The Hans algorithm is not prognostic in patients with diffuse large B-cell lymphoma treated with R-CHOP

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ABSTRACT

Our objective was to evaluate the non-germinal center (GC) profile as a marker for response and survival in DLBCL and to compare the characteristics of patients with GC and non-GC DLBCL treated with rituximab-containing regimens. In this patient-level meta-analysis, retrospective data from 712 newly diagnosed DLBCL patients treated with chemoimmunotherapy from 7 centers were analyzed. GC and non-GC profiles were defined according to the Hans algorithm. Although the non-GC profile showed a trend towards worse overall survival (HR 1.24, 95% CI 0.92–1.66; $p = 0.15$) and progression-free survival (HR 1.29, 95% CI 0.96–1.73; $p = 0.09$), it did not retain its value in the multivariate survival analysis. Additionally, the non-GC profile was independently associated with worse complete response rates (OR 0.55, 95% CI 0.37–0.83; $p < 0.01$) in the multivariate logistic regression analysis. Interestingly, Asian patients had higher proportion of GC DLBCL ($p = 0.01$).

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1. Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma (NHL), accounting for approximately 30–40% of the cases seen worldwide [1]. Despite its aggressive clinical course, DLBCL is considered a curable disease and, within the last years, the addition of rituximab to chemotherapy has further increased the rates of complete response (CR) as well as the median overall survival (OS) of patients with newly diagnosed DLBCL [2,3]. The combination of rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP) is considered the standard of care for patients with DLBCL. However, given the molecular heterogeneity of DLBCL [4], it is not surprising

there is also a marked clinical variability. In fact, the prognosis of patients with DLBCL varies greatly depending on their risk strata.

Since 1993, the International Prognostic Index (IPI) score has largely been considered the standard for risk-stratification of patients with aggressive variants of DLBCL [5]. More recently, with further refinement of molecular diagnostic methods, at least two variants of DLBCL have been identified as germinal center B-cell-like and activated B-cell-like DLBCL; the latter been associated with a worse outcome in patients treated with chemotherapy [6,7]. There have been significant obstacles to translate this finding into clinical practice since molecular diagnostic testing such as gene array analysis require specialized laboratory personnel and fresh tissue. In 2004, Hans and colleagues proposed an immuno-histochemical (IHC) method to classify patients with DLBCL into germinal center (GC) and non-GC subtypes [8]. In that study, the non-GC subtype was associated with a worse prognosis as compared to the GC subtype in patients treated with chemotherapy; however, the role of the non-GC subtype in the rituximab era has not been completely elucidated.

The main purpose of this study was to evaluate the predictive and/or prognostic role that the non-GC profile, as described by Hans, could have in newly diagnosed patients with DLBCL treated with R-CHOP. A secondary objective was to compare clinical and pathological characteristics between patients with GC vs. non-GC DLBCL.

2. Patients and methods

A literature search from January 2004 to June 2010 was performed using the search key: *rituximab and (immunophenotyping or "germinal center" or "activated B-cell")*, looking for retrospective studies evaluating the role of the non-GC profile, as defined by Hans, in newly diagnosed adult patients with DLBCL who were treated with R-CHOP given every 3 weeks. Once studies were identified, the authors of each study were contacted to obtain pertinent patient-level data. The data requested included age at diagnosis, sex, performance status according to the Eastern Cooperative Oncology Group (ECOG) scale, lactate dehydrogenase (LDH) levels, number of extranodal sites, Ann Arbor clinical stage, IPI score, expression of CD10, BCL6 and MUM1/IRF4 by the tumor cells, response to chemotherapy, OS in months and final outcome. In addition to the cases submitted by outside researchers, previously untreated adult patients diagnosed with DLBCL from January 2002 to December 2008 and received therapy with R-CHOP were identified from the medical records of The Miriam and Rhode Island Hospitals in Providence, RI. Clinical data as mentioned above were obtained through a retrospective chart review. Patients with primary CNS lymphoma and primary cutaneous, transformed or HIV-associated DLBCL were not included in this study. Pathological samples of the selected patients were retrieved and immunohistochemical staining was performed after deparaffinization in each case. Antibodies, dilution and manufacturers of the immunostains used by each institution are shown in Table 1. According to the classification proposed by Hans [8], each case was assigned as GC or non-GC; CD10, BCL6 and MUM1/IRF4 expression was considered positive if at least 30% of the tumor cells stained with the antibody. A second look study was performed to assess potential inter-observer variability of evaluating the expression of CD10, BCL6 and MUM1/IRF4. A total of 65 cases (9%), 20 from Finland, 18 from Korea and 27 from the US, were independently evaluated by another investigator and correlation coefficients (r coefficient) were calculated; r-values > 0.75 represent a strong correlation level. The institutional review boards at each of the participating institutions approved the present study.

Clinicopathological characteristics were compared using Mann–Whitney and Chi-square tests for continuous and categorical variables, respectively. Multiple logistic regression analysis was used to evaluate the predictive value of the non-GC profile for response to R-CHOP. OS was defined as the time elapsed between date of diagnosis and date of death or last follow-up. Progression-free survival (PFS) was defined as the time elapsed between date of diagnosis and date of progression, death or last follow-up. The Kaplan–Meier method was used to calculate the survival curves which were compared using the log-rank test. For the multivariate survival analysis, the Cox proportional hazard regression test was used including age, performance status, LDH levels, number of extranodal sites, clinical stage and immunohistochemical profile. All graphics and calculations were obtained using the statistical software MedCalc® version 11 (Mariakerke, Belgium).

3. Results

Out of 13 contacted research groups, six submitted patient-level data [9–14] accounting for 663 patients. Three patients were excluded because they were < 18 years. Additionally, 52 patients were identified at our institution for a total of 712 newly diagnosed R-CHOP-treated patients with DLBCL.

3.1. Clinicopathological characteristics

The median age was 64 years (range 18–90 years), and 386 cases (54%) were men. Regarding adverse clinical variables, 433 patients (61%) were >60 years, 160 (22%) had a performance status ECOG >1, 381 (54%) had elevated LDH levels, 112 (16%) had >1 extranodal site involved and 372 (52%) had advanced stage at presentation. Based on the IPI score, 241 patients (34%) had high or high-intermediate scores. According to the Hans classification, 379 patients (53%) had a GC and 333 (47%) had a non-GC profile. There were no major differences between the characteristics of GC versus non-GC DLBCL patients with the exception of a higher proportion of GC DLBCL in Asian than in Western patients (68% vs. 59%; p = 0.01).
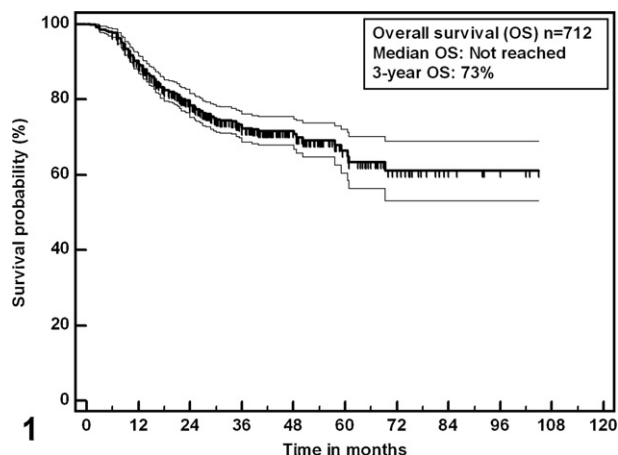

Fig. 1. Overall survival estimates in 712 patients with diffuse large B-cell lymphoma treated with R-CHOP.

The comparison between GC and non-GC DLBCL patients treated with R-CHOP is shown in Table 2.

3.2. Response analyses

Response data were available in 708 patients; 576 patients (81%) achieved a complete response (CR), 83 (12%) a partial response and 49 (7%) did not respond to R-CHOP. By using multivariate logistic regression, we evaluated age >60, male sex, ECOG >1, elevated LDH levels, >1 extranodal sites, advanced stage and non-GC profile as predictors for CR (n = 700). The non-GC profile was associated with an odds ratio (OR) of 0.55 (95% CI 0.37–0.83; p < 0.01). ECOG > 1 and advanced stage were also independent negative predictive factors for CR with ORs of 0.5 (95% CI 0.33–0.78; p < 0.01) and 0.44 (95% CI 0.29–0.68; p < 0.01), respectively. We evaluated the association with overall response rate (ORR) in a similar manner (n = 700); ECOG > 1, elevated LDH levels and advanced stage were independently associated with ORR with ORs of 0.52 (95% CI 0.27–0.97; p = 0.03), 0.45 (95% CI 0.24–0.85; p = 0.01) and 0.41 (95% CI 0.21–0.82; p = 0.01), respectively. The non-GC profile was not retained in the model.

3.3. Survival analysis

For the entire group (n = 712), the 3-year and 5-year OS were 73% and 66%, respectively, and the median OS has not yet been reached (Fig. 1). At the univariate level, the non-GC profile showed statistical trends towards being prognostic for OS and PFS with hazard ratios (HRs) of 1.24 (95% CI 0.92–1.66; p = 0.15, Fig. 2) and 1.29 (95% CI 0.96–1.73; p = 0.09, Fig. 3). At the multivariate level, the non-GC profile was not retained as an independent factor for OS or PFS when compared against the components of the IPI score (data not shown). In subgroup analyses, the non-GC profile was significantly associated with a worse OS in patients with advanced stage with OR of 1.47 (95% CI 1.05–2.07; p = 0.03; Fig. 4) and showed a statistical trend towards a worse OS in men and in patients < 60 years with OR of 1.33 (95% CI 0.92–1.94; p = 0.1) and OR 1.48 (95% CI 0.92–2.38; p = 0.1), respectively.

3.4. Pathological correlation

Our second-look study showed a strong correlation between pathologists when evaluating CD10 with an r-value 0.83 (95% CI

Table 1
List of antibodies, dilutions and manufacturers of the immunostains used by each institution.

Institution	Independent validation?	CD10	BCL6	MUM1/IRF4
Present	Yes	56C6; 1:10, Novocastra	PG-B6P; 1:10; Dako	MUM1p; 1:200; Dako
Song [9]	Yes	56C6; 1:20; Lab Vision	PG-B6P; 1:40; Dako	MUM1p; 1:50; Dako
Seki [10]	Yes	56C6; 1:20; Novocastra	P1F6; 1:20; Novocastra	MUM1p; 1:100; Dako
Ilic [11]	Yes	56C6; 1:20; Novocastra	P1F6; 1:20; Novocastra	MUM1p; 1:100; Dako
Uccella [12]	Yes	56C6; 1:20; Novocastra	GI191E/A8; undiluted; Cell Marque	MUM1p; 1:50; Dako
Nyman [13]	Yes	56C6; 1:20; Novocastra	PG-B6P; 1:40; Dako	MUM1p; 1:100; Dako
Xia [14]	Yes	56C6; 1:80; Novocastra	PG-B6P; 1:10; Dako	MUM1p; 1:40; Dako

Table 2
Clinicopathological comparison between GC and non-GC DLBCL patients treated with R-CHOP.

	All patients (%)	GC DLBCL (%)	Non-GC DLBCL (%)	p-Value
Total	712 (100%)	379 (53%)	333 (37%)	–
Age (n = 712)				
Median age (range)	64 (18–90)	65 (18–89)	63 (18–90)	0.4
<60 years	279 (39%)	153 (40%)	126 (38%)	0.54
60 years or older	433 (61%)	226 (60%)	207 (62%)	
Sex (n = 712)				
Female	326 (46%)	179 (47%)	147 (44%)	0.45
Male	386 (54%)	200 (53%)	186 (56%)	
Ethnicity (n = 712)				
Asian	455 (64%)	259 (68%)	196 (59%)	0.01
Western	257 (36%)	120 (32%)	137 (41%)	
Performance (n = 709)				
ECOG < 2	549 (77%)	298 (79%)	251 (76%)	0.47
ECOG 2 or higher	160 (23%)	81 (21%)	79 (24%)	
LDH levels (n = 707)				
Normal	326 (46%)	171 (46%)	155 (47%)	0.83
Elevated	381 (54%)	204 (54%)	177 (53%)	
Extranodality (n = 709)				
<2 sites	597 (84%)	328 (86%)	269 (81%)	0.08
2 sites or more	112 (16%)	52 (14%)	61 (19%)	
Stage (n = 712)				
Early stage	340 (48%)	192 (51%)	148 (44%)	0.11
Advanced stage	372 (52%)	187 (49%)	185 (56%)	
IPI score (n = 712)				
Low	332 (47%)	186 (49%)	146 (44%)	0.15
Low-intermediate	139 (20%)	72 (19%)	67 (20%)	
High-intermediate	104 (15%)	54 (14%)	50 (15%)	
High	137 (19%)	67 (18%)	70 (21%)	

DLBCL, diffuse large B-cell lymphoma; ECOG, Eastern Cooperative Oncology Group; GC, germinal center; IPI, International Prognostic Index; LDH, lactate dehydrogenase.

0.73–0.89). The level of correlation for MUM1/IRF4 and BCL6 was less strong with r-values 0.67 (95% CI 0.51–0.79) and 0.58 (95% CI 0.46–0.86), respectively. However, when evaluating the Hans algorithm, there was a strong inter-observer correlation with an r-value 0.85 (95% CI 0.76–0.90).

4. Discussion

Molecular differentiation by microarray analysis of patients with DLBCL between GC and non-GC subtypes are of prognostic value in the rituximab era [4]. However, a reliable differentiation of these two subtypes using IHC has historically been challenging
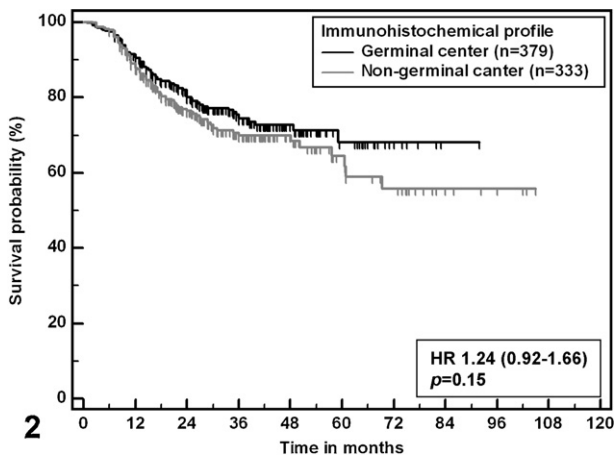

Fig. 2. Overall survival estimates in 712 patients with diffuse large B-cell lymphoma treated with R-CHOP, according to the Hans algorithm.
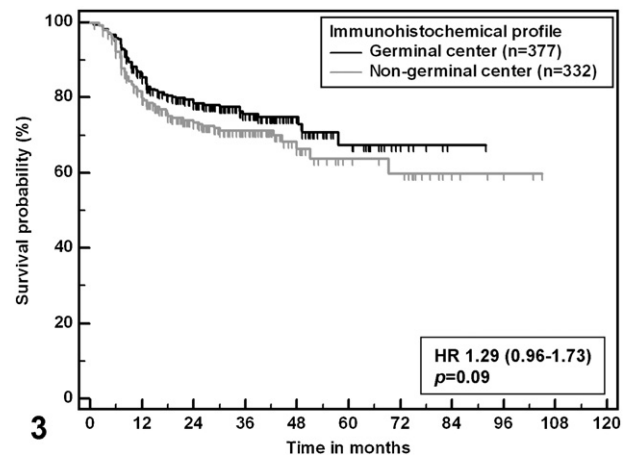

Fig. 3. Progression-free survival estimates in 712 patients with diffuse large B-cell lymphoma treated with R-CHOP, according to the Hans algorithm.
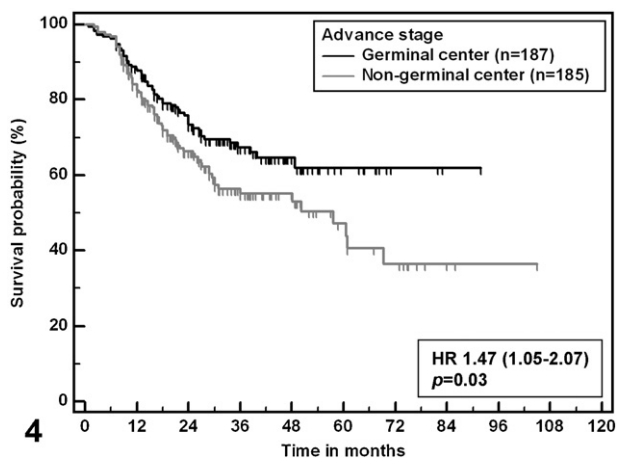
Fig. 4. Overall survival estimates in 372 patients with advanced stage diffuse large B-cell lymphoma treated with R-CHOP, according to the Hans algorithm.

[15]. One of the methods most commonly used is the Hans algorithm, which uses the expression of CD10, BCL6 and MUM1/IRF4 by the malignant DLBCL cells to classify patients as GC or non-GC [8]. However, the clinicopathological differences between GC and non-GC DLBCL patients as well as the prognostic and predictive value of the Hans algorithm have not been fully evaluated in the rituximab era.

Of most interest is that, according to the results of this study, there is a statistical association between GC profile and Asian ethnicity. Potential explanations for this finding include a true association, a systematic misclassification of GC or non-GC profile in Western or Asian patients, selection bias of Western or Asian patients or chance. Systematic misclassification and selection bias are unlikely, although not impossible, given that all the centers enrolled consecutive patients and followed the same criteria for classification of GC vs. non-GC profile. Two previous studies have investigated the racial differences between the incidence, presentation and outcome in patients with DLBCL [16,17]. However, none of these studies evaluated the IHC profile of these patients. The association between Asian race and GC DLBCL is a novel finding that should be further evaluated by molecular techniques.

Our study shows that the non-GC profile as defined by the Hans algorithm is associated with lower CR rates to R-CHOP in DLBCL patients. However, this potential predictive value does not translate into a prognostic one; although non-GC profile showed a statistical trend towards prognostic value for PFS and OS in patients with DLBCL treated with R-CHOP, it did not prevail in the multivariate analyses. The lack of prognostic value of the Hans algorithm is in concordance with other large studies in DLBCL patients [18], suggesting that more reliable tools need to be developed to sub-classify DLBCL patients. In fact, a recent study evaluated a series of algorithms, including Hans, and showed that a newer method, the Tally algorithm, was associated with a higher degree of concordance with genomic microarrays [19].

The predictive role of the Hans algorithm in the rituximab era has been previously evaluated in small studies with conflicting results [11,20,21]. A recent meta-analysis has shown a statistical trend towards an association between IHC profile and overall response rate [22]. However, a criticism is that this meta-analysis used study-level data and therefore could have been underpowered to identify smaller differences. Patient-level data, which were used in the present study, are intrinsically associated with less potential of bias introduction. Furthermore, although it is the authors claim, none of the studies included was a randomized controlled trial.

In the present study, we have attempted to critically evaluate the Hans algorithm in relationship to clinical and pathological characteristics as well as response to therapy and survival in newly diagnosed patients with DLBCL treated with R-CHOP. The large sample size of 712 patients in this study, the uniform therapeutic approach (all our patients received R-CHOP), and the multinational collaboration permitting the generalization of our findings, are all important strengths of our study. Although this is a retrospective study, which increases the risk of case selection bias, we have minimized it by contacting research groups that have evaluated consecutive patients. Furthermore, retrospective studies classically suffer from biases introduced by incomplete data. In this study, however, the clinicopathological data was complete in 99% of the cases included.

A weakness of this study is that there is a possibility our cases were misclassified, since there was not a centralized pathology review. However, our second look study evaluating 9% of our cases showed a strong inter-observer correlation on using the Hans algorithm ($r = 0.85$). The authors are aware of the reproducibility issues associated with IHC-based classification of DLBCL. In a recent study, the Lunenburg Lymphoma Biomarker Consortium evaluated 36 cases of DLBCL using an extensive battery of immunostains with the primary objective of evaluating inter-observer variation [23]. Such study, similar to ours, showed a high degree of reproducibility for CD10 but lower levels for BCL6 and MUM1/IRF4. It is important to notice that in our study there was a high variability among the reagents used to evaluate BCL6 expression among our institutions, which is likely the responsible of our lower rates of inter-observer correlation. Since there are data supporting prognostic value of BCL6 expression in DLBCL in the rituximab era [24], it is imperative to recognize that IHC-based risk-stratification of DLBCL, specifically with regard to BCL6 expression, should be evaluated in the context of studies with centralized pathology review. We believe the results of our study support the need of further evaluation and validation of markers for the IHC-based classification of DLBCL.

5. Conclusions

This study suggests that the Hans algorithm does not have prognostic value in DLBCL patients treated with R-CHOP; however, inter-observer reproducibility issues for IHC-based classification should be taken into account. An unexpected finding was that Asian populations might have a higher proportion of GC DLBCL. However, the latter should be confirmed by molecular analyses. Additional efforts should be directed at elucidating more reliable easy-to-use IHC-based algorithms to identify DLBCL subtypes in the rituximab era with future aims of not only risk-stratifying patients but also directing therapy to improve outcomes.

Conflict of interest statement

The authors have no conflict of interest to disclose.

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