y	D+39	Bilirubin, weight gain, ascites	2	90	Ascites	12.7	7.6	8	Skin and gut GvHD	Not done	IS	Death from refractory GvHD	**GvHD**
9	Female, 67y	D+36	Bilirubin, weight gain, ascites	12	420	Ascites	2.4	F	4.6	Skin and gut GvHD	Not done	IS	Improvement on IS	**GvHD**
10	Female, 67y	D+5	Bilirubin, weight gain, ascites, painful HMG	1	250	HMG, ascites, CHF signs	7.4	28.3	D	Skin and gut GvHD	Not done	IS Furosemide	Initial improvement then death from refractory GvHD	**GvHD / CHF**
11	Female, 25y	D+24	Bilirubin, weight gain, ascites	3	53	Ascites	4.8	4.9	F	Graft failure	Not done	Antibiotics	Death from sepsis	**Sepsis**
12	Female, 33y	D+11	Bilirubin, weight gain, ascites	1,5	113	HMG, ascites	4.4	4.4	4.8	Septic shock	Not done	Antibiotics	Improvement on antibiotics	**Septic shock**
13	Female, 27y	D+8	Bilirubin, ascites, painful HMG	6	53	HSMG, ascites	4.1	3.5	5.3	HVPG 4 mmHg, positive HVE PCR	Inflammatory lesions	None	Decreased of IS and resolved without specific treatment	**HVE infection**
14	Male, 34y	D+9	Bilirubin, weight gain, painful HMG	3,5	135	HMG	4.9	7.4	3.9	No GvHD sign	Not done	None	Improved after the withdrawal of cyclosporin without any treatment	**Cyclosporin cholestasis**
15	Male, 26y	D+35	Bilirubin, ascites	3	49	HMG, ascites, velocity, portal vein	12	9.5	7.6	relapse of hepatitis post-AA, candidemia, and aspergillosis treated by caspofungin and posaconazole	Not done	Prednisone Switch antifungal	Improved after prednisone treatment and withdrawal of posaconazole	**Lobular hepatitis / Drug injury**

AA: aplastic anemia, B: baseline, CHF: congestive heart failure, D: deceased before measure, F: measure failure, Flow: pseudo portal flow, GvHD: Graft-versus-host-disease, HMG: hepatomegaly, HSCT: hematopoietic stem cell transplantation, HSMG: hepatosplenomegaly, HVPG: hepatic venous pressure gradient, IS: immunosuppressive treatment, MAC: myeloablative conditioning, MUD: Matched unrelated donor, MMUD: Mismatch unrelated donor, PBSC: peripheral blood stem cells, Portal vein: increase portal vein diameter, PS: performance status, SOS: sinusoidal obstruction syndrome, TBI: total body irradiation, Velocity: decrease in velocity or reversal of the portal flow. 2D-SWE: 2D real-time shear wave
EBMT criteria used: bilirubin > 34 µmol/L, weight gain > 5%, ascites and painful HMG, hemodynamical (HVPG > 10 mmHg), ultrasound/doppler (HMG, ascites and velocity)