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Original Article

Peripheral blood lymphocyte-to-monocyte ratio is a useful prognostic factor in patients with newly diagnosed diffuse large B-cell lymphoma receiving chemoimmunotherapy

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Abstract: Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of lymphoid neoplasm. This study aims to investigate whether the immunologically relevant lymphocyte-to-monocyte ratio (LMR) can predict outcome of newly diagnosed DLBCL in the rituximab era. We analyzed retrospective data from 53 newly diagnosed DLBCL patients treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) therapy. Receiver operating characteristic (ROC) curve analysis was used to generate a cutoff value for LMR with an area under the curve =0.728 (P=0.021). The sensitivity and specificity for LMR were 69% and 73%, respectively. We found that a low LMR (<2.2) correlates with higher biological marker-adjusted International Prognostic Index (B-IPI) score (P=0.011). Patients with a low LMR (39.6% of all cases) were found to have significantly prolonged overall survival (OS) and progression-free survival (PFS). This study suggests that the LMR, a surrogate biomarker of host immune, can be used as a marker to assess prognosis of DLBCL after standard first-line chemotherapy.

Keywords: Diffuse large B-cell lymphoma, lymphocyte-to-monocyte ratio, survival

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of lymphoid neoplasm, accounting for 25-30% of all new non-Hodgkin lymphoma (NHL) diagnoses in the west and developing countries each year [1]. In spite of important advances in treatment, a significant proportion of patients will still be refractory to therapy or will relapse as a result. Several factors assessed at diagnosis have been proposed as predictors of clinical outcome in patients with DLBCL. Many efforts have been made to improve survival model's discrimination [2-8]. Biological marker-adjusted International Prognostic Index (B-IPI) was a reliable and clinically applicable tool to predict prognosis in our previous study [3, 4]. The peripheral blood lymphocyte-to-monocyte ratio (LMR) at diagnosis in HL [9-11] and NHL [12-16] patients were reported to be a prognostic factor for clinical outcomes. Tumour-associated macrophages are derived from circulating monocytes and are recruited to the tumour site by soluble tumour-derived chemotactic factors [17]. The LMR were calculated at diagnosis by a standard automated complete blood counter in these studies. This study aimed to use Giemsa staining of peripheral blood film to investigate the impact of peripheral blood lymphocyte-to-monocyte ratio (LMR) on survival in newly diagnosed DLBCL patients receiving rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) therapy.

Patients and methods

Patients

To participate in this study, 53 patients with newly diagnosed DLBCL who were treated with R-CHOP (rituximab, cyclophosphamide, hydroxydaunorubicin, vincristine, prednisone) were followed up at the Drum Tower Hospital between

Table 1. The relationship between LMR and clinicopathologic parameters

Clinicopathologic parameters	N	LMR		χ^2	*P*
		<2.2 (n=21)	≥ 2.2 (n=32)		
Sex					
Male	31	11	20	0.535	0.572
Female	22	10	12		
Age, years					
<60	28	8	20	3.03	0.099
>60	25	13	12		
Stage					
I-II	20	6	14	1.243	0.386
III-IV	33	15	18		
B symptom					
Yes	24	3	21	2.576	0.187
No	29	9	20		
ECOG					
0-1	45	17	28	3.481	0.111
2-4	8	1	7		
LDH					
Normal	27	7	20	4.316	0.051
High	26	14	12		
COO					
GCB	15	5	10	2.216	0.203
Non-GCB	38	16	12		
MYC					
Low	33	10	23	3.175	0.090
High	20	11	9		
BCL-2					
Low	24	7	17	2.004	0.174
High	29	14	15		
MYC and BCL-2 coexpression					
Yse	15	9	6	3.631	0.070
No	38	12	26		
IPI Scores					
0-2	24	6	18	3.920	0.056
3-5	29	15	14		
B-IPI Scores					
0-3	25	5	20	7.616	0.011
4-7	28	16	12		

the years 2009 and 2014. This study was approved by the Institutional Review Board (IRB) of the Drum Tower Hospital, and was performed in accordance with the principles expressed in the Declaration of Helsinki. All patients provided written informed consent. Clinical characteristics were obtained from medical records. Routine follow-up imaging analyses were performed every 3 months for the first 2 years, then every 6 months for the next 3 years, and then annually or whenever clinically indicated. Rate of bands, segments, lymphocytes, and monocytes of peripheral blood was determined by the manual differential counts by using peripheral blood film (Giemsa staining) prior to treatment. The lymphocyte-to-monocyte ratio was calculated by dividing the rate of lymphocytes by the rate of monocytes.

Immunohistochemistry

Immunohistochemistry (IHC) was carried out using a peroxidase-conjugated labeled dextran polymer method. The following primary monoclonal antibodies were used: CD10 (Santa Cruz, USA), BCL6 (Santa Cruz, USA), MUM1 (Santa Cruz, USA) , BCL-2 (Dako, Denmark), MYC (EPitoMics, USA). The cases were considered positive as previous reported [3, 4]. GCB and non-GCB subtypes were classified according to the algorithm described by Hans *et al* [18].

Statistical analysis

The selection of cutoff values of peripheral blood lymphocyte-to-monocyte ratio was determined using receiver operating characteristics (ROC) curve analysis. Survival outcomes were dichotomized into alive versus death in the ROC curve analysis. Associations of LMR with clinical characteristics of patients were described by the chi-square test or Fisher's exact test. The Kaplan-Meier method was used to determine overall survival (OS) and progression-free survival (PFS). OS was defined as the duration from diagnosis until the date of death from any cause, or date of the last follow-up. PFS and OS rates were estimated using the Kaplan-Meier method, and differences were assessed with the log-rank (Mantel-Cox) test. The prognostic impact of different variables on survival was determined by multivariate Cox proportional hazards model. All data were analyzed using SPSS version 13.0. Differences were considered significant when the *P*-value was < 0.05.

Results

Patient characteristics

We retrospectively analyzed data from a total of 53 DLBCL patients in this study. The median

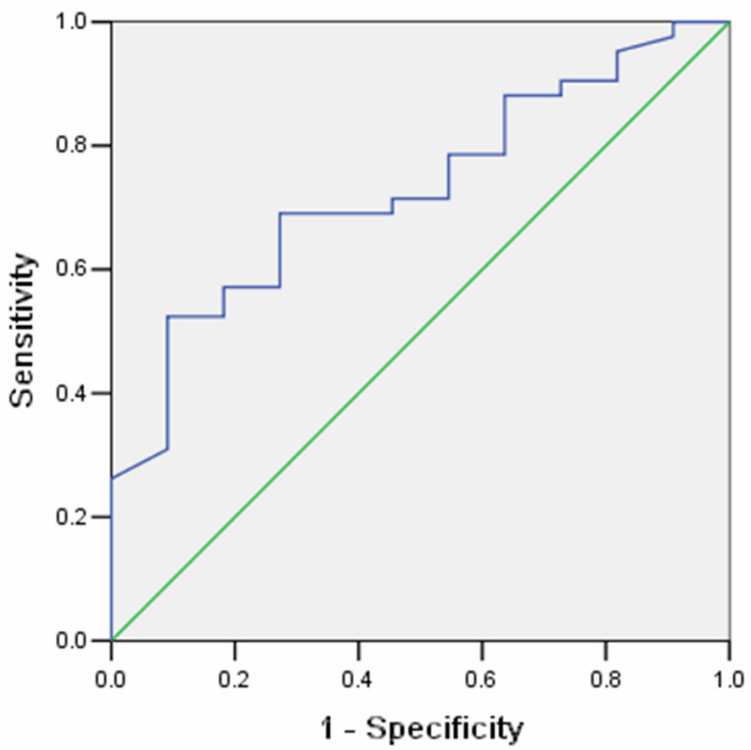

Figure 1. Receiver operating characteristic curve (ROC) and area under the curve (AUC) for the LMR at diagnosis.

Table 2. Univariate and multivariate analysis for OS and PFS outcomes

Covariate	OS			PFS		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Univariate analysis						
Age	1.01	0.96-1.05	0.795	1.01	0.97-1.04	0.881
Gender	1.38	0.42-4.52	0.601	0.81	0.26-2.41	0.698
Stage	3.18	0.69-14.74	0.140	2.74	0.76-9.88	0.121
B symptom	1.80	0.55-5.97	0.334	2.13	0.73-6.21	0.165
ECOG	1.68	0.36-7.89	0.510	1.02	0.23-4.58	0.981
LDH	2.89	0.76-10.93	0.117	2.06	0.69-6.16	0.194
COO	1.36	0.39-4.66	0.626	1.35	0.45-4.06	0.585
IPI	3.59	1.58-8.19	0.002	1.77	0.97-3.26	0.064
B-IPI	6.51	1.71-24.67	0.006	2.50	1.32-4.76	0.005
LMR	0.20	0.05-0.75	0.018	0.31	0.10-0.91	0.034
Multivariate analysis						
IPI	1.38	0.42-4.48	0.586	1.46	0.75-2.81	0.258
B-IPI	5.66	1.41-22.84	0.015	3.01	1.04-8.72	0.041
LMR	0.56	0.13-2.43	0.446	0.69	0.19-2.52	0.579

follow-up was 25 months (range 5-73 months). The male-to-female ratio was 1.41:1 and the median age of patients at diagnosis was 59 years old (range 18-81 years old). Twenty-five patients (47.2%) were ≥ 60 years old. Twenty-six patients (49.0%) had an elevated LDH level. The GC sub-type was applied to 15 of 53 cases (28.3%); the other 38 were of the non-GC subtype (**Table 1**).

Cutoff value for the LMR

ROC curve analysis established 2.190 as the cutoff point of LMR for survival with an AUC of 0.728 (95% CI, 0.577-0.879, P=0.021) (**Figure 1**). The LMR of 2.19 corresponded to the maximum joint sensitivity and specificity on the ROC curve (69% sensitivity and 73% specificity). Based on these results, we selected LMR =2.2 as the optimal cut-off point for survival analysis in our study.

Associations of LMR with clinical characteristics

On the basis of whether patients had an LMR ≥ 2.2 *vs.* LMR <2.2 at the time of diagnosis, the characteristics for patients (n=53) are presented in **Table 1**. 32 patients (60.3%) had an LMR ≥ 2.2 and 21 (39.7%) had an LMR <2.2. A lower LMR (<2.2) was significantly correlated with the B-IPI (P=0.011) (**Table 2**).

Survival analysis

Univariate survival analysis showed that patients in the low LMR group had significantly shorter overall survival (OS) and progression-free survival (EFS) rate than those in the high LMR group, as analyzed and compared in all the patients (P=0.024, with 4-year OS of 39.6% versus 90.6%, **Figure 2A**; P <0.001, with 4-year PFS of 43.8% versus 83.9%, **Figure 2B**). The multivariate survival analysis is shown in **Table 2**.

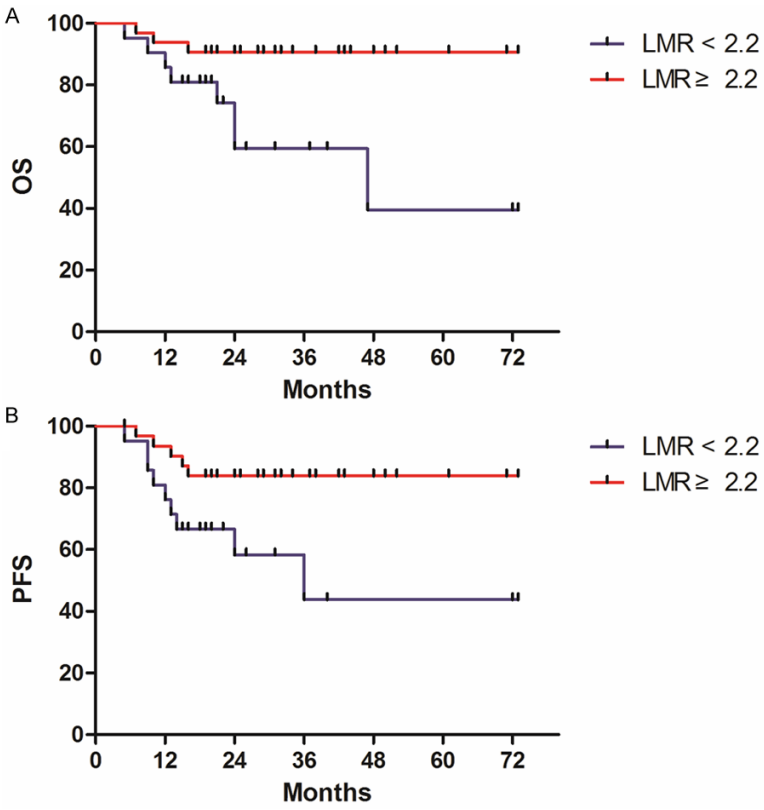

Figure 2. Survival according to LMR. A. Overall survival (P=0.008). B. Progression-free survival (P=0.024).

Discussion

Recent studies indicate that the low peripheral blood lymphocyte-to-monocyte ratio at diagnosis in DLBCL patients is associated with poor clinical out-come [14-16]. These findings indicate the role of host immune in DLBCL patients. To our knowledge, this is the first report demonstrating that a decreased LMR at diagnosis is associated with inferior clinical outcome in patients with newly diagnosed DLBCL by the manual differential counts.

In contrast to conventional prognostic variables, LMR does not incorporate patient and tumour characteristics, because it is detected by manual leukocyte differential count related to a patient's adaptive immune response. Survival outcomes in lymphoma patients have been shown to be influenced by immune infiltrates in the tumors, including lymphocytes and monocytic lineages [19, 20]. We subdivided patients into groups based on arbitrarily selected LMR cut-offs, using ROC curve analysis, and selected the cut-off value that best discriminated between two prognostic groups.

In most clinical laboratories, it is performed by using automated hematology analyzers, and the results are superior to manual differential counts. However, automated hematology analyzers are relatively ineffective in properly recognizing abnormal cells. Manual differential counts by microscopic examination remains the gold standard for this reason. To reduce the uncertainty of the results obtained from the standard automated complete blood counts, the manual differential counts was used thereby eliminating the risk of leukocyte misclassification mostly in those cases with marked peripheral blood infiltration.

We evaluated the prognostic impact of the LMR at diagnosis on the outcomes of patients with DLBCL treated with chemoimmunotherapy. Based on our findings, LMR obtained by manual differential counts provided prognostic information. Indeed, a LMR value of ≥ 2.2 at diagnosis in patients with DLBCL was significantly associated with inferior clinical outcome, suggesting that lymphocytes and monocytes may play a role in patients with DLBCL receiving chemoimmunotherapy. As anticipated, a LMR may reflect host immunity to tumour cells. The results of this study should be interpreted with caution because of its limitations, including the small population size with a relatively short median follow-up time. It is possible that distinct molecular signatures may have an influence on the immunological response. Indeed, human DLBCLs originating from activated B cell-like tumour cells are associated with a poorer prognosis and a more severe alteration of the immune system when compared with DLBCLs originating from the germinal centre tumour cells [21].

In conclusion, LMR at diagnosis showed promise as a prognostic factor of survival outcomes

in DLBCL patients receiving R-CHOP therapy. This biomarker can be used as a simple surrogate indicator of host immune. Further studies are required to more fully understand the relationship between systemic immune response and prognosis of DLBCL in the rituximab era.

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Disclosure of conflict of interest

None.

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References

[1] Cultrera JL, Dalia SM. Diffuse large B-cell lymphoma: current strategies and future directions. Cancer Control 2012; 19: 204-13.

[2] Zhou M, Wang J, Ouyang J, Xu JY, Chen B, Zhang QG, Zhou RF, Yang YG, Shao XY, Xu Y, Chen YM, Fan XS, Wu HY. MYC protein expression is associated with poor prognosis in diffuse large B cell lymphoma patients treated with RCHOP chemotherapy. Tumour Biol 2014; 35: 6757-62.

[3] Wang J, Zhou M, Xu JY, Yang YG, Zhang QG, Zhou RF, Chen B, Ouyang J, Li C. MYC and BCL-2 adjusted-International Prognostic Index (A-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP. Histol Histopathol 2016; 31: 285-92.

[4] Wang J, Zhou M, Xu JY, Chen B, Ouyang J. Combination of Bcl-2 and MYC protein expression improves high-risk stratification in diffuse large B-cell lymphoma. Onco Targets Ther 2015; 8: 2645-2650.

[5] Sehn LH, Berry B, Chhanabhai M, Fitzgerald C, Gill K, Hoskins P, Klasa R, Savage KJ, Shenkier T, Sutherland J, Gascoyne RD, Connors JM. The revised International Prognostic Index (R-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP. Blood 2007; 109: 1857-61.

[6] Advani RH, Chen H, Habermann TM, Morrison VA, Weller EA, Fisher RI, Peterson BA, Gascoyne RD, Horning SJ; Eastern Cooperative Oncology Group; Cancer and Leukemia Group B; Southwest Oncology Group. Comparison of conventional prognostic indices in patients older than 60 years with diffuse large B-cell lymphoma treated with R-CHOP in the US Intergroup Study (ECOG 4494, CALGB 9793): consideration of age greater than 70 years in an elderly prognostic index (E-IPI). Br J Haematol 2010; 151: 143-151.

[7] Zhou Z, Sehn LH, Rademaker AW, Gordon LI, Lacasce AS, Crosby-Thompson A, Vanderplas A, Zelenetz AD, Abel GA, Rodriguez MA, Nademanee A, Kaminski MS, Czuczman MS, Millenson M, Niland J, Gascoyne RD, Connors JM, Friedberg JW, Winter JN. An enhanced International Prognostic Index (NCCN-IPI) for patients with diffuse large B-cell lymphoma treated in the rituximab era. Blood 2014; 123: 837-42.

[8] Cox MC, Nofroni I, Ruco L, Amodeo R, Ferrari A, La Verde G, Cardelli P, Montefusco E, Conte E, Monarca B, Aloe-Spiriti MA. Low absolute lymphocyte count is a poor prognostic factor in diffuse-large-B-cell-lymphoma. Leuk Lymphoma 2008; 49: 1745-51.

[9] Porrata LF, Ristow KM, Habermann TM, Macon WR, Witzig TE, Colgan JP, Inwards DJ, Ansell SM, Micallef IN, Johnston PB, Nowakowski G, Thompson CA, Markovic SN. Peripheral blood absolute lymphocyte/monocyte ratio recovery during ABVD treatment cycles predicts clinical outcomes in classical Hodgkin lymphoma. Blood Cancer J 2013; 3: e110.

[10] Porrata LF, Ristow K, Habermann TM, Witzig TE, Colgan JP, Inwards DJ, Ansell SM, Micallef IN, Johnston PB, Nowakowski GS, Thompson C, Markovic SN. Peripheral blood lymphocyte/monocyte ratio at diagnosis and survival in nodular lymphocyte-predominant Hodgkin lymphoma. Br J Haematol 2012; 157: 321-30.

[11] Porrata LF, Ristow K, Colgan JP, Habermann TM, Witzig TE, Inwards DJ, Ansell SM, Micallef IN, Johnston PB, Nowakowski GS, Thompson C, Markovic SN. Peripheral blood lymphocyte/monocyte ratio at diagnosis and survival in classical Hodgkin's lymphoma. Haematologica 2012; 97: 262-9.

[12] Belotti A, Doni E, Bolis S, Rossini F, Casaroli I, Pezzatti S, Pogliani EM, Pioltelli PE. Peripheral blood lymphocyte/monocyte ratio predicts outcome in follicular lymphoma and in diffuse large B-cell lymphoma patients in the rituximab era. Clin Lymphoma Myeloma Leuk 2015; 15: 208-13.

[13] Li YL, Gu KS, Pan YY, Jiao Y, Zhai ZM. Peripheral blood lymphocyte/monocyte ratio at the time

of first relapse predicts outcome for patients with relapsed or primary refractory diffuse large B-cell lymphoma. BMC Cancer 2014; 14: 341.

[14] Porrata LF, Ristow KM, Habermann TM, Witzig TE, Colgan JP, Inwards DJ, Ansell SM, Micallef IN, Johnston PB, Nowakowski G, Thompson CA, Markovic SN. Peripheral blood absolute lymphocyte/monocyte ratio during rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone treatment cycles predicts clinical outcomes in diffuse large B-cell lymphoma. Leuk Lymphoma 2014; 55: 2728-38.

[15] Yan-Li L, Kang-Sheng G, Yue-Yin P, Yang J, Zhi-Min Z. The lower peripheral blood lymphocyte/monocyte ratio assessed during routine follow-up after standard first-line chemotherapy is a risk factor for predicting relapse in patients with diffuse large B-cell lymphoma. Leuk Res 2014; 38: 323-8.

[16] Watanabe R, Tomita N, Itabashi M, Ishibashi D, Yamamoto E, Koyama S, Miyashita K, Takahashi H, Nakajima Y, Hattori Y, Motohashi K, Takasaki H, Ohshima R, Hashimoto C, Yamazaki E, Fujimaki K, Sakai R, Fujisawa S, Motomura S, Ishigatsubo Y. Peripheral blood absolute lymphocyte/monocyte ratio as a useful prognostic factor in diffuse large B-cell lymphoma in the rituximab era. Eur J Haematol 2014; 92: 204-10.

[17] Ribatti D, Nico B, Crivellato E, Vacca A. Macrophages and tumor angiogenesis. Leukemia 2007; 21: 2085-9.

[18] Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, Müller-Hermelink HK, Campo E, Braziel RM, Jaffe ES, Pan Z, Farinha P, Smith LM, Falini B, Banham AH, Rosenwald A, Staudt LM, Connors JM, Armitage JO, Chan WC. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 2004; 103: 275-82.

[19] Dave SS, Wright G, Tan B, Rosenwald A, Gascoyne RD, Chan WC, Fisher RI, Braziel RM, Rimsza LM, Grogan TM, Miller TP, LeBlanc M, Greiner TC, Weisenburger DD, Lynch JC, Vose J, Armitage JO, Smeland EB, Kvaloy S, Holte H, Delabie J, Connors JM, Lansdorp PM, Ouyang Q, Lister TA, Davies AJ, Norton AJ, Muller-Hermelink HK, Ott G, Campo E, Montserrat E, Wilson WH, Jaffe ES, Simon R, Yang L, Powell J, Zhao H, Goldschmidt N, Chiorazzi M, Staudt LM. Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. N Engl J Med 2004; 351: 2159-69.

[20] Ansell SM, Stenson M, Habermann TM, Jelinek DF, Witzig TE. Cd4+ T-cell immune response to large B-cell non-Hodgkin's lymphoma predicts patient outcome. J Clin Oncol 2001; 19: 720-6.

[21] Mehta-Shah N, Younes A. Novel targeted therapies in diffuse large B-cell lymphoma. Semin Hematol 2015; 52: 126-37.