ResearchTJH-2017-0437.R2
Submitted: 6 December 2017
Accepted: 28 March 2018

Anemia associated with worse outcome in diffuse large B-cell lymphoma patients: a single-center retrospective study

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Running head: Hemoglobin is a useful biomarker of DLBCL

No conflict of interest
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Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin lymphoma (NHL) subtype, which accounts for 30%–40% of newly diagnosed malignant lymphoma.[1]. Standard immunochemotherapy, such as R-CHOP containing rituximab, cyclophospamide, hydroxydaunorubicin, oncovin, and prednisolone has been proven to be beneficial for the outcome of DLBCL patients; however, approximately one-third of patients with advanced-stage disease still experience relapse or are refractory to therapy. [2, 3]. There are many well-known established prognostic models to identify patients at high risk of disease progression, relapse, or refractory to therapy because it is an urgent necessity to improve outcome. The International Prognostic Index (IPI), well-known and useful tool for predicting the clinical outcomes of DLBCL patients, comprises age, serum lactate dehydrogenase (LDH) level, performance status, Ann Arbor stage, and number of extra-nodal lesions (EN) [4]. Variants are reported in elderly patients (age-adjusted IPI) and in patients treated with rituximab (R-IPI) [5]. Recently, the National Comprehensive Cancer Network (NCCN) published a reformed IPI, which weighted scoring for increasing age and lactate dehydrogenase levels [6]. Unfortunately, clinical risk stratification models, such as IPI, R-IPI, and NCCN-IPI, are not completely accurate in identifying patients who will not be sufficiently cured by first-line R-CHOP therapy. There is an urgent necessity for new prognostic biomarkers. In particular, anemia is commonly associated with lymphoma , even in chemotherapy-naive patients, and in the absence of bone marrow(BM) involvement [7]. The pathogenesis of lymphoma-associated anemia is multifactorial and may include BM dysfunction, problems with iron reutilization, and an inadequate erythropoietin response. In this study, we evaluated the significance of pretreatment hemoglobin (Hb) levels.

Material and Methods

This study was approved by the Yokohama City University Medical Center Clinical Research Ethics Board. All procedures used in this study were in accordance with the Declaration of Helsinki.

2.1 Patients: We reviewed the records of 226 patients diagnosed with DLBCL at Yokohama City University Medical Center during 2004–2014. Forty-one patients were excluded due to the following reasons: transfer to another hospital after diagnosis (n = 12), other regimens (n = 18), radiation only (n = 5), received supportive care only because of poor performance status (n = 3), double cancer (n = 1), early death within 30 days (n = 1), and lack of information (n = 1). The following clinical data on 185 patients were collected from medical records and pathology reports: histological confirmation of diagnosis, gender, age, Ann Arbor clinical stage, presence of B symptoms, LDH levels, serum albumin levels, Eastern Cooperative Oncology Group (ECOG) performance status, and bone marrow involvement data. Hematology data, including full blood count, were obtained at diagnosis, 1–7 days before initiating treatment. The institutional lower limit normal (LLN) of Hb in complete blood count was set as 13.8 g/dl for male patients and 11.3 g/dl for female patients. Severity of anemia was graded by Hb levels according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0, as follows: G1, <LLN – 10.0 PubMed g/dl; G2, <10.0 –≤8.0 g/dl; G3, <8.0 g/dl or transfusion indicated; and G4, life threatening. Bone marrow involvement was detected by either aspiration or biopsy of bone marrow, except of PET imaging.

Patients were treated with standard immunochemotherapy containing rituximab, cyclophosphamide, doxorubin, vincristine, and prednisolone (R-CHOP) regimen. R-CHOP regimen comprised 6–8 cycles of 750 mg/m^2 cyclophosphamide, 50 mg/m^2 doxorubicin, and 1.4 mg/m^2 (maximum 2.0 mg/kg body weight) vincristine on day 1,100 mg/body prednisolone on days 1–5, and 375 mg/m^2 rituximab per cycle for 21 days.

Progression-free survival (PFS) was defined as the time from R-CHOP therapy initiation to lymphoma progression, death from any cause, or last follow-up. Overall survival (OS) was defined as the time from diagnosis to death from any cause or till time of last follow-up for patients who remained alive.

2.2 Statistical analyses: Kaplan –Meier analysis was used to calculate PFS. The log-rank test was used to assess univariate associations between PFS and prognostic variables. A forward – backward stepwise variable selection for the Cox proportional hazards model was used for multivariate analysis. A *P* value < 0.05 was considered statistically significant. All statistical analyses were performed with EZR (version 1.10) [8], which is a graphical user interface for R (The R Foundation for StatisticalComputing, version 2.13.0)using the modified version of R commander (version 1.6-3) designed to add statistical functions frequently used in biostatistics.

Results

3.1 Patient characteristics: Among the 226 patients diagnosed with DLBCL at Yokohama City University Medical Center during 2000–2014, 185 patients satisfied the inclusion criteria. The characteristics of the study participants are summarized in Table 1. The study included 121 males and 64 females, with a median age of 66 years (range, 21–83 years).

3.2 Patient outcomes and prognostic factors: The median observation period in surviving patients was 55.3 months (range, 4.8–117 months). The estimated PFS rate of all patients at 3 and 5 years was 76.1% and 72.0%, respectively. The OS rate at 3 and 5 years was 85.0 and 80.1, respectively (Figure 1).

The mean baseline Hb was 12.1 ± SD 2.2 g/dl in male patients and 11.4 ±SD 1.8 g/dl in female patients. Eight-seven patients (47%) had G ≥ 1, and 33 patients (18%) had G ≥ 2 baseline anemia. Patients with G ≥ 2 anemia showed inferior PFS compared with those with no (G0) or G1 anemia ($p < 0.0029$; Figure 2)

On univariate analysis, factors associated with worse PFS included ECOG performance status ≥ 2 (vs. ≤1:$p = 0.041$), Ann Arbor clinical stage ≥ 3 (vs. ≤2:$p<0.001$) , G≥ 2 anemia (vs. G≤1:$p = 0.001$), serum albumin < 3.5g/dl (vs. ≥3.5 g/dl:$p = 0.008$), and BM involvement (vs. negative:$p<0.001$).

Multivariate analysis showed that AnnArbor clinical stage (CS) ≥3 [hazard risk (HR)= 3.0; 95% confidence interval (CI)= 1.4–6.4; $p = 0.005$), G ≥2 anemia (HR= 2.3; 95% CI= 1.2 –4.3; $p = 0.012$), and BM involvement (HR= 1.9; 95% CI= 1.0-3.6;$p = 0.037$) were identified (Table 2). Because CS IV criteria includes BM involvement, CS ≥3 and G ≥2 anemia remained as independent determinants. These factors were each assigned a score and the sum was tested as a prognostic index for PFS. The 3-year PFS in the patients with an R-CHOP regimen of score 0 (n = 79), score 1 (n = 81), and score 2 (n = 27) were 89.1%, 73.9%, and 35.5%, respectively ($p < 0.001$), and the 3-year OS in the patients with an R-CHOP regimen of score 0 (n = 79), score 1 (n = 81), and score 2 (n = 27) were 94.6%, 82.0%, and 61.4%, respectively ($p < 0.001$:Figure 3 A, B).

Discussion

Anemia is commonly encountered in patients with malignant lymphoma or lymphoproliferative disorders. Incidence was reported approximately 39% previously[7]. The purpose of the current study was to determine whether anemia has a prognostic value in DLBCL, for which a number of prognostic tools have recently been devised. Because Hb status is a standard laboratory parameter, easy to measure, cheap, and highly reproducible in the clinical setting, Hb level could be readily incorporated into a newly differentiated prognostic index.

In follicular lymphoma, Hb < 12 g/dl was known to be prognostic [9, 10], butChen et. al. showed that Hb < 12 g/dl did not show a significant association with inferior PFS or OS in DLBCL patients treated with rituximab-containing immunochemotherapy [11]. In a recent study by Hong et al, G>2 anemia showed an association with inferior event-free survival [12], which was the same with our result.

Baseline anemia may not be an independent factor, but a consequence of bone marrow involvement, which in turn may actually just be a part of various factors causing anemia. Tisi et. al. showed that lymphomatous bone marrow (BM) involvement is independent to the occurrence of anemia, with no difference of Hb level observed according to the BM status (median, 11.8 g/dl for patients without BM infiltration vs 10.9 g/dl for those with BM infiltration, $p = 0.27$) [13]. They also concluded that an elevated level of interleukin-6, a pro-inflammatory cytokine, was the dominant factor affecting anemia, and reduced erythropoietin synthesis may result in anemia.

This study had some limitations, including its observational retrospective design and the analysis on a small number of patients.

In conclusion, anemia assessed by pre-treatment Hb < 10.0g/dl was an overall prognostic factor, and Hb is very easy to analyze in the clinical settings, with almost no additional cost. For patients with DLBCL treated with R-CHOP, our new prognostic index, which consists of Hb and clinical stages may be helpful for selecting the treatment strategy, including investigational salvage therapy, although the effectiveness of our index should be validated in a larger cohort.

5. Conflict of Interests

The authors declare that they have no conflict of interest. A summary of relevant information will be published with the manuscript.

6. References

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Figure legends

Figure.1. (A) Progression-free survival (PFS) and (B) Overall survival (OS) in 185 patients with diffuse large B-cell lymphoma. The median observation period in surviving patients was 55

months (range, 4.8–117 months). The 3-year PFS rate was 76.1%, and the 3-year OS rate was 80.1%.

Figure.2. Analysis of the impact of anemia on treatment outcomes in patients with diffuse large B-cell lymphoma treated with R-CHOP therapy (n = 185). Kaplan–Meier plots for progression-free survival according to the grade of baseline anemia.

Figure.3. (A) Progression-free survival (PFS) and (B) Overall survival (OS) in 185 patients with diffuse large B-cell lymphoma according to prognostic index. Kaplan–Meier plots for event-free survival according to the grade of baseline anemia and clinical stage.

Figure 1

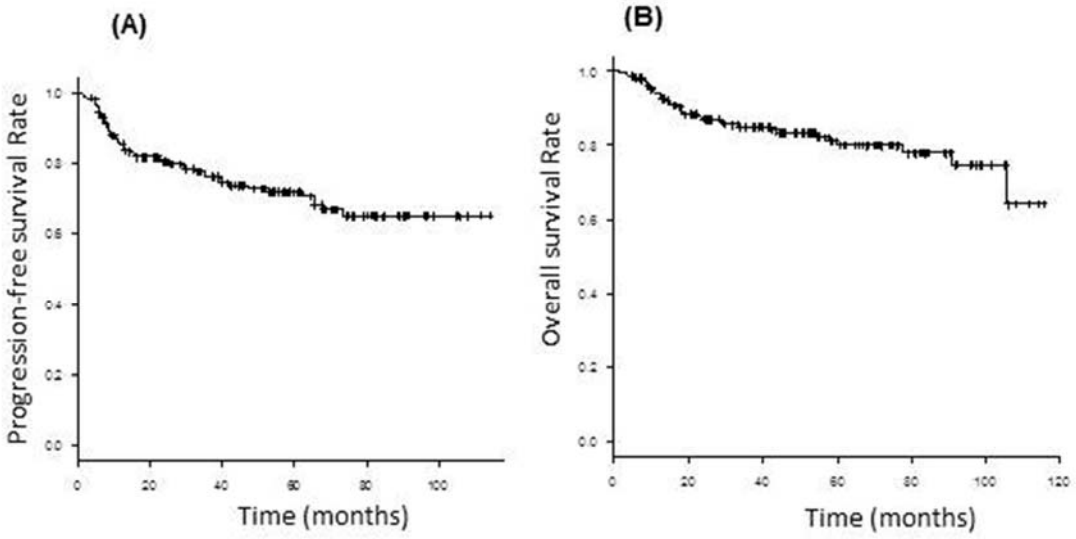
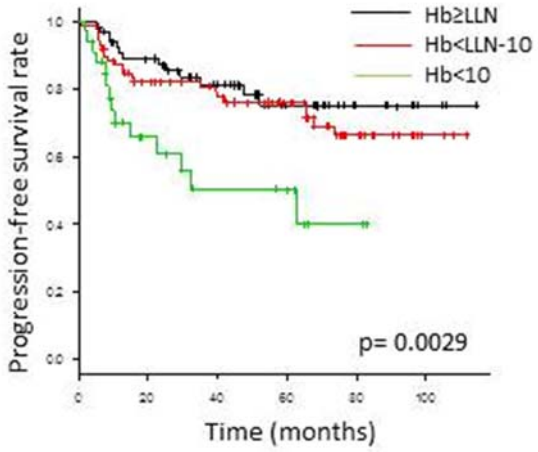


Figure 2



LLN:lower limit normal

Figure 3

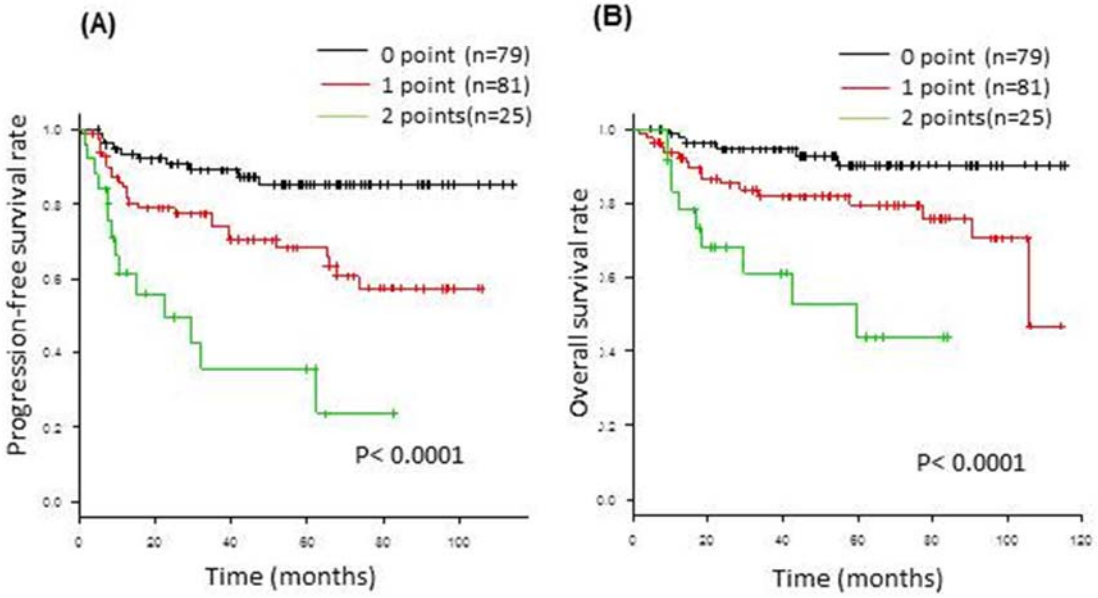


Table 1 Clinicopathological characersitics of patients with DLBCL

	total (n=185)
Gender	
Male	121 65%
Age at diagnosis	
Median	66
Range	21-83
>60 years	140 75%
Performance status	
≥2	49 26%
LDH	
elevated	136 73%
Clinical stage (Ann Arbor)	
≥3	104 56%
Extranodal sites	
≥2	54 29%
B sympton	
Present	56 30%
BM involvement	
Present	37 20%
Hemoglobin	
>LLN (grade 0)	65 35%
LLN-10 (grade 1)	87 47%
<10g/dl (grade 2)	33 18%
Serum albumin	
>3.5g/dl	121 65%

DLBCL, diffuse large B-cell lymphoma; LDH, lactate dehydrogenase; BM, bone marrow

Table 2. Univariate and multivariate analysis for progression free survival in all patients with diffuse B cell lymphoma (n=185)

Parameters	HR(95% CI)	P-value
Univariate analysis		
Male	0.89(0.49-1.51)	0.606
Age>60	1.16(0.62-2.15	0.643
ECOG performance status ≥2	1.84(1.03-3.30	0.041
Elevated lactose dehydrogenase	1.84(0.92-3.68)	0.084
Clinical stage≥3	4.31(2.15-8.63	<0.001
Extranodal sites ≥2	1.27(0.70-2.30	0.432
Presence of B symptom	1.44(0.80-2.60)	0.226

Grade ≥2 anemia	2.78(1.49-5.19)	0.001
Serum albumin ＜3.5	1.64(0.94-2.87)	0.008
BM involvement	3.27(1.84-5.79)	<0.001
Multivariate analysis		
Clinical stage ≥3	3.00（1.40-6.42）	0.005
Grade ≥2 anemia	2.27(1.19-4.30)	0.012
BM involvement	1.94(1.04-3.63)	0.037

CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio