Identification of a clinicopathologic prognostic index for newly diagnosed large B-cell lymphoma patients treated with R-CHOP

While the International Prognostic Index (IPI),[1] as well as the revised IPI (R-IPI),[2] are predictive of survival outcomes in newly diagnosed large B-cell lymphoma (LBCL) patients treated with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP), differences in survival outcomes may also be explained by heterogeneity of tumor features. Results from assays performed in clinical laboratories can identify pathologic subgroups of LBCL with an unfavorable prognosis following treatment with R-CHOP, including non-germinal center B (GCB) cell of origin,[3] *MYC* rearrangement,[4,5] double-hit,[6,7] double-expressor[8,9] and *TP53* mutation.[10,11] However, tumor features have not been routinely incorporated into a prognostic index for patients with newly diagnosed LBCL treated with R-CHOP. Through analysis of features of LBCL tumors obtained from newly diagnosed LBCL patients treated with R-CHOP, we developed a novel Clinicopathologic Prognostic Index (CPPI), which outperformed the R-IPI in identifying a smaller cohort of patients who experienced inferior survival outcomes. The CPPI may be useful in risk-stratifying newly diagnosed LBCL patients receiving standard-of-care therapies and identifying high-risk patients who may benefit from treatment with investigational regimens.

Clinicopathologic data from eligible newly diagnosed LBCL patients obtained from three publicly available datasets[12,14] were retrospectively compiled and analyzed. Included patients were those treated with R-CHOP with available International Prognostic Index (IPI) score, cell of origin (COO) and double expressor (DE) status (*MYC* ≥40% and BCL2 ≥ 50%) by immunohistochemistry (IHC), *MYC* rearrangement and *MYC-BCL2* double-hit status by fluorescence *in situ* hybridization (FISH), genomic variant reporting of *TP53* and follow-up time of over 1 year from diagnosis if not experiencing disease progression before that time. Excluded patients were those whose tumors demonstrated *MYC-BCL2* double-hit status as they are not routinely treated with R-CHOP in clinical practice. *TP53* mutations were classified as loss of function (LOF) as previously described,[12] defined as either as a missense mutation designated as non-functional and/or LOF via query of the TP53 Database or any nonsense, frameshift, or splice site mutation as per ClinVar and/or PVS1 criteria for predicted LOF variants, and only those classified as LOF were included in the analysis. Progression-free survival (PFS) was determined from diagnosis to documented disease progression, death from any cause or date of last follow-up in remission. Overall survival (OS) was calculated from the date of diagnosis until death due to any cause or date last known alive. Hazard ratios (HR) were calculated via Cox regression analysis, and those demonstrating statistically significant HR for progression or death events, defined as two-tailed *P* value <0.05, on univariable Cox regression analysis were included in the multivariable Cox regression analysis. Concordance Index (C-Index), Akaike Information Criterion (AIC), and Net Reclassification Index (NRI) measurements comparing the two models. All analyses were conducted in R version 4.3.0. This study was approved by the Institutional Review Board of the University of Pennsylvania.

Selection of patients is depicted in the *Online Supplementary Table S1* and individual characteristics of the 409 included patients are listed in in *Online Supplementary Table S2*. IPI score distribution was 0 for 69 (17%), 1 for 125 (31%), 2 for 87 (21%), 3 for 88 (21%), 4 for 35 (9%) and 5 for (5) 1%. Pathologic features were non-GCB COO for 215 (53%) and GCB COO for 194 (47%), DE lymphoma for 104 (25%), *MYC* rearrangement for nine (2%) and *TP53* LOF mutation for 79 (19%). For the entire cohort, the estimated 2-year PFS was 73% (95% confidence interval [CI]: 68-77) and estimated 2 year OS was 87% (95% CI: 84-90). As listed in Table 1, univariate Cox regression analysis performed using the aforementioned characteristics (with IPI score categorized dichotomously as <3 *vs*. ≥3) demonstrated that IPI score ≥3, DE lymphoma and *TP53* LOF mutation were each significantly associated with disease progression and death events at 2 years, and these all remained significantly or nearly significantly associated with progression and death events at 2 years on multivariate analysis.

Based on the significance of these three identified risk factors, the CPPI was designed to account for the presence of one additional risk factor with each unit increase in the severity tier. The small 3-factor cohort (N=10) was included with the 2-factor cohort which resulted in the combined 2-factor cohort which was used for analysis. This resulted in a distribution of CPPI scores as follows: N=182 (45%) for score 0, N=153 (37%) for score 1 and N=74 (18%) for score 2. Estimates of 2 year PFS and OS by CPPI score were as follows: 85% (95% CI: 79-89) and 96% (95% CI: 91-98) for score 0, 67% (95% CI: 59-74) and 81% (95% CI: 73-86) for score 1 and 54% (95% CI: 41-65) and 79% (95% CI: 67-87) for score 2, which differed significantly (*P*<0.01 for both PFS and OS). Comparatively, the distribution of R-IPI scores (used for comparison instead of IPI scores given the similar 3-tiered classification) for the cohort were as follows: N=68 (17%) for score 0, N=213 (52%) for score 1 and N=128

(31%) for score 2. Estimates of 2 year PFS and OS by R-IPI score were as follows: 88% (95% CI: 77-94) and 97% (95% CI: 87-99) for score 0, 75% (95% CI: 68-80) and 89% (95% CI: 83-92) for score 1 and 61% (95% CI: 52-69) and 80% (95% CI: 72-87) for score 2, which also differed significantly (P<0.01 for both PFS and OS). R-IPI to CPPI score reclassification is depicted in Figure 1. Kaplan Meier distributions of survival by CPPI and R-IPI score are depicted in Figure 2: PFS by CPPI (Figure 2A), OS by CPPI (Figure 2B), PFS by R-IPI (Figure 2C) and OS by R-IPI (Figure 2D).

CPPI was assessed on metrics of discriminative ability, model fit, and risk reclassification as compared to R-IPI using C-Index, AIC, and NRI respectively. As listed in *Online Supplementary Table S3*, CPPI demonstrated moderate improvements as compared to R-IPI as demonstrated by higher corresponding C-Index values for both 2 year PFS (C-Index 0.63 *vs*. 0.59) and 2 year OS (C-Index 0.67 *vs*. 0.63). Additionally, CPPI demonstrated improved model fit as compared to R-IPI as demonstrated by lower AIC values for both 2 year PFS (AIC: 1,237.39 *vs*. 1,248.4) and 2 year OS (AIC: 550.13 *vs*. 555.79). Finally, CPPI demonstrated improved reclassification as compared to R-IPI by NRI for both 2 year PFS (NRI: 0.14, NRI^{+}: -0.31, NRI^{-}: 0.45) and 2 year OS (NRI: 0.16; NRI^{+}: -0.28, NRI^{-}: 0.44).

The diversity of LBCL tumor features has become apparent in clinical practice with the increased utilization of IHC, FISH and next generation sequencing (NGS) as part of the diagnostic workup of cases, and features identified by these assays can predict response to first-line treatment with R-CHOP for newly diagnosed patients. However, it has not

Table 1. Regression analysis of clinicopathologic features predictive of survival outcomes.

Feature	HR	95% CI low	95% CI high	*P*
Univariate Cox regression - progression at 2 years				
International Prognostic Index score ≥3	1.85	1.27	2.71	<0.01
Germinal center cell of origin	0.69	0.47	1.01	0.06
Double expressor lymphoma	2.22	1.51	3.26	<0.01
MYC rearrangement	1.7	0.63	4.62	0.3
TP53 loss of function mutation	1.68	1.09	2.58	0.02
Multivariate Cox regression - progression at 2 years				
International Prognostic Index score ≥3	1.63	1.1	2.39	0.01
Double expressor lymphoma	2.07	1.4	3.06	<0.01
TP53 loss of function mutation	1.55	1	2.39	0.048
Univariate Cox regression - death at 2 years				
International Prognostic Index score ≥3	2.21	1.26	3.9	<0.01
Germinal center cell of origin	0.66	0.37	1.19	0.17
Double expressor lymphoma	2.23	1.26	3.97	<0.01
MYC rearrangement	0.92	0.13	6.67	0.93
TP53 loss of function mutation	2.05	1.11	3.78	0.02
Multivariate Cox regression - death at 2 years				
International Prognostic Index score ≥3	1.91	1.07	3.39	0.03
Double expressor lymphoma	1.98	1.11	3.54	0.02
TP53 loss of function mutation	1.84	1	3.4	0.05

HR: hazard ratio; CI: confidence interval.

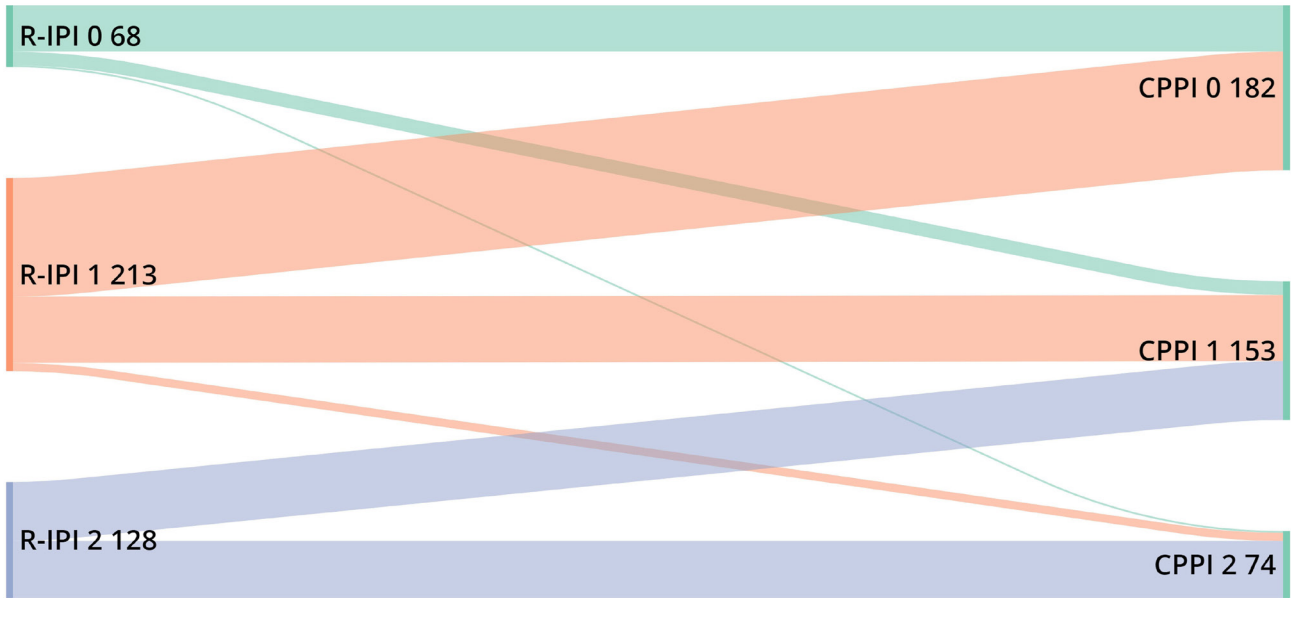

Figure 1. Reclassification of cases from revised International Prognostic Index to Clinicopathologic Prognostic Index. R-IPI: revised International Prognostic Index; CPPI: Clinicopathologic Prognostic Index.

been clear if tumor features can be practically incorporated into LBCL prognostic models, nor how a prognostic model incorporating LBCL tumor features would compare to the IPI or R-IPI.

In a cohort of >400 newly diagnosed LBCL patients treated with R-CHOP, we identified three statistically significant risk factors - IPI score of ≥3, DEL, and *TP53* LOF mutation - that are independently predictive of inferior 2 year PFS and OS. The novel three-tiered CPPI, which was developed through incorporation of these three risk factors, predicted for survival outcomes in this patient population. In our analysis of CPPI as a predictor of PFS and OS in comparison with the R-IPI, PPI identified patients with lower estimated 2-year PFS and OS rates at each level of classification. CPPI also demonstrated a better model fit for PFS and OS prediction as per C-Index and AIC as compared to R-IPI. This is also supported by the positive NRI score for PFS and OS for CPPI as compared to R-IPI, with this being driven by a greater ability to predict for non-event cases (lower false-negative rate) over a lesser ability to predict for event cases (higher false-positive rate). Taking these findings into account as well as the fact that the CPPI identifies a smaller proportion of higher risk (score 1 or 2) and larger proportion of lower risk patients (score 0) as compared to the R-IPI, the CPPI may be more accurate in identifying newly diagnosed LBCL patients who will experience inferior survival outcomes following treatment with R-CHOP.

Strengths of our analysis are evaluation of a large population of patients with available IHC, FISH and NGS data across three cohorts including patients treated in the USA, Europe and Asia, as well as advance statistical modeling to compare the CPPI to the R-IPI. Weaknesses include lack of analysis of an independent cohort of patients which the prognostic ability of the CPPI could be validated, so it is unknown if our results would be replicated in an independent patient cohort. However, this not possible to obtain based on the paucity of publicly available IHC and genomic data from tumors of newly diagnosed LBCL patients. Additionally, it should be noted that the R-IPI was also not applied to a validation cohort in the original publication[2] but remains widely utilized. Furthermore, data were collected retrospectively for two of the datasets, although these

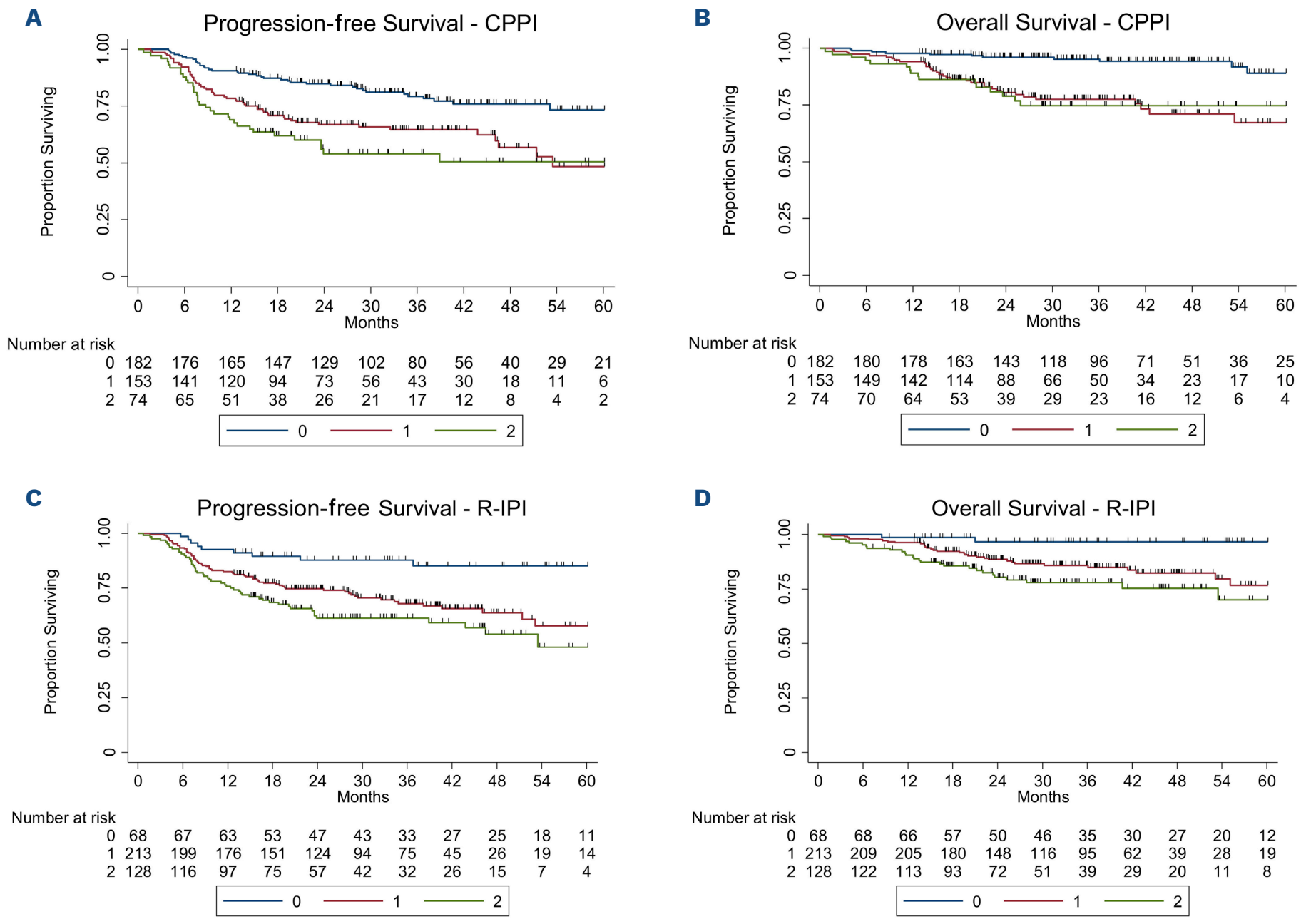

Figure 2. Survival. (A) Progression-free survival by Clinicopathologic Prognostic Index (CPPI). (B) Overall survival by only CPPI. (C) Progression-free survival by Revised International Prognostic Index (R-IPI). (D) and overall survival by R-IPI.

data sets were large and there was no obvious selection bias for included cases. Finally, although consistent with previously published definitions of DEL,[8,9] ours may not be universally accepted, noting that at least one other analysis recommended a *MYC IHC* cutoff of ≥70% for improved prognostication of cases of newly diagnosed DEL.[15]

A related issue raised by our analysis is whether *MYC* IHC can reliably serve as a surrogate for MYC rearrangement. Our study was not designed to evaluate for this due to exclusion of cases of MYC-BCL2 double-hit lymphoma, although an analysis using MYC IHC cutoff of ≥40% reported that 21% of cases of newly diagnosed LBCL with overexpression of MYC harbored *MYC* rearrangement,[16] and the aforementioned analysis using MYC IHC cutoff of ≥70% reported that 44% of cases with overexpression of MYC harbored *MYC* rearrangement.[15] A similar question can be posed regarding the utility of p53 expression by IHC (which was not analyzed in our study), for prediction of *TP53* LOF mutation. Increased expression of p53 by IHC has been associated with inferior survival outcomes in newly diagnosed LBCL patients.[17] However, a recently published report suggests that p53 IHC ≤3% identified 53% of tumors harboring *TP53* LOF mutation, while p53 IHC ≥3% identified only 1% of tumors harboring *TP53* LOF mutation.[18] While consensus on appropriate IHC cutoffs for MYC and p53 may provide a standardized answer to the relationship between IHC and genomic alterations, it appears that not all MYC rearrangements,[19] nor *TP53* mutations[12] carry the same prognostic significance for newly diagnosed LBCL patients, and perhaps molecular assays should be routinely used to detect these and other genomic alterations of prognostic value.

In conclusion, the CPPI is predictive of 2 year PFS and OS in patients with newly diagnosed LBCL patients treated with R-CHOP and may refine the prognosis offered by the R-IPI in this patient population. The CPPI should be applied to other cohorts of newly diagnosed LBCL patients treated with first-line therapy, and potentially be used to identify patients at increased risk for inferior survival outcomes as candidates for investigational therapies.

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Contributions

DJL performed research. VN and DJL performed data analysis. VN and DJL wrote the manuscript. All other authors reviewed the manuscript.

Data-sharing statement

All data analyzed have been made available in the *Online Supplementary Appendix.*

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