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Cell of origin (COO), BCL2/MYC status and IPI define a group of patients with Diffuse Large B-cell Lymphoma (DLBCL) with poor prognosis in a real-world clinical setting

1. Introduction

Multiple studies have shown that cell of origin (COO) has an impact on overall prognosis in diffuse large B cell lymphoma (DLBCL), with the activated B-cell (ABC) as determined by gene expression profile (GEP) or non-GCB (germinal center B-cell) by immunocytochemistry (IHC) subgroup experiencing worse outcomes when treated with standard R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) chemo immunotherapy [1–5]. In contrast, the German Study group, failed to identify any differences in outcome between GCB and ABC subtypes of DLBCL [6]. Furthermore, two recent retrospective analyses also found that the non-GCB origin of this malignancy had no prognostic impact in patients with limited-stage DLBCL [7,8]. More, results from the recent Goya study once again confirmed that the assignment to COO subsets by NanoString has a prognostic impact with a significantly longer 3-year PFS for the GCB subtype [9]. In addition, a recent retrospective study has also confirmed that COO, determined by both GEP and IHC, is a strong predictor of survival in DLBCL [10].

Some investigators have suggested that the poor outcome of patients with ABC DLBCL may in part be attributed to the higher incidence of MYC and BCL2 expression (double expressers, DE) encountered in this subgroup [11–14]. Because of this conflicting data, we planned the present study with the primary aim of evaluating the prognostic impact of COO and MYC/BCL2 status, followed by an assessment of whether the combination of COO, BCL2/MYC status and IPI could define a group of patients with poor prognosis in a real-world clinical setting.

2. Patients and methods

This retrospective study of previously untreated patients with stage I-IV DLBCL/NOS (according to WHO criteria), included all patients treated with R-CHOP / CHOP-like regimens, between January 2009 and December 2017. After approval by local institutional review boards, patient data were collected from academic/university and community centres in Modena, Rome, Pisa, Rionero in Vulture, and Perugia in Italy and Haifa in Israel. The study was performed in accordance with the Declaration of Helsinki. The inclusion criteria were: histopathological diagnosis of DLBCL/NOS, no previous therapy, age > 18 years, HIV negativity, staging and response evaluation by PET-CT, minimum follow-up 24 months, complete clinical availability of all clinical and laboratory data and survival outcome. To assign COO subset we used both Hans algorithm (CD10, BCL6 and MUM-1, cutoff 30 % reactivity) [3] and Lymph2Cx assay on RNA extracted from FFPE samples [15]. For DE status we utilized IHC staining for MYC (positive ≥40 %) and BCL2 (positive ≥50 %), while double-hit (DH) and triple-hit (TH) cases were defined by FISH testing for rearrangements in MYC, BCL2 and BCL6. As FISH was not routinely performed at diagnosis in all cases and was too expensive, we retrospectively and arbitrarily limited testing for GCB subset of patients with Ki 67 positivity of > 85 % of neoplastic cells. IHC cut offs were made

Table 1
Clinical features of the 213 eligible patients with DLBCL.

Variable	N	Median (range)	Mean (SD)
Age, years	213	64 (18−90)	65 (15)
Variable	N	Status	n (%)
Age	213	>60	127 (60)
Gender	213	M	103 (48)
LDH	149	>ULN	73 (49)
Stage	207	III-IV	104 (50)
ECOG PS	203	>1	29 (14)
ENS	149	>1	26 (17)
B-symptoms	203		68 (34)
IPI	188		
		0−1	53 (28)
		2	50 (27)
		3−5	85 (45)
Treatment	207		
		R-CHOP	160 (77)
		R-COMP	41 (20)
		Other regimens[a]	6 (3)
COO by Lymph2Cx	155		
		ABC	58 (37)
		GCB	76 (49)
		Unclassified	21 (14)
Hans algorithm	207		
		GCB	83 (40)
		non-GCB	124 (60)
Bcl2	206	+	139 (67)
Bcl6	206	+	158 (77)
MUM1	203	+	114 (56)
CD10	206	+	46 (22)
c-myc	201	+	31 (15)
Bcl2/c-myc	199		
		Bcl2-	66 (33)
		Bcl2+/c-myc-	108 (54)
		Bcl2+/c-myc+	25 (12)
FISH	12	No translocations	6
		Myc	3 (50)
		Bcl2	3 (50)
		DH	*2*
Response	210	CR	151 (72)

LDH: lactate dehydrogenase; ECOG/PS: Eastern Cooperative Oncology Group-Performance Status; ENS: extranodal sites; ULN: upper limit of normality; COO: Cell of Origin; IPI: International Prognostic Index; R: Rituximab; CR: complete response. R-CHOP: Rituximab plus cyclophosphamide, doxorubicin, vincristine and prednisolone; R-COMP: (rituximab plus cyclophosphamide, non-pegylated liposome-encapsulated doxorubicin, vincristine and prednisone. FISH: Fluorescence In Situ Hybridization.

[a] Other regimens: other anthracycline containing regimens + R.

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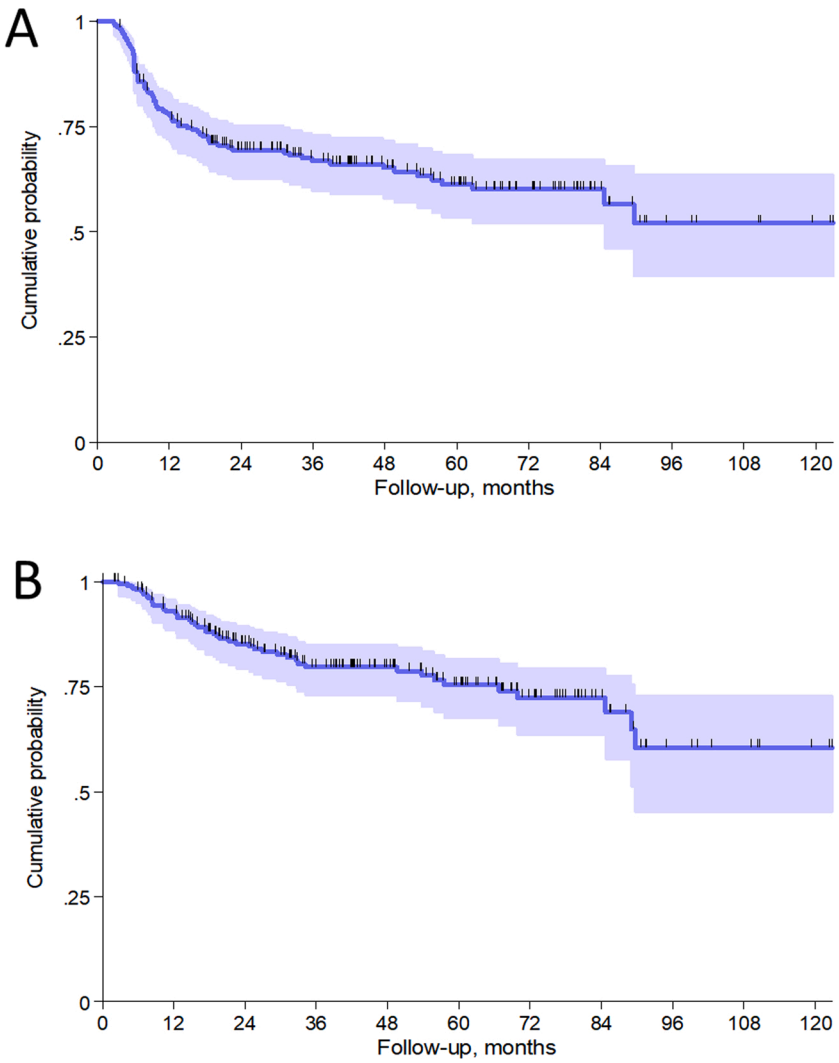

Fig. 1. A: Progression Free-Survival (PFS) and B: Overall Survival (OS) in the 213 eligible patients with DLBCL.
fx1

by the pathology team after a consensus meeting in June 2019. PET-CT imaging was interpreted locally using Deauville criteria.

3. Statistical analysis

Continuous variables were dichotomized according to literature data and categorical variables were reported as absolute and percent frequency. Groups were compared using Fisher's exact test or Chi2 test. Overall survival (OS) was defined as time from diagnosis to date of death or last contact. Progression-free survival (PFS) was defined as time from diagnosis to date of progression, death or last contact. PFS and OS were assessed by Kaplan-Meier estimates and risk groups were compared using log-rank test. Covariate effect was performed by Cox proportional hazard (PH) [16] regression and expressed as hazard ratio (HR) with 95 % confidence interval (95 %CI). Probability values less than 0.05 were considered statistically significant. All analyses done with Stata 14.2 software (StataCorp LLC, College Station, TX, USA).

4. Results

4.1. Patient characteristics

Clinical characteristics of our cohort of 213 patients are summarized in Table 1. Median follow-up was 48 months and PFS and OS were 61 % and 75 %, respectively at 5 years (Fig. 1). Hans algorithm identified 40 % of cases as GCB and 60 % as non-GCB DLBCL, while Lymph2Cx revealed 49 % GCB, 37 % ABC and 14 % Unclassified (Table 1). After excluding 14 % of Unclassified patients, we observed a k-statistic between COO determined by the two methods of 0.719. GCB subtype patients had better survival compared to ABC or non-GCB subsets (Fig. 2A, B).

4.2. FISH analyses

Of the 76 patients in the GCB subgroup (by Lynph2Cx), 15 had Ki67 higher than 85 %. In 12 cases, there was enough material to perform FISH. No translocations were observed in 6 patients, while in 6 we recorded : 3 cases with MYC translocation and 3 with translocation in BCL2; 2 patients were DH, with translocations in MYC / BCL2. As the aim of our research was to evaluate the impact of COO subsets and of MYC / BCL2 expression in a real world experience, we considered these observations as non-essential, and then these 6 patients were included together with all the others in the assessment of survival parameters.

4.3. Association between baseline characteristics with COO subsets and PFS

Table 2A and B shows the association of COO subsets and baseline characteristics. Covariate analysis had a strong significant association of

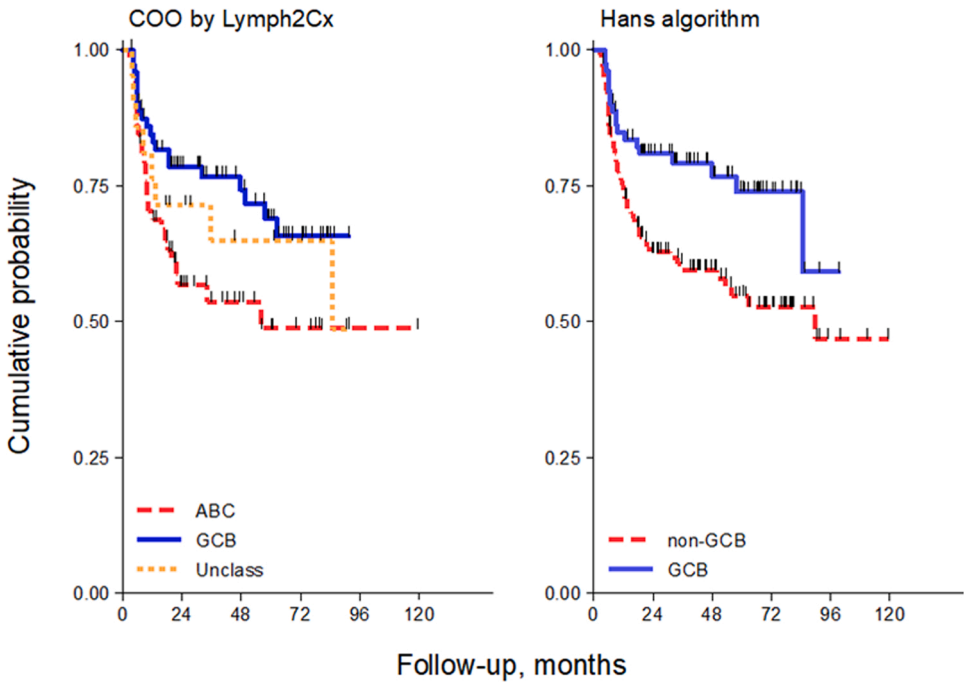

Fig. 2. Progression Free Survival (PFS) by Cell of Origin (COO).
fx2

ABC/non-GCB subtype with IPI 3-5 and BCl2+ and only marginally significance with MYC+. Thereafter we evaluated the association between variables and PFS. Univariable analysis showed significant differences between IPI 3-5 vs 0-2, stage III-IV vs I-II, ABC/non-GCB vs GCB subtype, BCl2+ vs BCl2-, BCl2+/MYC + vs BCl2- and BCL2+/MYC- vs BCL2- (Table 1, Supplemental). No significant differences were noted between double expresser and BCL2+/MYC- (P = 0.989). IPI 3-5, ABC subtype and BCL2 expression predicted poor outcome in both stage I / II and stage III / IV patients.

4.4. IPI, ABC/non-GCB and BCL2 based score

Seeing that IPI and its subsequent revisions fail in clearly recognizing truly high-risk patients we attempted to establish a score by combining both clinical and biological parameters. Considering the strong negative prognostic significance of IPI 3-5, ABC/non-GCB subtype and BCL2 expression, we evaluated a prognostic score based on these parameters. Fig. 3 and Table 3 show the scores when non-GCB subtype, BCL2+ and IPI 3-5 are all given weight 1; 26 % of patients have score 3 and PFS and OS at 5 years of 30 % and 39 %, respectively. These survival figures are significantly different from those of patients with scores 1 and 2. Using Lymph2Cx to determine COO, similar results were obtained.

5. Discussion

This study confirms the significant prognostic role of COO and shows that BCL2 positivity significantly correlated with worse PFS. We also noted, both in ABC/non-GCB and GCB subgroups, that double expresser patients had significantly worse PFS than BCL2- patients, but not with BCL2+/MYC- patients. Thus, it is apparent that BCL2 positivity determines the unfavorable trend and not MYC positivity. Our results agree with those reported for elderly DLBCL patients enrolled in the LNH03-6B trial [17], which is in contrast to results recorded by other authors [5,11–14,18,19]. In addition, unlike other studies [7,8] we observed that ABC subtype and BCL2 expression predicted poor outcome both in stage I/II and in stage III / IV. Thus, our results highlight the importance of BCL2 positivity as a poor prognostic factor and provide support for the use of BCL2 inhibitors in treating patients with BCL2+ DLBCL

In our series of patients, IPI maintains a strong prognostic impact. However, 5-year PFS is 61 % (Fig. 1A), and 45 % of cases are classified as high risk by IPI (Table 1) with a 5 years PFS of 40 % (Table 1, Supplemental). Thus, we feel, like other authors [20], that IPI probably overestimates the percentage of high-risk patients. Using our model which combines IPI 3-5 with ABC/non-GCB subtype and BCL2 expression, we show that 26 % of patients have the highest score of 3 and a 5-year PFS of 30 %. In the light of these findings, we believe that we more accurately identify a sub-group (26 % of all patients) who are truly at

Table 2
Association between baseline characteristics and response to treatment and COO by Lymph2Cx (A) and by Hans Algorythm (B).

A

Variable [n]	COO, n (%)			Total	p-value
	ABC (n = 58)	GCB (n = 76)	Uncl. (n = 21)		
Age >60 [155]	39 (67)	36 (47)	11 (52)	86 (55)	0.065
Gender M [155]	28 (48)	44 (58)	10 (45)	82 (53)	0.402
B symptoms [145]	30 (53)	17 (25)	6 (32)	53 (37)	**0.005**
IPI 3-5 [134]	35 (66)	17 (27)	6 (35)	58 (43)	**<0.001**
c-myc + [153]	14 (25)	7 (9)	4 (18)	25 (16)	0.064
MUM-1 [155]	49 (84)	18 (24)	15 (68)	82 (53)	**<0.001**
Bcl2 + [156]	49 (84)	40 (53)	13 (59)	102 (65)	**<0.001**

B

Variables [n]	HANS algorithm		Total	p-value
	GCB (n = 83)	non-GCB (n = 124)		
Age >60 [207]	45 (54)	77 (62)	122 (59)	0.313
Gender M [207]	38 (46)	65 (52)	103 (50)	0.396
B symptoms [197]	20 (26)	48 (40)	68 (35)	0.065
IPI 3-5 [182]	23 (32)	57 (52)	80 (44)	**0.006**
c-myc + [201]	7 (9)	24 (20)	31 (15)	**0.045**
MUM-1 [202]	53 (60)	82 (73)	135 (67)	0.070
BCL-2 [206]	49 (59)	90 (73)	139 (67)	**0.048**

COO: Cell of Origin; bold indicates statistically significant results.

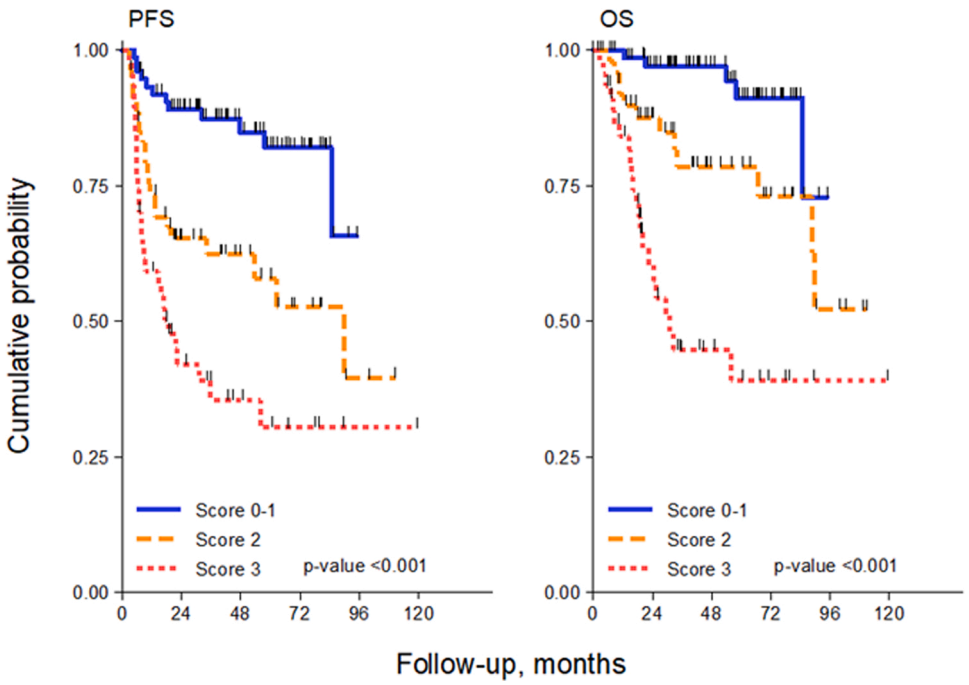

Fig. 3. Kaplan-Meier curves of PFS and OS by proposed score.

Table 3
Prognostic score by PFS and OS.

Score	n (%)	5-yr PFS (95 %CI)	HR (95CI)	p-value
0–1	77 (43)	82 (69–90)	1.00	
2	56 (31)	58 (41–71)	3.16 (1.56–6.40)	0.001
3	48 (26)	30 (16–46)	6.01 (3.05–11.8)	<0.001
		3 vs 2	1.90 (1.09–3.31)	0.024
Score	n (%)	5-yr OS (95 %CI)	HR (95 %CI)	p-value
0–1	77 (43)	91 (77–97)	1.00	
2	56 (31)	79 (62–88)	3.77 (1.32–10.8)	0.013
3	48 (26)	39 (22–56)	11.4 (4.28–30.1)	<0.001
		3 vs 2	3.01 (1.48–6.15)	0.002

The score considers ABC and non-GCB by GEP and Hans algorithm, respectively, BCL2 positivity and IPI 3-5 all having weight 1.

high-risk. Our score also predicts poor outcome in both stage I/II as well as stage III/IV and works well when COO is correlated with GEP array and the Hans algorithm. Obviously, we consider GEP analysis as the gold standard for the determination of COO, however, taking into consideration the good correlation between the two methods and the higher costs linked to the newer technologies, we believe that the more simple and less expensive IHC method can also be utilized to determine the score.

In conclusion our model can identify a group of clinically high-risk patients. Given the aggressive nature of DLBCL, important and significant prognostic information needs to be readily available within a short time frame in order to be clinically relevant. Our model, which combines clinical prognostic factors and new biomarkers such as the COO subset, is very easy to apply, quick to perform and can therefore be efficiently utilized in routine clinical practice.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.leukres.2021.106552.

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