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Evaluating cell-of-origin subtype methods for predicting diffuse large B-cell lymphoma survival: A meta-analysis of gene expression profiling and immunohistochemistry algorithms

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Abstract

Background—Patients with diffuse large B-cell lymphoma (DLBCL) exhibit widely divergent outcomes despite harboring histologically identical tumors. Currently, gene expression profiling (GEP) and immunohistochemistry algorithms (IHC) assign patients to one of two main subtypes: germinal center B-cell-like (GCB), or activated B-cell-like (ABC), the latter of which historically carries a less favorable prognosis. However, it remains controversial as to whether these prognostic groupings remain valid in the era of rituximab therapy.

Materials and Methods—A systematic literature review identified 24 articles from which meta-analyses were conducted, comparing survival outcomes for patients assigned to either GCB or ABC/non-GCB subtype by GEP and/or Hans, Choi or Muris IHC algorithms.

Results—Patients designated as GCB DLBCL by GEP fared significantly better in terms of overall survival than those with ABC DLBCL (HR = 1.85, $P < .0001$). In contrast, the Hans and Choi algorithms failed to identify significant differences in overall survival ($P = .07$ and $P = .76$ respectively) between GCB and non-GCB groups.

Conclusions—Our study illustrates a lack of evidence supporting the use of the Hans and Choi algorithms for stratifying patients into distinct prognostic groups. Rather, GEP remains the preferred method for predicting the course of a patient's disease and informing decisions regarding treatment options.

Keywords

non-Hodgkin lymphoma; rituximab; chemoimmunotherapy; prognosis; systematic review

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Introduction

While diffuse large B-cell lymphoma (DLBCL) is the most common form of non-Hodgkin's lymphoma (NHL), clinical presentation, response to therapy, and long-term prognosis varies considerably across patients, suggesting underlying differences in the biology of this disease despite a uniform histologic appearance.¹ Alizadeh *et al.* classified DLBCL tumors into germinal center B-cell-like (GCB) and activated B-cell-like (ABC) subtypes based on distinct patterns of gene expression profiling (GEP) that share features with corresponding normal B-cell counterparts.² These subtypes employ distinct mechanisms of oncogenesis and exhibit divergent expected outcomes with standard treatment.^{2,3}

Given that GEP has not yet been adopted as a standard approach in most clinical settings, researchers have attempted to recapitulate the prognostic grouping of GEP with immunohistochemistry (IHC). Several IHC algorithms have been developed⁴⁻⁸ to assign patients into GCB and non-GCB/ABC subtypes.⁸⁻¹¹ The first such algorithm was described by Hans *et al.*, in which staining for CD10, BCL6 and MUM1 is performed in a stepwise manner. The Choi and Muris methods, among others, were devised using additional cell surface markers in an attempt to improve the prognostic value of IHC. One report measured the concordance of the Hans, Choi and Muris algorithms with GEP to be 86%, 87% and 77% respectively.⁸

While classifying DLBCL as GCB or ABC lends insight into lymphoma biology, clinically useful classification schemas must identify subgroups with meaningful differences in survival. Historically, patients with DLBCL have been treated with chemotherapy regimens containing cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP), resulting in a 5-year overall survival (OS) and failure-free survival of 57% and 39%, respectively. The inclusion of rituximab with CHOP (R-CHOP) improved 5-year OS and failure-free survival to 67% and 52%, respectively.¹² In the pre-rituximab era, patients with ABC subtype had a 5-year OS of 35% compared to 60% for those with GCB DLBCL ($P < .001$).³ Since the inclusion of rituximab with standard therapy, outcomes for both GCB and ABC subtypes have improved. In one study, the 3-year OS rates for R-CHOP treated patients were 92% and 44% for GEP-defined GCB and ABC cases, respectively ($P < .001$), and 87% and 44% for Choi IHC-defined GCB and ABC cases, respectively ($P < .001$).⁶ However, the prognostic significance of DLBCL subtype designation has been called into question, particularly when IHC is used.^{13,14} To further examine whether GEP and IHC are appropriate prognostic tools in the era of rituximab therapy, we performed a systematic review of the literature and meta-analyses evaluating OS and progression-free survival (PFS) in patients assigned to either GCB or ABC/non-GCB subtype by GEP and/or IHC.

Materials and Methods

Systematic Literature Review

Studies that directly compared OS and PFS for GCB and ABC/non-GCB subtypes were identified in the MEDLINE database through a series of searches using combinations of the medical subject heading (MeSH) terms "Lymphoma, large B-cell, diffuse," "gene expression profiling," "immunohistochemistry," "survival rate," "survival analysis" and

“prognosis,” generating a pool of 361 papers that were assessed for suitability as shown in Figure 1. Four reviewers (JR, JW, JK, and JC) independently performed study selection and quality assessment using a predefined format. Any disagreement was resolved by another reviewer (LN or CF), each of whom independently confirmed that inclusion criteria were met for all selected studies and that exclusion criteria were met in a random sample (15%) of the remaining studies. Studies were carefully screened for possible duplication of study population based on the author list, participating institutions, and period of patients’ diagnosis and accrual.

Meta-Analysis Inclusion Criteria, Study Selection, and Data Extraction

Criteria for inclusion in the meta-analysis were: 1) articles published in English between January 1st, 2007 and November 30th, 2013, with a full set of experimental details; 2) a cohort of patients with *de novo* DLBCL, analyzed separately, who had been treated exclusively with a regimen including rituximab and anthracycline-based chemotherapy; 3) a direct comparison between GCB and ABC/non-GCB subtypes reported as Kaplan-Meier survival data with the outcome expressed in terms of hazard ratio (HR), or data from which the HR could be calculated; and 4) patients included in analysis not duplicated in any other article included in our meta-analysis. When more than one article featured patients from the same study or reported the same authors and institutions during the same recruitment period, then only the study with the largest number of participants was included in the meta-analysis. We extracted data for patient characteristics (age, sex, Ann Arbor stage, and International Prognostic Index [IPI] score), follow-up time, and HR. HRs compared PFS and OS for patients in the GCB group to those in the ABC (GEP data) or non-GCB (IHC data) group. When multiple IHC algorithms were used to analyze survival data, each set of data was extracted and analyzed separately.

In cases for which HR was not reported, it was calculated indirectly from the data available, according to the methods described by Parmar.¹⁵ In cases in which the only available data were presented in the form of graphical survival curves, the freely available Engauge Digitizer software version 4.1 (SourceForge, <http://digitizer.sourceforge.net/>) was used to extract survival rates at specified time points, assuming that the rate of patient censoring was constant throughout the follow-up period. HR was then calculated using data points for each group.

Data Analysis and Statistical Methods

We used the freely available Review Manager (RevMan) software version 5.2 (The Cochrane Collaboration, <http://ims.cochrane.org/revman/download>) to perform all statistical analyses. Forest plots were generated using the rmeta package in R (The R Foundation for Statistical Computing, <http://cran.r-project.org/package=rmeta>). Individual estimates of HR for both OS and PFS were combined using a fixed effects or random effects model based on the degree of heterogeneity detected. To test for the presence of study heterogeneity, a χ^2 -test was performed and the corresponding I^2 value calculated.¹⁶ Significant heterogeneity was detected when the χ^2 value was greater than the degrees of freedom, and in these cases a random effects model was used rather than a fixed effects model. Forest plots were generated for each analysis to display the result of combining individual study estimates. To

test for the presence of bias in the studies collected, funnel plots were generated for each analysis and visually evaluated for asymmetry. To assess the sensitivity of the results to the effect of individual studies, the meta-analyses were iteratively repeated with each study omitted.

Results

Characteristics of the study population

The search strategy generated an initial pool of 361 papers, which was narrowed to 24 using the inclusion criteria depicted in Figure 1. Of these, 3 papers included only GEP data^{17–19}, 18 included only IHC data^{8,13,20–36} and 3 included both GEP and IHC data^{9,37,38}. The included reports were published between 2008 and 2013. In total, 1120 patients with GEP data and 2618 patients with IHC data were included in the meta-analyses. Patient characteristics, treatment protocols and experimental details from each study are presented in Table 1. For each meta-analysis, the results were unchanged when repeated with each study removed iteratively (data not shown), indicating that the results were insensitive to the effects of individual studies. Funnel plots of OS and PFS endpoints were symmetric for each meta-analysis and did not demonstrate evidence of publication bias (supplemental figures). In each meta-analysis, the χ^2 measure was greater than the degrees of freedom, and thus each analysis was performed using a random effects model.

Meta-analysis of GEP data

Six papers were included in the meta-analysis for GEP data. Forest plots summarizing results of the meta-analyses comparing survival rates in individuals with GCB and ABC DLBCL are presented in Figure 2. Patients diagnosed with GCB DLBCL by GEP had significantly better survival than patients diagnosed with ABC subtype. The pooled HR was 1.85 (95% CI = 1.46–2.35, $P < .0001$) for OS and 1.80 (95% CI = 1.36–2.38, $P < .0001$) for PFS. The I^2 values for the OS and PFS data were calculated as 3% and 25%, respectively, indicating no heterogeneity by the criteria recommended by Higgins et al.¹⁶

Meta-analysis of IHC data

Twenty-one papers were included in the meta-analyses of IHC data. Forest plots summarizing results of the meta-analyses comparing survival rates in individuals with GCB and non-GCB DLBCL as defined by IHC methods (Hans, Choi or Muris algorithms) are shown in Figure 3. There was no significant difference in OS between patients designated as GCB or non-GCB subtype by the Hans method ($n = 1970$, Figure 3A), although a significant difference in PFS was observed between the two groups (Figure 3B). The pooled HR was 1.27 (95% CI = 0.98–1.64, $P = .07$) for OS and 1.49 (1.09–2.03, $P = .01$) for PFS. The I^2 values for the OS and PFS data were calculated as 37% and 39% respectively, implying low heterogeneity. The results of these meta-analyses were not dominated by any single study; the study by Meyer et al. exerted the greatest influence on the results with a weight of 12.3%.⁸

For patients assigned to GCB or ABC subtype by the Choi method ($n = 594$), there was no significant difference in OS or PFS (Figure 3C and 3D). The pooled HR was 1.04 (95% CI =

0.69–1.58, $P = .85$) for OS and 1.12 (0.54–2.32, $P = .76$) for PFS. The I^2 values for the OS and PFS data were calculated as 26% and 71% respectively, indicating low to moderate heterogeneity.

When patients assigned as GCB subtype by the Muris method were compared with ABC patients ($n = 640$), significant differences in OS and PFS were observed (Figure 3E and 3F). The pooled HR was 2.12 (95% CI = 1.50–3.00, $P < .0001$) for OS and 2.07 (1.32–3.24, $P = .002$) for PFS. The I^2 values for the OS and PFS data were calculated as 0% and 51% respectively indicating zero to moderate heterogeneity.

Discussion

The meta-analyses conducted based on this systematic review assess the ability of GEP and the most commonly used IHC algorithms to classify DLBCL patients into subtypes with divergent PFS and OS. Our results demonstrate that the prognostic differences first described between ABC and GCB subtypes in the pre-rituximab era remain consistent across more recent studies using GEP to classify patients treated with rituximab and anthracycline-based chemotherapy regimens. Patients assigned to the GCB group by GEP exhibited significantly better OS and PFS when compared to those assigned to the ABC group. In contrast, the IHC methods produced widely variable results. When patients were stratified into GCB and non-GCB subtypes by the Hans algorithm, no significant difference was observed for OS, and only a small difference in PFS was seen. These results echo those of a recent meta-analysis performed with individual patient data on a more limited set of studies.¹⁴ Our analysis identified low heterogeneity between studies using the Hans algorithm, which directly opposes the idea that IHC methods predominantly suffer from inconsistency when applied by different pathologists or in different patient populations. Similarly, pooled estimates of DLBCL subtype using the Choi algorithm failed to demonstrate significant difference in OS or PFS.

The Muris method divided DLBCL patients into GCB and ABC subtypes with significant differences in HRs for PFS and OS, as seen with corresponding subtypes assigned by GEP. Although the Muris algorithm appears comparable to GEP in terms of prognostic ability, it is clear that the two are not equivalent. GEP and the Hans algorithm both assign patients to GCB or ABC/non-GCB subtypes in an approximately 1:1 ratio. In contrast, the Muris algorithm assigns more patients to the GCB than the ABC subtype, in a ratio of 1.9:1, while the Choi algorithm assigns more patients to the ABC subtype, in a ratio of 1:1.5. However, these ratios are aggregates drawn from the meta-analyses, and an inspection of individual studies reveals inconsistency in the distribution between subtypes. This likely reflects both the variability in the population being studied as well as the variability inherent in the IHC methods. Overall, these findings support the notion that GEP remains the most robust method for assigning patients to distinct prognostic groups based on underlying DLBCL biology. Furthermore, the Hans and Choi algorithms lack prognostic value, at least in part due to misclassification of subtype.

The potential limitations of the current study are those inherent to carrying out any meta-analysis and include: publication bias, study selection bias and synthesis of data derived

from studies with heterogeneous methodology. To avoid the latter issue, our search strategy was focused to include only studies that compared survival data from patients treated with R-CHOP or similar immunochemotherapy regimens. It remains possible that our narrow inclusion criteria biased our results; however, from the qualitative examination of funnel plots produced from the meta-analyses (supplemental figures), any publication bias appears to be minimal.

Much research has been conducted in order to better understand the genetic aberrations that drive the difference in survival between ABC and GCB subtypes. It is clear that GCB and ABC subtypes of DLBCL employ distinct mechanisms of oncogenesis. For instance, ABC subtypes are dependent on NF- κ B signaling as indicated by more frequent mutations in *TNFAIP3*, *CARD11*, *CD79B* and *MYD88*, while recent studies have demonstrated that mutations to *BCL2*, *MYC* and *EZH2* occur more frequently in patients with the GCB subtype.^{39–42} Consequently, therapies targeting the NF- κ B pathway appear more likely to be effective in patients with ABC DLBCL.⁴³ Examples of such therapies include bortezomib (a proteasome inhibitor that may weakly inhibit NF- κ B activation), ibrutinib (an upstream BCR signaling pathway inhibitor), and lenalidomide, which acts by downregulating transcription factors IRF4 and SPIB, thus enhancing B-cell interferon- β production.^{1,44} The findings of this study indicate that GEP approaches should be used to define the optimal subgroup of DLBCL patients who require interventions that may improve upon outcomes expected with R-CHOP.

Recent studies have argued that the prognostic difference between GCB and ABC subtypes may be attributed to concomitant MYC and BCL2 overexpression and/or translocation.^{17,20,43} While it has been firmly established that patients with translocations affecting both *MYC* and *BCL2* genes (so-called “double-hit” lymphoma) have an extremely poor prognosis, these patients are thought to represent only 5% of all cases of DLBCL.²⁰ In contrast, simultaneous overexpression of MYC and BCL2 by IHC is more common, representing approximately 46% of patients with ABC DLBCL and 22% of those with GCB subtype.¹⁷ In a study by Hu et al., patients were divided into two groups: one group whose lymphoma was found to overexpress both MYC and BCL2 by IHC and a second that did not overexpress both gene products.¹⁷ When the authors examined patients within the MYC⁺/BCL2⁺ group, they found no difference in survival between patients who were further divided into GCB and ABC subtypes ($P = .3163$ for OS; $P = .4291$ for PFS). Similarly, there was no difference in survival between patients with GCB and ABC subtype lymphomas in the group that did not express both MYC and BCL2 ($P = .4114$ for OS; $P = .7020$ for PFS). Further study will be necessary to determine how best to integrate *MYC* and *BCL2* expression with ‘cell of origin’ subtyping when risk-stratifying newly diagnosed patients with DLBCL. Unfortunately, the impact of *MYC* and *BCL2* expression could not be assessed in our meta-analysis due to limitations in the number of available IHC studies.

Conclusion

The meta-analyses performed in this study demonstrate that the Hans and Choi methods for classifying DLBCL subtypes do not hold significant prognostic value for patients treated with R-CHOP. Although these algorithms may be subject to inconsistency in their

application by individual pathologists, the low to moderate heterogeneity observed for these studies suggests that the lack of prognostic value may relate to the IHC methods themselves rather than variability in the study populations or implementation of the IHC algorithms. While the Muris method is able to classify DLBCL patients into subtypes with distinct prognoses, its accuracy is called into question since it assigns 2/3 of all patients to the GCB group. Thus, further study is necessary to determine whether the Muris method remains relevant in terms of predicting response to new therapies developed on the basis of GEP analysis.

At present, GEP remains the gold standard for classifying DLBCL into biologically and prognostically meaningful groups. A recent study evaluated the Lymphoma/Leukemia Molecular Profiling Project's Lymph2Cx assay, a digital gene expression test that employs 20 genes to assign GCB and ABC DLBCL subtypes using formalin-fixed paraffin-embedded tissue.⁴⁵ This test had high concordance with GEP performed on frozen tissue from the same cases and >95% concordance between 2 independent laboratories. This test and similar approaches may promote the integration of GEP into broader clinical practice as technological advances make GEP faster, simpler, and more affordable.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Clinical Practice Points

- Gene expression profiling can divide DLBCL patients treated with anthracycline-based chemoimmunotherapy into biologically distinct subtypes that have significant differences in survival
- The Hans and Choi immunohistochemistry algorithms divide DLBCL into subgroups that lack prognostic significance when studies of chemoimmunotherapy are combined

Figure 1.
Selection of articles for meta-analysis

Figure 2. Forest plots summarizing hazard ratios (HRs) for the meta-analysis of A) progression free survival (PFS), and B) overall survival (OS) for patients assigned to GCB or ABC subtypes by gene expression profile (GEP)

Figure 3.

Forest plots summarizing hazard ratios (HRs) for meta-analysis of progression free survival (PFS) and overall survival (OS) for patients assigned to GCB or non-GCB subtypes by the Hans algorithm [A–B]; the Choi algorithm [C–D]; and the Muris algorithm [E–F]. Articles that were weighted less than 0.1% for the analysis were omitted from figure A): Wilson *et al.*, 2012 and B) Varoczy *et al.*, 2012 and Wilson *et al.*, 2012

Table 1

Summary of patient characteristics from studies determining cell of origin through gene expression profiling (GEP) and/or immunohistochemistry (IHC).

Author	Number of patients	Median age (years)	Male (%)	Median follow-up (years)	Stage III/IV (%)	IPI >2 (%)	Method	Treatment protocol
Barrans, 2012	140	67	88 (63)	-	70 (56)	3-5 (%)	GEP, Hans	R-CHOP
Green, 2012	193	64	113 (59)	4.7	92 (48)	65 (34)	Hans	R-CHOP
Gutierrez-Garcia, 2011	157	65	77 (49)	4.3	94 (60)	-	GEP, Hans, Choi, Muris	R-CHOP
Hong, 2012	70	59	33 (47)	2.5	34 (49)	20 (29)	Hans, Muris	R-CHOP
Hu, 2013	466	-	272 (58)	4.8	228 (51)	75 (47)	GEP	R-CHOP
Huang, 2012	97	59	60 (62)	4.7	40 (41)	10 (10)	Hans	R-CHOP
Ilic, 2009	92	51	41 (45)	3.1	63 (68)	40 (43)	Hans	R-CHOP or R-CHOP like
Jais, 2008	67	69	33 (49)	3.3	-	148 (36)	GEP	R-CHOP
Koh, 2013	120	60	75 (63)	3.5	59 (49)	39 (33)	Choi	R-CHOP
Kojima, 2013	100	67	56 (56)	4.2	40 (40)	33 (33)	Hans, Choi, Muris	R-CHOP or R-CHOP like
Lanic, 2012	57	65	30 (53)	2.3	-	28 (64)	GEP, Hans	R-CHOP
Lenz, 2008	233	-	-	2.1	121 (54)	-	GEP	R-CHOP or R-CHOP like
Meyer, 2011	262	62	-	-	-	-	Hans, Choi, Muris	R-CHOP or R-CHOP like
Niitsu, 2011	101	51	54 (53)	3.5	72 (71)	-	Hans	R-CycloBEAP
Nyman, 2009a	117	63	62 (53)	2.4	74 (63)	45 (39)	Hans	R-CHOP or R-CHOEP
Nyman, 2009b	88	67	42 (48)	3.1	47 (53)	33 (38)	Muris	R-CHOP
Ott, 2010	313	68	171 (55)	-	161 (51)	132 (42)	Hans	R-CHOP
Porrata, 2012	99	61	54 (55)	2.7	67 (68)	54 (55)	Hans	R-CHOP
Seki, 2009	279	68	142 (51)	2.1	(50)	(36)	Hans	R-CHOP
Song, 2010	136	61	72 (53)	3	58 (43)	54 (40)	Hans	R-CHOP
Varoczy, 2012	51	53	21 (41)	-	-	-	Hans	R-CHOP
Wilson, 2012	43	60	24 (56)	5.2	29 (67)	18 (43)	Hans	DA-EPOCH-R
Wilson, 2008	63	50	40 (63)	4.5	45 (71)	25 (40)	Hans	DA-EPOCH-R

Abbreviations: IPI: International Prognostic Index, R-CHOP: rituximab, cyclophosphamide, doxorubicin, vincristine & prednisone; R-CycloBEAP: rituximab, cyclophosphamide, vincristine, bleomycin, etoposide, doxorubicin & prednisolone; RCHOEP: rituximab, cyclophosphamide, doxorubicin, vincristine, etoposide & prednisone; DA-EPOCH-R: dose accelerated etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin & rituximab