Original Paper

Prognostic Significance of Monocytes and Monocytic Myeloid-Derived Suppressor Cells in Diffuse Large B-Cell Lymphoma Treated with R-CHOP

Chongyang Wu^a Xiangyang Wu^b Xiaoning Liu^c Peiqi Yang^d Juan Xu^a Ye Chai^a
Qi Guo^e Zhiping Wang^e Liansheng Zhang^a

^aHematology Department, The Second Hospital of Lanzhou University, Lanzhou, ^bCardiac Surgery Department, The Second Hospital of Lanzhou University, Lanzhou, ^cInstitution of Epidemiology and Health Statistics, School of Public Health, Lanzhou University, Lanzhou, ^dLaboratory Department, The Second Hospital of Lanzhou University, Lanzhou, ^eInstitute of Urology, The Second Hospital of Lanzhou University, Key Laboratory of Urological Diseases in Gansu Province, Lanzhou, Gansu Nephro-Urological Clinical Center, Lanzhou, China

Key Words

Monocytic myeloid-derived suppressor cells • Diffuse large B-cell lymphoma • Flow cytometry • Chemotherapy

Abstract

Background/Aims: To evaluate the prognostic significance of monocytes and monocytic myeloid-derived suppressor cells (M-MDSCs) for patients with diffuse large B-cell lymphoma (DLBCL) under R-CHOP chemotherapy. **Methods:** Flow cytometry (FCM) was applied to measure M-MDSCs (CD14⁺ HLA-DR^{low/-} M-MDSCs). **Results:** Analysis of 144 patients with DLBCL under R-CHOP treatment showed that the 5-year overall survival rate was 61.09% (95% CI: 43.72%-72.56%) and the average survival time of patients with monocytes (%) $\geq 8\%$ was shorter than those with monocytes (%) $< 8\%$ ($P = 0.0036$). Further stratified analysis suggested that the average survival time of patients with monocytes (%) $\geq 8\%$ was shorter than patients with monocytes (%) $< 8\%$ in the moderate outcome group ($R\text{-IPI} = 1, 2$) ($P = 0.0168$) and in the poorest outcome group ($R\text{-IPI} > 2$) ($P = 0.0397$), meanwhile, there was no significant difference in survival of patients with monocytes (%) $\geq 8\%$ compared to patients with monocytes (%) $< 8\%$ in the best outcome group ($R\text{-IPI} = 0$) ($P = 0.3106$). Both of monocytes (%) and M-MDSCs were decreased in different R-IPI groups after 4-course of R-CHOP chemotherapy ($P < 0.05$). **Conclusion:** Our results indicated that monocytes (%) and M-MDSCs combined with R-IPI may be a simple and efficient immunological index to evaluate prognosis.

© 2016 The Author(s)
Published by S. Karger AG, Basel

C. Wu and X. Wu are the co-first authors of this paper.

Zhiping Wang and Liansheng Zhang

Institute of Urology, The Second Hospital of Lanzhou University, Key Laboratory of Urological Diseases in Gansu Province, Lanzhou, Gansu Nephro-Urological Clinical Center, Lanzhou, Gansu Province, No. 82 Cuiyingmen, 730030 (China); Hematology Department, The Second Hospital of Lanzhou University, Lanzhou, Gansu Province, 730030, (China)
E-Mail erylwzp@lzu.edu.cn / zhanglsh@lzu.edu.cn

Introduction

Diffuse large B-cell lymphoma (DLBCL) is a mostly common type of malignant lymphoma, its morbidity is up to 30%-40% in non-Hodgkin's lymphoma (NHL) [1, 2]. The clinical presentation, pathomorphology, immunophenotype, therapeutic reaction and prognosis of DLBCL significantly varied [3]. The accurate pathologic diagnosis, type, stratification and prognostic indexes are useful to promote the efficacy for patients with DLBCL. It has been proved that R-CHOP (rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristinesulfate, prednisone) chemotherapy improved the outcome of patients with DLBCL [2, 4-6]. Now revised international prognostic index (R-IPI) submitted by Sehn LH, et al in 2007 is widely used to identify the prognosis accurately for patients with DLBCL treated with R-CHOP in clinic [1]. Patients are divided into three groups according to R-IPI based on multiple clinical indexes: the best outcome group (R-IPI = 0), the moderate outcome group (R-IPI = 1, 2) and the poorest outcome group (R-IPI > 2) [1]. Up to now, it's well known that the clinical progression and biologic characteristics of tumor is not only dependent on tumor malignancy, but also determined to the immunologic status and microenvironment of organism. The immunocytes including tumor invasive lymphocytes (TIL), tumor associated macrophages (TAM) and MDSCs play an important role in the tumor pathogenesis, and the related primary and secondary immunity may affect the efficacy and outcome of patients [7-11]. MDSCs were significantly elevated in pathological process and became a hot topic recently [12]. Long-term clinical studies showed that patients diagnosed lymphoma (Hodgkin lymphoma, follicular lymphoma (FL), DLBCL) with elevated peripheral absolute lymphocyte count (ALC) and absolute monocyte count (AMC) or low lymphocyte/monocyte ratio (LMR) tended to have worse survival and tumor progression [13-17]. However, the type and threshold of related immunocytes were different among different studies so that it is in doubt that the immunologic index could be a factor to identify prognosis in lymphoma. This study analyzed retrospective data from 144 patients with DLBCL under R-CHOP treatment in the Second Hospital of Lanzhou University. We measured peripheral monocytes (%) and M-MDSCs in patients with DLBCL at new diagnosis and after 4-course of R-CHOP chemotherapy, analyzed the variation of monocytes (%) and M-MDSCs pre and post-therapy, compared the survival time of patients with different monocytes (%) adjusted to R-IPI to investigate the prognostic significance of monocytes (%) and M-MDSCs in immunochemotherapy.

Materials and Methods

This study is approved by The Second Hospital of Lanzhou University Medical Ethics Committee.

Patients

We collected data from 144 patients with newly diagnosed DLBCL from January 2010 to January 2014 in the Second Hospital of Lanzhou University according to WHO 2008 the diagnostic criteria of B-cell lymphoma. Patients consist of 75 male and 69 female, aged from 20 to 79 years. The follow-up was done by clinic visit or telephone for all patients. The end point of follow-up was in December 2014. The overall survival (OS) is from the time of new diagnosis to death or the last time of follow-up.

R-IPI score and treatment

All patients were divided into three groups according to R-IPI system. There were 21, 57 and 66 patients in the best outcome group (R-IPI = 0), the moderate outcome group (R-IPI = 1, 2) and the poorest outcome group (R-IPI > 2), respectively. All patients received standard R-CHOP chemotherapy, once every 21 days, received assessment every 2-course of chemotherapy. If the patient got CR (complete response) or PR (partially response) after receiving 4-course of chemotherapy, he/she would be treated for total 6 or 8 courses of chemotherapy, respectively. If the patient got SD (stable disease) or PD (progressive disease) after receiving 4-course of chemotherapy rescue therapy or second-line therapy (ESHAP, ICE, GDP, allo-BMT) would be taken for them.

Measurement of Monocytes and M-MDSCs

We collected 5 ml blood in heparinized tube from patients at initial diagnosis or after receiving 4-course of R-CHOP treatment. M-MDSCs was marked by CD14⁺HLA-DR^{-/low} and measured by FCM. Automatic blood cell analyzer was used to count and classify blood cells and the percent of monocytes in leukocytes (monocytes (%)) represented the detection of monocytes.

Statistical analysis

The data was analyzed by Stata12.0 software. The comparison of survival rate between different groups was done by log-rank test of Kaplan-Meier. The data got from pre and post-therapy was compared by matched-pair design and the D-value obeyed normal distribution. The simple relative factor *r* was used in correlation test. *P* < 0.05 was defined as statistical significance.

Results

Patient characteristics

All 144 patients with newly diagnosed DLBCL met the inclusion criteria were enrolled into this study. The median age was 61 years, aged from 20 to 79 years. 89 patients (61.8%) were older than 60 years and other 55 patients (38.2%) were younger than or equal to 60 years. All patients were divided into two groups including 63 germinal center

Table 1. The baseline characteristics of patients with DLB-CL

Variable	N	%
Age, years		
> 60	89	61.8
≤ 60	55	38.2
Gender		
Male	75	52.1
Female	69	47.9
Hans classification		
GCB	63	43.8
Non-GCB	81	56.2
Origin		
Primary lymphoma	86	59.7
Extranodal lymphoma	58	40.3
Lymph nodes		
Invaded	98	68.1
Non-invaded	46	31.9
Extranodal lymph nodes		
Invaded	73	50.7
Non-invaded	71	49.3
Monocytes (%)		
≥ 8%	62	43.1
< 8%	82	56.9
R-IP1		
The best outcome group(0)	21	14.6
The moderate outcome group (1, 2)	57	39.6
The poorest outcome group (> 2)	66	45.8
Median		
Age, years	61(20-79)	

B-cell (GCB) patients and 81 non-germinal center B-cell (non-GCB) patients according to the Hans classification [18]. 86 cases (59.7%) are primary lymphoma and 58 cases (40.3%) are primary extranodal lymphoma. The lymph nodes had been invaded in 68.1% (98/144) cases, especially cervical lymph nodes invasion in 60.2% (59/98) cases. 50.7% (73/144) cases had extranodal invasion, especially bone marrow invasion in 67.1% (49/73) cases. All patients were subdivided into two groups according to monocytes (%): monocytes (%) ≥ 8% group and monocytes (%) < 8% group. (Table 1)

After receiving 4-course of standard R-CHOP, 81 cases (56.3%), 40 cases (27.8%), 19 cases (13.2%) and 4 cases (2.8%) got CR, PR, SD and PD respectively. To the end point of follow-up, 26 patients (18.1%) died (5 cases died of treatment and 21 cases died of progressive disease), the median follow-up time was 37.4 months (ranged from 12.1 to 93.0 months) (Table 2).

Survival analysis of different subtypes and prognosis of patients

The follow-up data was analyzed by Kaplan-Meier. The 5-year overall survival was 61.09% (95%CI: 43.72%-72.56%) (Fig. 1). Sub-group analysis showed that the mean survival time of non-GCB and GCB patient was 43.9 months (95%CI: 41.3-46.6 months) and 47.8 months (95%CI: 45.6-50.0 months) respectively and there was no significant difference (*P* = 0.1692) (Fig. 2A). The mean survival time of patients in the best outcome group,

the moderate outcome group and the poorest outcome group was 51.1 months (95%CI: 50.2-52.1 months), 43.7 months (95%CI: 42.4-45.1 months) and 30.2 months (95%CI: 29.4-31.1 months), respectively and the mean survival time of patients in each of two groups was significantly different (All $P < 0.0001$) (Fig. 2B).

Effect of peripheral Monocytes (%) on survival of patients at new diagnosis

The normal value of monocytes (%) ranges from 3% to 8% in our hospital and the upper limit value (8%) was used as the threshold in this study. With R-IPI for the diagnostic criteria of prognosis and monocytes (%) for the result of prognostic diagnosis, AUC (area under the curve) was 0.8246 (95%CI: 0.7025-0.9466) and 0.7067 (95%CI: 0.6215-0.7919) in the moderate outcome group and poorest outcome group, respectively (Fig. 3A, 3B). Meanwhile, there was no corresponding cutoff value in the best outcome group because of the irrelevance of monocytes (%). Patients were divided into two groups based on the value of monocytes (%). The mean survival time of patients newly diagnosed with monocytes (%) $\geq 8\%$ was 42.3 months (95%CI: 39.3-45.2 months) while the mean survival time of patients with monocytes (%) $< 8\%$ was 48.6 months (95%CI: 46.6-50.6 months), and it was significantly different between these two groups ($P = 0.0036$) (Fig. 4A). In the best outcome group consisting of 21 cases, the mean survival time between the group with monocytes (%) $\geq 8\%$ (49.2 months (95%CI: 48.2-50.3 months)) and the group with monocytes (%) $< 8\%$ (51.4 months (95%CI: 50.3-52.5 months)) was similar ($P = 0.3106$) (Fig. 4B). In the moderate outcome group consisting of 57 cases, the mean survival time in the group with monocytes (%) $\geq 8\%$ (40.6 months (95%CI: 38.4-42.7 months)) was shorter compared to the group with monocytes (%) $< 8\%$ (44.8 months (95%CI: 43.5-46.1 months)) ($P = 0.0168$) (Fig. 4C). In the poorest outcome group made up of 66 cases, the mean survival time in the group with monocytes (%) $\geq 8\%$ (29.5 months (95%CI: 28.1-31.0 months)) was shorter than the group with monocytes (%) $< 8\%$ (35.9 months (95%CI: 29.9-36.9 months)) ($P = 0.0397$) (Fig. 4D). These results suggested that monocytes (%) may be a predictor for outcome of patients with DLBCL.

Table 2. The follow-up of patients with DLBCL after R-CHOP treatment

Variable	N	%
Response		
Complete response	81	56.3
Partially response	40	27.8
Stable disease	19	13.2
Progressive disease	4	2.8
Monocytes (%)		
$\geq 8\%$	56	38.9
$< 8\%$	88	61.1
Survival		
Survive	118	81.9
Death	26	18.1
Related to treatment	5	19.2
Related to progressive disease	21	80.8
Median		
Follow-up, months	37.4(12.1-93)

Fig. 1. Kaplan-Meier survival analysis of patients with diffuse large B-cell lymphoma treated with R-CHOP. The 5-year OS of patients with DLBCL treated with R-CHOP was 61.09% (95%CI: 43.72%-72.56%).

Fig. 2. Kaplan-Meier survival analysis of different subtypes and prognosis of patients with DLBCL. (A) The overall survival of patients with non-GCB-DLBCL and GCB-DLBCL. The mean survival time of GCB patients was similar to non-GCB patients ($P = 0.1692$). (B) The overall survival of different groups based on R-IPI. The mean survival time among the best prognosis group, the moderate prognosis group and the poorest prognosis group was significantly different ($P < 0.0001$).

Fig. 3. ROC curves analysis for the cutoff value of monocytes (%). (A) ROC curve analysis for the cutoff value of monocytes (%) with $AUC = 0.8246$ in the moderate outcome group. (B) ROC curve analysis for the cutoff value of monocytes (%) with $AUC = 0.7067$ in the poorest outcome group.

Comparison of peripheral monocytes (%) and M-MDSCs pre and post-therapy from different R-IPI groups

The data was analyzed from 144 patients with DLBCL pre-therapy and after 4-course of R-CHOP. We compared the variation of peripheral monocytes (%) in different groups based on R-IPI. In the best outcome group, the monocytes (%) after therapy ($6.43\% \pm 1.25\%$ (95%CI: 5.86%-6.70%)) was decreased significantly compared with those of pre-therapy ($7.62\% \pm 1.53\%$ (95%CI: 6.92%-8.32%)) ($t = 5.294$, $P < 0.0001$); In the moderate outcome group, the monocytes (%) after therapy ($6.70\% \pm 1.65\%$ (95%CI: 6.27%-7.14%)) was decreased significantly compared with those of pre-therapy ($7.65\% \pm 2.48\%$ (95%CI: 6.99%-8.31%)) ($t = 5.4244$, $P < 0.0001$); In the poorest outcome group, the monocytes (%) after therapy ($9.18\% \pm 3.61\%$ (95%CI: 8.29%-10.07%)) was decreased obviously compared with those of pre-therapy ($10.39\% \pm 4.68\%$ (95%CI: 9.24%-11.55%)) ($t = 4.8403$, $P < 0.0001$) (Table 3).

The variation of peripheral M-MDSCs pre-therapy and post-therapy was compared in different groups based on R-IPI. In the best outcome group, the M-MDSCs after therapy ($6.98\% \pm 1.39\%$ (95%CI: 6.35%-7.61%)) was decreased significantly compared with those of pre-therapy ($7.7\% \pm 1.35\%$ (95%CI: 7.08%-8.32%)) ($t = 3.1993$, $P = 0.0023$); In the moderate outcome group, the M-MDSCs after therapy ($7.90\% \pm 1.24\%$ (95%CI: 7.57%-8.23%)) was

Fig. 4. Effect of peripheral Monocytes (%) on survival of patients with DLBCL at new diagnosis. (A) The mean survival time of patients newly diagnosed with monocytes (%) $\geq 8\%$ was shorter than those with monocytes (%) $< 8\%$ ($P = 0.0036$). (B) In the best prognosis group composed of 21 cases, there was no difference in survival between the patients with monocytes (%) $\geq 8\%$ and the patients with monocytes (%) $< 8\%$ ($P = 0.3106$). (C) In the moderate prognosis group composed of 57 cases, the mean survival time of patients with monocytes (%) $\geq 8\%$ was shorter than those with monocytes (%) $< 8\%$ ($P = 0.0168$). (D) In the poorest prognosis group composed of 66 cases, the mean survival time of the group with monocytes (%) $\geq 8\%$ was shorter than the group with monocytes (%) $< 8\%$ ($P = 0.0397$).

Fig. 5. The correlation analysis of monocytes (%) and M-MDSCs in patients with DLBCL newly diagnosed or treated R-CHOP. (A) Peripheral monocytes (%) was positively related to M-MDSCs in patients with DLBCL at new diagnosis ($r = 0.6172$, $P < 0.0001$). (B) Peripheral monocytes (%) was positively related to M-MDSCs in patients with DLBCL after R-CHOP chemotherapy ($r = 0.7241$, $P < 0.0001$).

decreased significantly compared with those of pre-therapy ($8.21\% \pm 1.38\%$ (95%CI: $7.84\%-8.58\%$)) ($t = 2.9874$, $P = 0.0021$); In the poorest outcome group, the M-MDSCs after therapy ($10.59\% \pm 1.61\%$ (95%CI: $10.19\%-10.98\%$)) was decreased significantly compared with those of pre-therapy ($12.24\% \pm 1.16\%$ (95%CI: $11.95\%-12.52\%$)) ($t = 10.9424$, $P < 0.0001$) (Table 3).

Table 3. Comparison of monocytes (%) and M-MDSCs pre-therapy and post-therapy

Variable	Pre-therapy	Post-therapy	T-test	P value
Monocytes (%)				
R-IPI = 0, n = 21	7.62±1.53	6.43±1.25	5.2940	< 0.0001
R-IPI = 1, 2, n = 57	7.65±4.28	6.70±1.65	5.4244	< 0.0001
R-IPI > 2, n = 66	10.39±4.68	9.18±3.61	4.8403	< 0.0001
M-MDSCs				
R-IPI = 0, n = 21	7.70±1.35	6.98±1.39	3.1993	0.0023
R-IPI = 1, 2, n = 57	8.21±1.38	7.90±1.24	2.9874	0.0021
R-IPI > 2, n = 66	12.24±1.16	10.59±1.61	10.9424	< 0.0001

The correlation analysis of monocytes (%) and M-MDSCs in patients newly diagnosed or treated with R-CHOP

We tried to found the link of monocytes (%) and M-MDSCs in patients with DLBCL at new diagnosis or after 4-course of R-CHOP treatment by correlation analysis. The correlation analysis of peripheral monocytes (%) and M-MDSCs demonstrated that there was a positive correlation between peripheral monocytes (%) and M-MDSCs pre-therapy ($r = 0.6172$, $P < 0.0001$) or post-therapy ($r = 0.7241$, $P < 0.0001$) (Fig. 5).

Discussion

With the application of molecular targeted drugs, the 10-year overall survival rate of patients with DLBCL with R-CHOP treatment was improved [19-21]. To date, R-CHOP chemotherapy is still the standard treatment for patients with DLBCL. Fu, K et al. showed that the 3-year OS of patients with DLBCL was improved after receiving R-CHOP chemotherapy (42% VS 77%, $P < 0.001$) and the prognosis of GCB patients was better than non-GCB patients (85% VS 69%, $P = 0.032$) [22]. However, it also demonstrated that there was no obviously different OS between GCB and non-GCB subtype patients with DLBCL who received R-CHOP chemotherapy [23]. Our results also suggested that the 5-year OS of patients with DLBCL who were treated with R-CHOP chemotherapy was 61.09% and the mean survival time was not related to the pathological subtypes ($P = 0.1692$). Prognostic indexes for lymphoma are very profitable to select best treatment for patients and improve their prognosis [24, 25]. A systematic review and meta-analysis enrolled 2643 patients demonstrated that low serum 25-hydroxyvitamin D levels can be a prognostic factor for patients with hematological cancer [26]. R-IPI is a better clinical prognostic index than international prognostic index (IPI) in patients with DLBCL [1]. R-IPI was applied to divide all patients with R-CHOP treatment into three groups and we found that the mean survival time among different prognostic groups was significantly different ($P < 0.0001$). It coincided with the results of study executed by Hong J. et al. [27]. These results suggested that immunochemotherapy (R-CHOP) could improve the efficacy for patients with DLBCL with poor prognosis and R-IPI is still an efficient prognostic index for patients with DLBCL in immunochemotherapy era.

The non-malignant immunocytes in tumor microenvironment play a vital role in tumor heterogeneity [28]. Single-factor analysis or multi-factor analysis showed that AMC and ALC both were the independent poor prognostic factor and both of them adjusted to R-IPI may identify the high-risk patients in DLBCL and FL [15, 17]. A multi-center study enrolled 1017 patients showed that $AMC > 630 \text{ mm}^3$ at diagnosis was a predictor to identify the high-risk patients in rituximab era [16]. During the period of R-CHOP treatment for patients with DLBCL, peripheral ALC/AMC ratio recovery may predict prognosis and $ALC/AMC < 1.1$ was an independent parameter of OS and PFS [29]. We found that peripheral AMC usually was normal but monocytes (%) usually elevated in DLBCL from previous studies. Thus, we used an automatic blood cell analyzer to count and classify blood cells, monocytes (%) represents

the result of monocytes and its threshold is the upper limit value (8%) based on the normal value (3%-8%) in our hospital. Our study showed that the 5-year OS was 61.09% among 144 patients with newly diagnosed DLBCL with R-CHOP treatment and the survival time of patients with monocytes (%) $\geq 8\%$ at new diagnosis was shorter than those with monocytes (%) $< 8\%$ in the poorest group and the moderate outcome group but not in the best outcome group based on R-IPI. ROC curves were done for the cutoff value of monocyte (%). In the best outcome group, there was no corresponding cutoff value because of the irrelevance of monocytes (%). In the moderate outcome group, AUC (area under the curve) was 0.8246 (95%CI: 0.7025-0.9466). The sensitivity is 94.74% and specificity is 66.67% when monocytes (%) $\geq 7\%$. The sensitivity is 54.39% and specificity is 80.95% when monocytes (%) $\geq 8\%$. Those results illustrated that the value of monocytes (%) of 8% had better prognostic value in the moderate outcome group. In the poorest outcome group, AUC was 0.7067 (95%CI: 0.6215-0.7919). The sensitivity is 60.61% and specificity is 55.13% when monocytes (%) $\geq 8\%$, and the 5-year OS and survival time of patients between the group with monocytes (%) $\geq 8\%$ and the group with monocytes (%) $< 8\%$ are different. But when monocytes (%) $\geq 10\%$, the sensitivity is 54.55% and specificity is 83.88%. It indicated that the value of 10% as a threshold may be more valuable, which may result from other prognostic indexes have even bigger weight. Therefore, a further research with more patients and fine sorted stratification should be done to clarify the more proficient cutoff value of monocytes (%) directed against patients with poor prognosis. These results suggested that the peripheral monocytes (%) could predict prognosis as well as R-IPI, even that the elevation of monocytes (%) could be a better predictor to evaluate poor prognosis in the poorest and moderate outcome groups. Blood test as a clinical routine test is economic, convenient and easy. Therefore, it is very necessary to apply monocytes (%) combined with R-IPI to identify the high risk of patients with poor prognosis so that we could adjust the corresponding therapy in time to improve therapeutic effect.

MDSCs are a heterogeneous group of cells derived from immature precursor cells that could change immunological microenvironment through multiple pathways and promote progressive disease [30, 31]. Monocytic MDSCs (M-MDSC CD14⁺HLA-DR^{-/low}) is one subtype of MDSCs. Previous studies showed that the elevation of monocytes expressing CD14⁺HLA-DR^{-/low}, TAM and tumor-associated MDSC all were related to the poor prognosis in different tumors including lymphoma [10, 32]. Tadmor et al. found that AMC of patients at new diagnosis may be related to M-MDSCs [33]. Monocytes (%) and M-MDSCs from different prognosis groups based on R-IPI significantly decreased after receiving 4-course of R-CHOP treatment compared to pre-therapy and there was significant difference in comparison of every two groups in our study. Our results indicated that monocytes and M-MDSCs may participate in the progression of DLBCL, R-CHOP could directly kill tumor cells and is related to immunity of organism. These findings may provide a new way to target monocytes (%) and M-MDSCs remedy and improve therapeutic effect for patients with DLBCL in the future. Correlation analysis showed that peripheral monocytes (%) was positively related to M-MDSCs at initial diagnosis or after 4-course of R-CHOP treatment, but the link of monocytes (%) and M-MDSCs still needs for further research. A further study enrolled more subjects should be done to investigate the derivation and function of M-MDSCs, the detailed link of monocytes (%) and M-MDSCs and prognostic significance in patients with DLBCL.

Acknowledgments

This work was supported by The National Natural Science Foundation of China (Grant No.81372733/H1619) and (Grant No.81470026/H0213).

Disclosure Statement

None.

References

- 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;109:1857-1861.
- Coiffier B, Lepage E, Briere J, Herbrecht R, Tilly H, Bouabdallah R, Morel P, Van Den Neste E, Salles G, Gaulard P, Reyes F, Lederlin P, Gisselbrecht C: CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. *N Engl J Med* 2002;346:235-242.
- Staudt LM, Dave S: The biology of human lymphoid malignancies revealed by gene expression profiling. *Adv Immunol* 2005;87:163-208.
- Feugier P, Van Hoof A, Sebban C, Solal-Celigny P, Bouabdallah R, Ferme C, Christian B, Lepage E, Tilly H, Morschhauser F, Gaulard P, Salles G, Bosly A, Gisselbrecht C, Reyes F, Coiffier B: Long-term results of the R-CHOP study in the treatment of elderly patients with diffuse large B-cell lymphoma: a study by the Groupe d'Etude des Lymphomes de l'Adulte. *J Clin Oncol* 2005;23:4117-4126.
- Halaas JL, Moskowitz CH, Horwitz S, Portlock C, Noy A, Straus D, O'Connor OA, Yahalom J, Zelenetz AD: R-CHOP-14 in patients with diffuse large B-cell lymphoma: feasibility and preliminary efficacy. *Leuk Lymphoma* 2005;46:541-547.
- Yamato H, Tsutsumi Y, Ehira N, Kanamori H, Shimoyama N, Tanaka J, Imamura M, Asaka M, Hasegawa T, Masauzi N: A case of non-Hodgkin's lymphoma and invasive thymoma treated with R-CHOP therapy. *Int J Hematol* 2006;83:464-466.
- Byers RJ, Sakhinia E, Joseph P, Glennie C, Hoyland JA, Menasce LP, Radford JA, Illidge T: Clinical quantitation of immune signature in follicular lymphoma by RT-PCR-based gene expression profiling. *Blood* 2008;111:4764-4770.
- Farinha P, Masoudi H, Skinnider BF, Shumansky K, Spinelli JJ, Gill K, Klasa R, Voss N, Connors JM, Gascoyne RD: Analysis of multiple biomarkers shows that lymphoma-associated macrophage (LAM) content is an independent predictor of survival in follicular lymphoma (FL). *Blood* 2005;106:2169-2174.
- Parker KH, Beury DW, Ostrand-Rosenberg S: Myeloid-Derived Suppressor Cells: Critical Cells Driving Immune Suppression in the Tumor Microenvironment. *Adv Cancer Res* 2015;128:95-139.
- Mantovani A, Sica A, Allavena P, Garlanda C, Locati M: Tumor-associated macrophages and the related myeloid-derived suppressor cells as a paradigm of the diversity of macrophage activation. *Hum Immunol* 2009;70:325-330.
- Noy R, Pollard JW: Tumor-associated macrophages: from mechanisms to therapy. *Immunity* 2014;41:49-61.
- Lim HX, Hong HJ, Cho D, Kim TS: IL-18 enhances immunosuppressive responses by promoting differentiation into monocytic myeloid-derived suppressor cells. *J Immunol* 2014;193:5453-5460.
- Coiffier B: Rituximab therapy in malignant lymphoma. *Oncogene* 2007;26:3603-3613.
- Wilcox RA, Wada DA, Ziesmer SC, Elsawa SF, Comfere NI, Dietz AB, Novak AJ, Witzig TE, Feldman AL, Pittelkow MR, Ansell SM: Monocytes promote tumor cell survival in T-cell lymphoproliferative disorders and are impaired in their ability to differentiate into mature dendritic cells. *Blood* 2009;114:2936-2944.
- Wilcox RA, Ristow K, Habermann TM, Inwards DJ, Micallef IN, Johnston PB, Colgan JP, Nowakowski GS, Ansell SM, Witzig TE, Markovic SN, Porrata L: The absolute monocyte and lymphocyte prognostic score predicts survival and identifies high-risk patients in diffuse large-B-cell lymphoma. *Leukemia* 2011;25:1502-1509.
- Tadmor T, Bari A, Sacchi S, Marcheselli L, Liardo EV, Avivi I, Benyamini N, Attias D, Pozzi S, Cox MC, Baldini L, Brugiattelli M, Federico M, Polliack A: Monocyte count at diagnosis is a prognostic parameter in diffuse large B-cell lymphoma: results from a large multicenter study involving 1191 patients in the pre- and post-rituximab era. *Haematologica* 2014;99:125-130.
- Wilcox RA, Ristow K, Habermann TM, Inwards DJ, Micallef IN, Johnston PB, Colgan JP, Nowakowski GS, Ansell SM, Witzig TE, Markovic SN, Porrata L: The absolute monocyte count is associated with overall survival in patients newly diagnosed with follicular lymphoma. *Leuk Lymphoma* 2012;53:575-580.

- 18 Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, Muller-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;103:275-282.
- 19 Coiffier B, Thieblemont C, Van Den Neste E, Lepeu G, Plantier I, Castaigne S, Lefort S, Marit G, Macro M, Sebban C, Belhadj K, Bordessoule D, Ferme C, Tilly H: Long-term outcome of patients in the LNH-98.5 trial, the first randomized study comparing rituximab-CHOP to standard CHOP chemotherapy in DLBCL patients: a study by the Groupe d'Etudes des Lymphomes de l'Adulte. *Blood* 2010;116:2040-2045.
- 20 Li X, Liu Z, Cao J, Hong X, Wang J, Chen F, Wang C, Zou S, Li J, Shen Z: Rituximab in combination with CHOP chemotherapy for the treatment of diffuse large B cell lymphoma in China: a 10-year retrospective follow-up analysis of 437 cases from Shanghai Lymphoma Research Group. *Ann Hematol* 2012;91:837-845.
- 21 Tomita N, Suzuki T, Ishiyama Y, Miyashita K, Takahashi H, Numata A, Ito S, Motohashi K, Tachibana T, Takasaki H, Kawasaki R, Hagihara M, Hashimoto C, Yamazaki E, Taguchi J, Fujimaki K, Sakai R, Fujisawa S, Motomura S, Ishigatsubo Y: R-CHOP therapy alone for limited-stage follicular lymphoma. *Leuk Res* 2015;39:582-585.
- 22 Fu K, Weisenburger DD, Choi WW, Perry KD, Smith LM, Shi X, Hans CP, Greiner TC, Bierman PJ, Bociek RG, Armitage JO, Chan WC, Vose JM: Addition of rituximab to standard chemotherapy improves the survival of both the germinal center B-cell-like and non-germinal center B-cell-like subtypes of diffuse large B-cell lymphoma. *J Clin Oncol* 2008;26:4587-4594.
- 23 Nyman H, Adde M, Karjalainen-Lindsberg ML, Taskinen M, Berglund M, Amini RM, Blomqvist C, Enblad G, Leppa S: Prognostic impact of immunohistochemically defined germinal center phenotype in diffuse large B-cell lymphoma patients treated with immunochemotherapy. *Blood* 2007;109:4930-4935.
- 24 Byrd JC, Jones JJ, Woyach JA, Johnson AJ, Flynn JM: Entering the era of targeted therapy for chronic lymphocytic leukemia: impact on the practicing clinician. *J Clin Oncol* 2014;32:3039-3047.
- 25 Ghanem H, Tank N, Tabbara IA: Prognostic implications of genetic aberrations in acute myelogenous leukemia with normal cytogenetics. *Am J Hematol* 2012;87:69-77.
- 26 Wang W, Li G, He X, Gao J, Wang R, Wang Y, Zhao W: Serum 25-hydroxyvitamin D levels and prognosis in hematological malignancies: a systematic review and meta-analysis. *Cell Physiol Biochem* 2015;35:1999-2005.
- 27 Hong J, Park S, Park J, Kim HS, Kim KH, Ahn JY, Rim MY, Jung M, Sym SJ, Cho EK, Shin DB, Lee JH: Evaluation of prognostic values of clinical and histopathologic characteristics in diffuse large B-cell lymphoma treated with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone therapy. *Leuk Lymphoma* 2011;52:1904-1912.
- 28 Lenz G, Wright G, Dave SS, Xiao W, Powell J, Zhao H, Xu W, Tan B, Goldschmidt N, Iqbal J, Vose J, Bast M, Fu K, Weisenburger DD, Greiner TC, Armitage JO, Kyle A, May L, Gascoyne RD, Connors JM, Troen G, Holte H, Kvaloy S, Dierickx D, Verhoef G, Delabie J, Smeland EB, Jares P, Martinez A, Lopez-Guillermo A, Montserrat E, Campo E, Braziel RM, Miller TP, Rimsza LM, Cook JR, Pohlman B, Sweetenham J, Tubbs RR, Fisher RI, Hartmann E, Rosenwald A, Ott G, Muller-Hermelink HK, Wrench D, Lister TA, Jaffe ES, Wilson WH, Chan WC, Staudt LM, Lymphoma/Leukemia Molecular Profiling P: Stromal gene signatures in large-B-cell lymphomas. *N Engl J Med* 2008;359:2313-2323.
- 29 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;55:2728-2738.
- 30 Gabrilovich DI, Ostrand-Rosenberg S, Bronte V: Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol* 2012;12:253-268.
- 31 Youn JI, Nagaraj S, Collazo M, Gabrilovich DI: Subsets of myeloid-derived suppressor cells in tumor-bearing mice. *J Immunol* 2008;181:5791-5802.
- 32 Lin Y, Gustafson MP, Bulur PA, Gastineau DA, Witzig TE, Dietz AB: Immunosuppressive CD14+HLA-DR(low)/- monocytes in B-cell non-Hodgkin lymphoma. *Blood* 2011;117:872-881.
- 33 Tadmor T, Fell R, Polliack A, Attias D: Absolute monocytosis at diagnosis correlates with survival in diffuse large B-cell lymphoma-possible link with monocytic myeloid-derived suppressor cells. *Hematol Oncol* 2013;31:65-71.