Outcome prediction of diffuse large B-cell lymphomas associated with hepatitis C virus infection: a study on behalf of the Fondazione Italiana Linfomi

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Appendix Supplementary Material

Supplementary Methods

HCV infection status

HCV serology was detected at the time of the initial staging of lymphoma using a second or third generation enzyme-linked immunosorbent assay (ELISAs). HCV immunoreactivity was confirmed by a third generation recombinant immunoblott assay (RIBA). In 332 patients qualitative detection of HCV-RNA was performed. Serum HCV-RNA load was determined by quantitative reverse-transcription polymerase chain reaction (detection value of 0.5-7500 KIU/ml) and was recorded at baseline and, when available, up to the maximum elevation during treatment. HCV genotype was determined by molecular assays (genotyping, LIPA).

Definition of hepatotoxicity

Definition of hepatotoxicity relied on the National Cancer Institute common toxicity criteria grading scale (NCI-CTC AE ver. 3.0) and severe hepatotoxicity was defined as a grade 3 (≥5 times the upper level of normal, ULN, and <20 ULN) or grade 4 (≥20 ULN) alanine-transaminase (ALT) level.

Hepatic evaluation

Baseline virologic and hepatic evaluation included HCV serology, HBV serology, qualitative and quantitative HCV-RNA, quantitative HBV-DNA, HCV genotype, laboratory tests of cholestasis and cytolysis, albumin and prothrombine time, cryoglobulins, liver imaging and liver histology. Liver function laboratory tests were collected also after each cycle of therapy and during follow-up period.

Hepatic histology was scored by histology activity index (Knodell-HAI) for inflammation (grading: 0-18) and METAVIR score for fibrosis assessment (staging: F0-F4), when available.

Treatments and response criteria

Patients suitable for curative-intent treatment underwent to chemotherapy or immuno-chemotherapy. Curative-intent chemotherapy regimens included anthracycline-based schemes as CHOP-like and IIIrd generation regimens with or without rituximab. Other less intensive regimen included parenteral or oral alkylators with or without rituximab. In unfit patients according to comprehensive geriatric assessment, palliative management options (surgery, radiotherapy or eventually steroids alone) or careful observation were adopted. Complete remission (CR) was defined according to response criteria for malignant lymphomas.[1]

For each patient, progression-free survival (PFS) was calculated as the time from the date of first line of therapy until date of relapse, progression or death from any cause. Overall survival (OS) was calculated as the time between the date of diagnosis and the date of death or last follow-up for censored cases.

Disease-specific survival (DSS) was calculated as the time between the date of diagnosis and the date of death for lymphoma progression or last follow-up for censored cases.

Parameters analyzed for prognostic influence

Variables analyzed for influence on OS and PFS were: age >60 years; LDH greater than normal value; liver, splenic and bone marrow involvement by lymphoma; extranodal sites (more than 1 *vs* 1); performance status (PS) (ECOG 2-3 *vs* 0-1); Ann Arbor stage (III-IV *vs* I-II); IPI and R-IPI and risk groups; albumin level less than 3.5 g/dL; HBsAg positivity; presence of B symptoms; baseline HCV-RNA load (≥1000 KIU *vs* <1000 KIU); HCV genotype (2 *vs* 1); baseline ALT (elevated *vs* normal); occurrence of SH during treatment; INR ≥1,7; total-bilirubin ≥2 mg/dl; presence of ascites; Child score (B+C *vs* A); hepatic histology (HAI ≥9 and/or stage ≥2); AVT with (peg-)interferon a ± ribavirin after 1st line treatment (yes vs no); reduction or suspension of steroids (yes *vs* no).

Supplemental statistical methods

Toxicity (SH) was analyzed by means of time-dependent Cox's proportional hazards regression.

All computations were performed using Statistica 7.1 (StatSoft, Tulsa, OK), STATA (StataCorpLP, College Station, TX) and Microsoft Excel 97 (Microsoft, Redmond, WA).

1.   Cheson BD, Horning SJ, Coiffier B, et al. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. J Clin Oncol. 1999;17:1244.
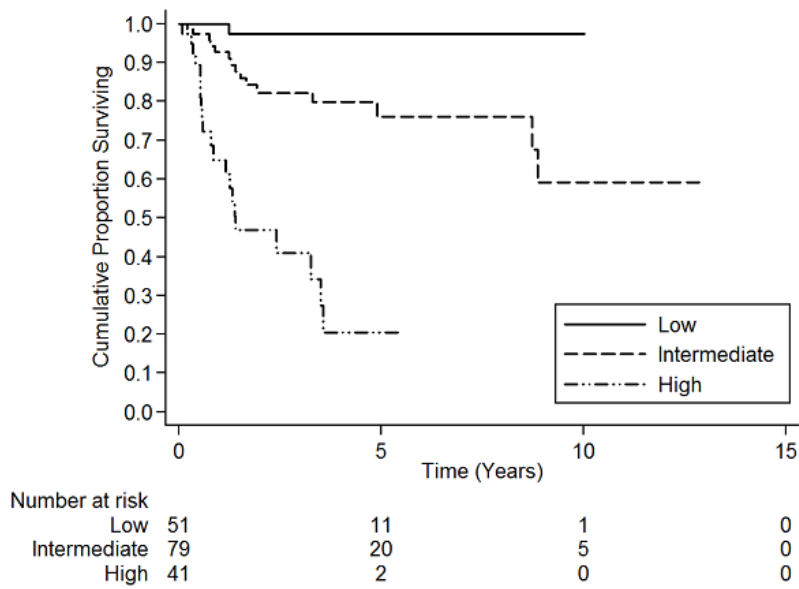
Supplementary figures
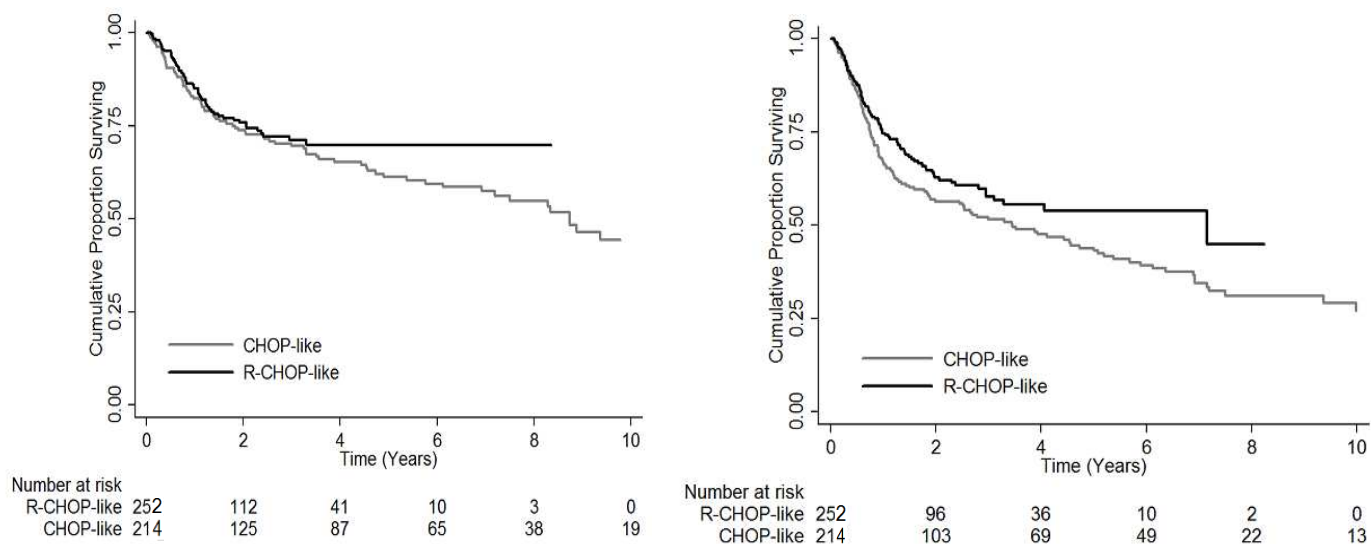
Supplementary figure 1. OS and PFS in the subgroup of patients treated with R-CHOP and CHOP (Panels A and B)
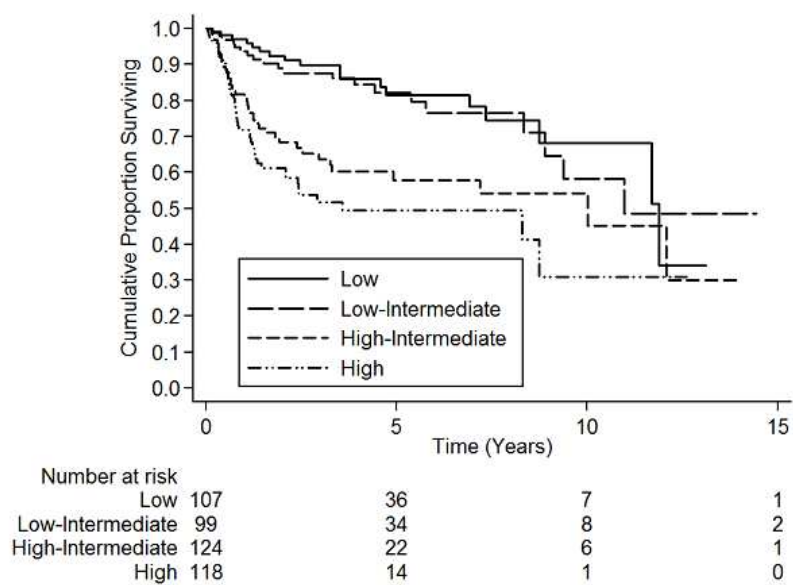
Supplementary figure 2. OS according to standard IPI in the entire series

Supplementary figure 3. DSS according to HCV prognostic score (HPS) categories in patients treated with curative-intent therapy (171 patients)
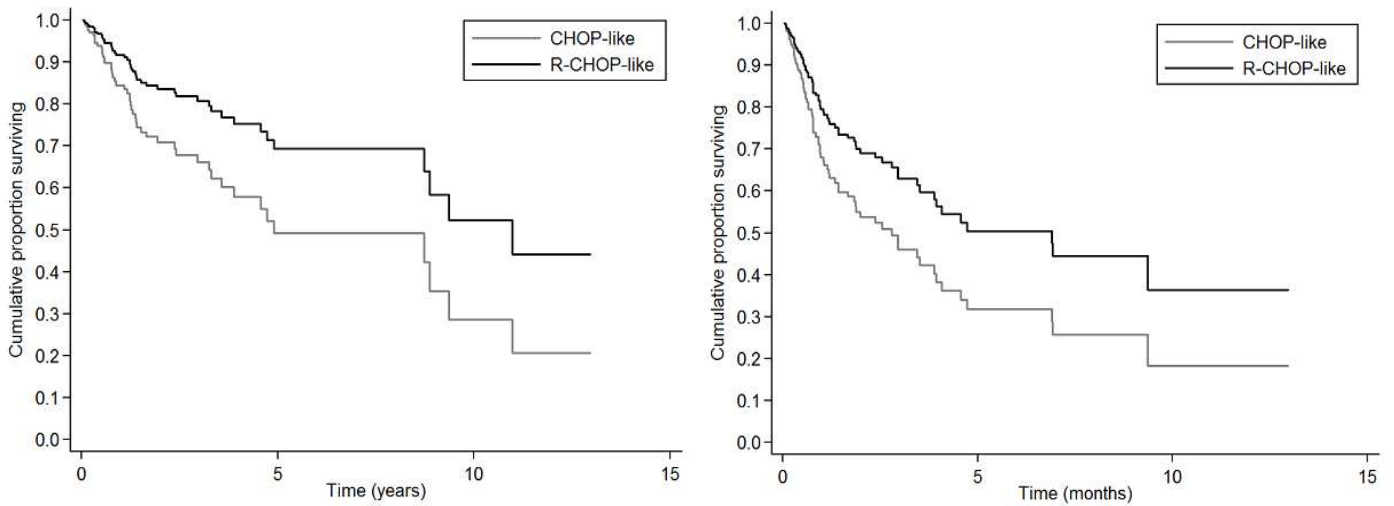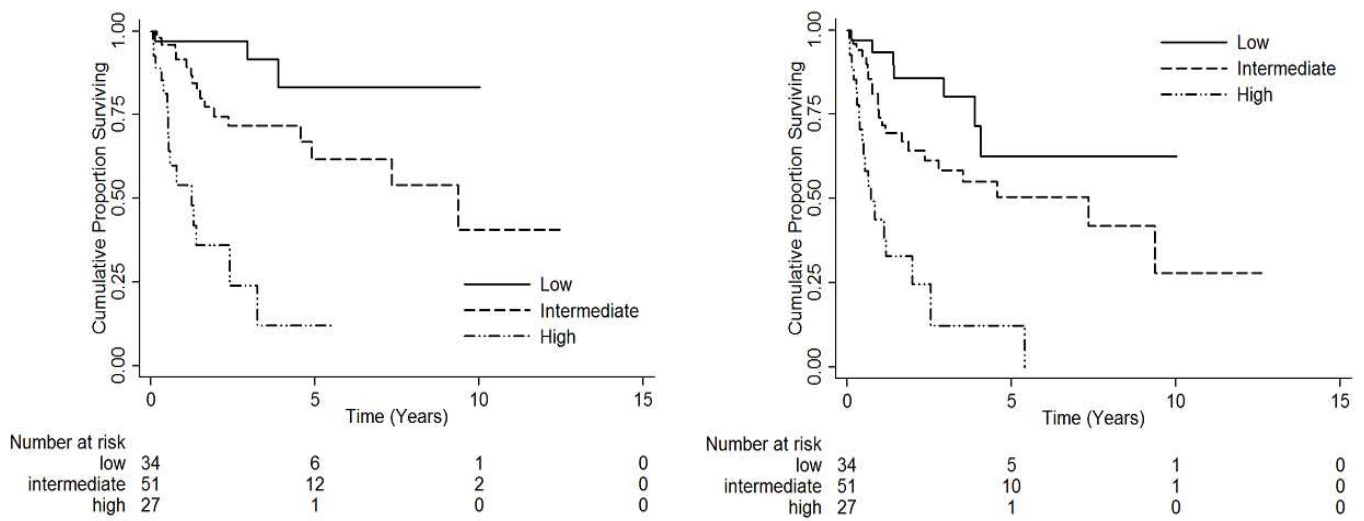

Supplementary figure 4. OS and PFS in the subgroup of patients treated with R-CHOP and CHOP after adjusting for HPS (Panels A and B)

Number at risk

low	34	6	1	0
intermediate	51	12	2	0
high	27	1	0	0

Number at risk

low	34	5	1	0
intermediate	51	10	1	0
high	27	1	0	0

Supplementary figure 5. OS (Panel A) and PFS (panel B) according to HCV prognostic score (HPS) categories in 2/3 of patients (testing sample)

Number at risk

low	17	5	0	0
intermediate	28	8	3	0
high	14	1	0	0
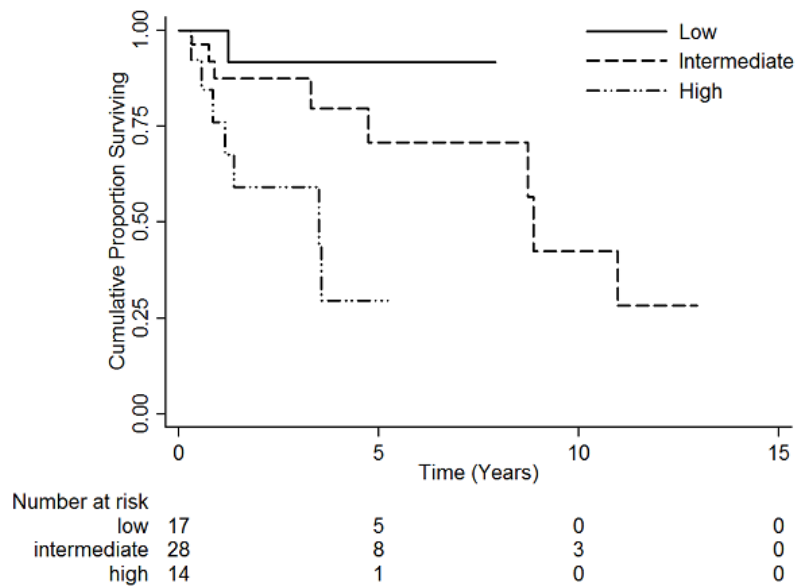
Supplementary figure 6. OS according to HCV prognostic score (HPS) categories in 1/3 of patients (validation sample)

Supplementary Tables

Supplementary Table 1 – Comparison of baseline clinical and virological features between 535 patients with HCV-positive DLBCL treated with curative-intent therapy and 77 patients managed with palliative intent options

	Curative intent (N=535)		Palliative intent (N=77)		
	n	%	N	%	p-value
Age>60	388	73	63	83	0.050
Males/ Females	262/273	49/51	41/36	53/47	0.544
Ann Arbor stage					0.437
I-II	171	32	28	37	
III-IV	363	68	49	63	
BM involvement	102	21	19	25	0.440
Splenic involvement	171	35	14	18	0.008
Liver involvement	74	15	11	15	>0.900
B symptoms	164	31	21	27	0.490
ECOG≥2	126	24	31	40	0.005
Extranodal sites ≥2	163	35	24	31	0.536
LDH elevated	279	55	41	54	0.828
IPI					0.799
Low	107	24	17	23	
Low-int	99	22	14	18	
High-int	124	28	21	27	
High	119	26	25	32	
R-IPI					0.754
Very good	26	5	4	5	
Good	197	41	28	36	
Poor	264	54	45	59	
HCV-RNA positive	303	91	31	91	>0.900
HCV-RNA>10^6 UI/ml	101	49	10	77	0.053
HCV genotype					0.281
1	71	49	8	54	
2	64	45	5	33	
3	4	3	2	13	
4	3	2	0	0	
6	1	1	0	0	
Cryoglobulins	34	8	2	4	0.413
HBsAg-positive	14	3	2	3	>0.900
AntiHBc-positive	132	34	15	31	0.677
Albumin <3.5 g/dl	107	28	19	32	0.484
Total bilirubin ≥2 mg/dl	27	9	5	11	0.784
INR>1.7	11	5	1	3	>0.900
ALT levels at baseline					0.265
≤ULN	241	54	38	62	
>ULN - 2.5 x ULN	140	32	20	32	
>2.5 – 5 x ULN	49	11	2	3	
>5 – 20 x ULN	15	3	2	3	
Child score					>0.900
A	189	93	39	93	
B/C	14	7	4	7	

Supplementary Table 2 – Comparison of the IPI distribution with respect to HPS scores classes in 171 HCV-positive DLBCL patients with all HPS parameters (concordance p<0.001)

IPI	HPS score n (%)			Total
	Low	intermediate	high	
Low	21 (41)	22 (28)	4 (10)	47 (27)
Low-intermediate	15 (29)	25 (32)	2 (5)	42 (25)
Intermediate-high	12 (24)	17 (21)	13 (31)	42 (25)
High	3 (6)	15 (19)	22 (54)	40 (23)
Total	51 (30)	79 (46)	41 (24)	171 (100) (100)