SUPPLEMENTARY MATERIAL

Residual burden of liver disease after HCV-clearance in hemophilia: a word of caution in the era of gene therapy

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Supplementary methods:

Joint evaluation in agreement to the protocol of the multidisciplinary program of the Joint ultrasound Evaluation in Hemophilia (JOINEM study, approved by the Milan Area 2 Ethics committee, number 199_2021bis):

Physical examination

The HJHS score was performed by a trained physiotherapist with several years of experience in the evaluation and management of patients with hemophilia. The HJHS is based on the physical examination of the six index joints (elbows, knees and ankles) and gait assessment. The items for this scale are scored as follows: swelling (0–3), duration of swelling (0–1), muscular atrophy (0–2), crepitus on motion (0–2), range of motion (flexion loss 0–3, extension loss 0–3), strength (0–4) and joint pain (0–2), for a total score of 0–20 points per joint. The global gait score ranges from 0 to 4. Higher scores indicate poorer joint condition and range from 0 to 124 overall.[1]

Musculoskeletal ultrasound imaging

The HEAD-US was performed by a rheumatologist with experience in musculoskeletal ultrasound and specifically trained in the use of the HEAD-US score. A Philips Affiniti 50 machine with a 5-12 MHz linear probe was employed. In the HEAD-US score, the elbows, knees, and ankles are evaluated and scored based on synovitis (0 - 2), articular cartilage damage (0 - 4), and subchondral bone damage (0-2). Possible scores range from 0 to 8 per joint with a total score ranging from 0 to 48. Higher scores indicate a more severe arthropathy.[2]

Reference list:

1. Feldman BM, Funk SM, Bergstrom B-M, et al. Validation of a new pediatric joint scoring system from the International Hemophilia Prophylaxis Study Group: validity of the hemophilia joint health score. *Arthritis Care Res.* 2011;63(2):223–230.
2. Martinoli C, Della Casa Alberighi O, Di Minno G, et al. Development and definition of a simplified scanning procedure and scoring method for Haemophilia Early Arthropathy Detection with Ultrasound (HEAD-US). *Thromb. Haemost.* 2013;109(6):1170–1179.

Supplementary Table 1: virological data

Viral etiology	**Total**
HCV	33 (28%)
HCV/HBV	53 (45%)
HCV/HIV	10 (8%)
HCV/HBV/HIV	23 (19%)
HCV genotype	
1a	47 (40%)
1b	32 (27%)
2	9 (8%)
3	12 (10%)
4	1 (1%)
Genotype not available	18 (15%)
Eradication regimen or spontaneous eradication	
IFN standard	5 (4%)
IFN standard + ribavirin	2 (2%)
PEG-IFN+ribavirin	33 (28%)
PEG-IFN+telaprevir+Ribavirin	1 (1%)
IFN lambda+ribavirin+daclatasvir	2 (2%)
DAA+ribavirin	11 (9%)
DAAs	53 (45%)
Spontaneous eradication	12 (10%)
Previous failure	
PEG-IFN+ribavirin	29 (24%)
PEG-IFN	1 (1%)
IFN standard	10 (8%)
DAAs	1 (1%)
HBV status	
HBsAg +, on antiviral	3 (3%)
HBsAg +, not on antiviral	1 (2%)
HBsAg +, DNA negative	4 (3%)
HBsAb +/HBcAb+	60 (50%)
HbsAb -/HBcAb+	14 (12%)
HBsAb +/HBcAb -	37 (31%)
HBsAb -/HBcAb-	7 (6%)

Supplementary Table 2: Age distribution by metabolic risk factors (RFs) and alcohol consumption

Alcohol consumption	**Age**	**p**
0-6 U/week (n=89)	52 (36-85)	0.345 (J-T)
7-14 U/week (n=16)	59 (39-73)	
≥14 U/week (n=14)	53 (39-87)	
Metabolic RFs	**Age**	**p**
0 (n=26)	48 (36-80)	0.007 (J-T)
1 (n=27)	51 (36-74)	
2 (n=40)	57 (39-87)	
≥3 (n=26)	53 (42-79)	
Combined RFs	**Age**	p
Alcohol consumption <14 U/week, Metabolic RFs < 3 (n=80)	54 (36-85)	0.375 (K-W)
Alcohol consumption ≥14 U/week, Metabolic RFs <3 (n=13)	51 (39-87)	
Alcohol consumption <14 U/week, Metabolic RFs ≥3 (n=25)	52 (42-79)	
Alcohol consumption ≥14, Metabolic RFs≥3 (n=1)	73	

RFs: Risk Factors; J-T: Jonckheere-Terpstra test; K-W: Kruskal Wallis test

<u>Supplementary Table 3</u>: Characteristics of patients who developed hepatocarcinoma and treatment strategies

Characteristics	**Patient 1**	**Patient 2**	**Patient 3**
Etiology	HCV	HCV	HCV
HBV serology	HBcAb neg/HBsAb neg	HBcAb pos/HBsAb pos	HBcAb pos/HBsAb pos
Alcohol intake *(units/week)*	7	0	0
Comorbidities	Arterial hypertension, ischemic heart disease, type 2 diabetes, cholelithiasis	Anxiety disorder, nephrolithiasis	None
Age *(years)* at HCV eradication	51	46	65
Age *(years)* at screening	57	51	69
Age *(years)* (and time) at HCC diagnosis	57 (Feb 2021)	51 (Apr 2021)	61 (Nov 2013)
Last LSM *(KPa)* before first HCC diagnosis	Not available	20	11
Already in semestral US surveillance	No	No	Yes
Peri-HCC histology	Non-alcoholic steatohepatitis	Micronodular cirrhosis	Micronodular cirrhosis
Milan criteria at diagnosis	Out	Out	In
First line of HCC treatment	Resection and MWTA (March 21)	Resection (May 21)	RFTA (December 2013)
Further treatments before liver transplant	TACE, atezolizumab-bevacizumab (April 2022)	MWTA (September 2021)	HCC relapse in 2022: TACE (total: 6 treatments) MWTA 1 treatment
Outcome after liver transplant	Relapse	Disease free at last visit (patients transplanted in other hospital)	Disease free at last visit

neg/pos: negative/positive; MWTA: macro-wave thermo-ablation; TACE: trans-arterial chemoembolization

Supplementary Table 4: Liver stiffness 8 kPa threshold matched with the most important clinical and US features suggestive of advanced fibrosis/cirrhosis

	Liver stiffness categories (kPA)		
	<8 *(n=66)*	**≥8** *(n=24)*	**p**
At least one US sign of cirrhosis	12 (18%)	20 (83%)	<0.001
Irregular or nodular margins	5 (8%)	12 (50%)	<0.001
Caudate lobe hypertrophy	1 (2%)	5 (21%)	0.002
Splenomegaly	9 (14%)	16 (68%)	<0.001
Portal vein dilatation	2 (3%)	4 (17%)	0.033
Previous events of decompensation	0	4 (17%)	<0.001
History of esophageal varices	0	8 (67%)	0.009
NAFLD	23 (35%)	7 (29%)	0.386
Alcohol consumption (7-14 U/week)	8 (12%)	2 (9%)	0.662
Alcohol consumption (>14U/week)	8 (12%)	4 (17%)	0.517
Any metabolic risk factor	45 (68%)	22 (92%)	0.020
Any metabolic risk factor or alcohol >7 U/week	48 (73%)	22 (92%)	0.052
Platelet count < 150.000/mcL	4 (6%)	6 (25%)	0.014
Platelet count < 100.000/mcL	0	4 (17%)	<0.001