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ORIGINAL ARTICLE

Prognostic significance of monocyte chemoattractant protein-1 and CC chemokine receptor 2 in diffuse large B cell lymphoma

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Abstract

Aberrant monocyte chemoattractant protein-1 (MCP-1) and CC chemokine receptor 2 (CCR2) expression in malignant tissues have been reported; however, their role in hematological malignancies prognosis remains little known. The aim of this study was to investigate the prognostic value of MCP-1 and CCR2 expression in patients with diffuse large B cell lymphoma (DLBCL). The study included 221 patients with DLBCL. MCP-1 and CCR2 expression was analyzed by immunohistochemical staining and its correlations with clinicopathologic features and prognosis were evaluated. High expression of MCP-1 or CCR2 was correlated with clinicopathological characteristics, and an adverse prognostic factor for overall survival (OS) and progression-free survival (PFS) of DLBCL patients. Also, significant positive correlation between MCP-1 and CCR2 expression was revealed ($r = 0.545$, $P < 0.001$). Patients with high MCP-1 or high CCR2 expression had significantly poorer OS and PFS than those with low MCP-1 or low CCR2 expression (OS: $P < 0.001$, $P < 0.001$; PFS: $P < 0.001$, $P < 0.001$), respectively, even in the rituximab era, and MCP-1 or CCR2 expression could further identify high-risk patients otherwise classified as low/intermediate risk by the International Prognostic Index (IPI) alone. Furthermore, incorporation of MCP-1 or CCR2 expression into the IPI score could improve prognostic value for OS. This is the first report describing the clinicopathological features and survival outcome according to expression of MCP-1 and CCR2 in DLBCL.

Keywords Diffuse large B cell lymphoma · Monocyte chemoattractant protein-1 · CC chemokine receptor 2 · Overall survival · Progression-free survival · Prognostic value

Introduction

Diffuse large B cell lymphoma (DLBCL) is the most common, accounts for 25–30% of all newly diagnosed cases of adult non-Hodgkin lymphoma (NHL). It is an aggressive lymphoma but is potentially curable [1]. Despite the improvements in overall survival of patients with DLBCL with the routine addition of rituximab therapy, approximately one-third of the patients will develop relapsed/refractory disease that remains a major cause of morbidity and mortality [2].

Chemokines and their receptors were found to be involved in metastasis and also to be direct targets of oncogene

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activation [3, 4]. MCP-1 (monocyte chemoattractant protein-1), the most representative member of the CC chemokine subfamily, was first purified by Matsushima et al from serum-free culture supernatant of human myelomonocytic cell line [5]. The receptor of MCP-1 is CC chemokine receptor 2 (CCR2) and it is expressed in various cell types, such as monocytes and memory T lymphocytes. Many recent studies have reported that MCP-1 is expressed by several malignant tumor cells, including prostate cancer, breast cancer, pancreatic cancer, lung cancer, gastric cancer, hepatocellular carcinoma, and clear-cell renal cell carcinoma [6–12]. MCP-1/CCR2 axis has been shown to induce angiogenic activation of endothelial cells along with inflammatory responses [13], facilitate tumor survival and invasion [14–17], mediate recruitment of myeloid-derived cell subset promoted metastasis [18–21] and polarization of an alternatively activated M2-phenotype thereby contributing to immunosuppression and enhanced tumor cell survival [10, 22, 23]. In this study, we sought to explore the expression patterns of MCP-1 and CCR2 in DLBCL, and their association with clinicopathologic features characteristics and also prognostic value of MCP-1/CCR2 pathway in this disease.

Patients and method

Patients and clinical database

We recruited 221 DLBCL patients undergoing pathological biopsy at the First Affiliated Hospital and the Second Affiliated Hospital of Anhui Medical University, between 2004 and 2015. This study was approved by the hospital's ethics committee and informed consent was obtained from each patient. This study was performed in accordance with the principles expressed in the Declaration of Helsinki. All patients met the following criteria: pathologically confirmed DLBCL; no previous treatment; no previous history of malignancy, transplantation, or immunosuppression; negativity for anti-HIV; treatment with combination chemotherapy with or without radiation treatment; and the availability of laboratory data and follow-up information. Histologic type was defined according to the current World Health Organization classification [24] and for prognostic purposes, International Prognostic Index (IPI) calculated [25].Consecutive 221 patients treated with cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisone (CHOP), or rituximab-cyclophosphamide, hydroxy-daunorubicin, vincristine, and prednisone (R-CHOP) every 3 weeks for 3 to 8 cycles as first-line therapy, and follow-up procedures usually included physical examination, laboratory studies, or imaging examination, every month for the first 3 months and every 3 months thereafter for 2 years, then every 6 months for 3 years in our hospitals.

Immunohistochemistry

Immunohistochemical staining was performed on tissue microarray (TMA). Tissue microarrays were established as previously described [26]. Primary antibodies against human MCP-1 (1:200 dilution, Cat No.ab9669, Abcam, USA) and CCR2 (1:300 dilution, Cat No.ab32144, Abcam, USA) were applied in the procedure. Four-micrometer-thick, formalin-fixed, paraffin-embeded specimen sections were deparaffinized in xylene and rehydrated in a series of grade alcohols. They were then protreated in citric acid antigen retrieval solution (pH 6.0) using heat-induced epitope retrieval technique. After inhibiting internal peroxidase activity with 3% hydrogen peroxide, the sections were incubated with anti-MCP-1 antibody and anti-CCR2 antibody overnight at 4 °C. The slides were then incubated with HRP-conjugated goat anti-mouse IgG secondary antibody for 10 min at 37 °C. Finally, the sections were visualized by DAB solution (DAKO, Carpinteria, CA, USA) and counterstained with hematoxylin (DAKO). Staining intensities and percentages of positive tumor cells were scored independently by two pathologists who were blind to the patients' outcome. The pathologists assessed the adequacy of immunostaining in test slides and in negative and positive control slides, and then selected representative areas of tumor for counting. Semi-quantitative analysis of staining in tumor cells was evaluated as grade 0, 1, 2, or 3 [27]. Grade 0 was as staining in < 1% of tumor cells, grade 1 as 1–33%, grade 2 as 34–66%, and grade 3 as > 67 %. Grade 0 corresponded to negative expression, and grades 1–3 corresponded to positive expression.

Study objective

For statistical analyses, MCP-1 and CCR2 staining were dichotomized into two groups [low (grade 0 and 1) and high (grade 2 or 3)]. The absolute monocyte count (AMC) at the time of diagnosis was obtained from routine automated complete blood count (CBC), and the cutoff value for the AMC at diagnosis (460/μl) was based on data from our reported study [28]. Response criteria were based on the criteria from the Lugano Classification [29]. Progression-free survival (PFS) was calculated from the time of diagnosis to disease progression, relapse, or death of any cause or the last date of follow-up. Overall survival (OS) was calculated from the date of diagnosis to the date of death or last follow-up. Patient and disease characteristics included in the IPI [age < 60 versus ≥ 60 years, Ann Arbor stage (I/II versus III/IV), Eastern Cooperative Oncology Group performance status (ECOG-PS) (≤ 1 versus > 1), lactate dehydrogenase (LDH) (normal

versus >normal), and number of extra nodal sites (ENS) involved (≤ 1 versus > 1)] were utilized.

Statistical analysis

We compared two groups using X^2 or Fisher's exact test for categorical variables. Survival curves were established using the Kaplan-Meier method, and log-rank test was applied to compare the difference between the curves. The Cox proportional hazards regression model was applied to perform univariate and multivariate analyses, and those parameters that demonstrated a statistically significant effect on OS and PFS in the univariate analysis were included in the multivariate analysis. The sensitivity and specificity for the prediction of OS and PFS were analyzed by receiver operating characteristic (ROC) curves. The area under the curve (AUC) was used to measure prognostic or predictive accuracy. Data were analyzed using SPSS Statistics 17.0 SPSS Inc., Chicago, IL, USA). All statistical tests were two-sided and statistical significance was set at 0.05.

Results

Immunohistochemical MCP-1 and CCR2 intensity and their associations with clinical pathological characteristics

The characteristics of patients are listed in Table 1. The median age at diagnosis for this cohort of 221 DLBCL patients was 56 years (range, 13–84 years). The median follow-up following diagnosis was 42 months for the entire cohort (range, 3 to 118 months). A total of 72.9% (161 of 221) and 77.4% (171 of 221) tumors were scored as high MCP-1 and CCR2 expression, respectively, and significant association was detected between MCP-1 and CCR2 expression in DLBCL tumor cells ($r = 0.545$, $P < 0.001$; Supplementary Table 1). MCP-1 immunostaining was exclusively observed in cytoplasm of cancer cells (Fig. 1a, b). Expression of CCR2 in tumor cells was found in either cytoplasm or membrane (Fig. 1c, d). The frequencies of MCP-1 and CCR2 expression level increased gradually accompanied with IPI score (IPI score = 0–1, 2–3, and 4–5) (Fig. 2a, b), and the correlations both reached significant difference ($P < 0.001$ and $P = 0.010$, respectively). MCP-1 expression was significantly associated with ECOG-PS, extranodal sites of disease, Ann Arbor stage, LDH, IPI score, and AMC, respectively ($P = 0.002$, $P = 0.023$, $P = 0.001$, $P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively; Table 1), while CCR2 expression was significantly associated with LDH and AMC, respectively ($P = 0.012$ and $P < 0.001$, respectively; Table 1).

High MCP-1 or high CCR2 expression is an adverse prognostic factor for overall survival and progression-free survival of DLBCL patients

To investigate whether MCP-1 and CCR2 expression was an independent prognostic predictor of OS and PFS, univariate and multivariate analyses were performed. As shown in Table 2, univariate analysis demonstrated that MCP-1 and CCR2 expression was significantly associated with OS (HR 19.709, 95% CI 9.389–41.375, $P < 0.001$ and HR 6.796, 95% CI 3.680–12.550, $P < 0.001$, respectively). Furthermore, multivariate analysis showed that MCP-1 or CCR2 expression remained an independent prognostic indicator for OS (HR 10.983, 95% CI 4.936–24.436, $P < 0.001$ and HR 2.713, 95% CI 1.401–5.256, $P = 0.003$, respectively), as did LDH ($P = 0.004$), AMC ($P = 0.024$), and first-line treatment ($P = 0.001$). Similarly, MCP-1 expression, LDH, AMC, first-line treatment, and Ann Arbor stage were independently significant predictors of PFS when adjusted for components of the IPI on multivariate analysis ($P < 0.001$, $P = 0.011$, $P = 0.008$, $P = 0.042$, and $P = 0.015$, respectively; Supplementary Table 2).

Table 1 Patient's demographics according to MCP-1 and CCR2 expression

Characteristics	Patients		MCP-1 expression			CCR2 expression		
	No.	%	Low	High	*P*	Low	High	*P*
Gender								
Female	105	47.5	29	76	0.881	22	83	0.631
Male	116	52.5	31	85		28	88	
Age (years)								
< 60	130	58.8	39	91	0.284	35	95	0.074
60	91	41.2	21	70		15	76	
ECOG-PS								
≤ 1	136	61.5	47	89	0.002	36	100	0.099
> 1	85	38.5	13	72		14	71	
Extranodal sites of disease								
≤ 1	176	79.6	54	122	0.023	41	135	0.695
> 1	45	20.4	6	39		9	36	
Ann Arbor stage								
I/II	127	57.5	45	82	0.001	34	93	0.104
III/IV	94	42.5	15	79		16	78	
LDH								
≤normal	159	71.9	56	103	< 0.001	43	116	0.012
>normal	62	28.1	4	58		7	55	
IPI score								
0–2	154	69.7	54	100	< 0.001	40	114	0.081
3–5	67	30.3	6	61		10	57	
AMC								
< 460/μl	118	53.4	47	71	< 0.001	40	78	< 0.001
≥ 460/μl	103	46.6	13	90		10	93	

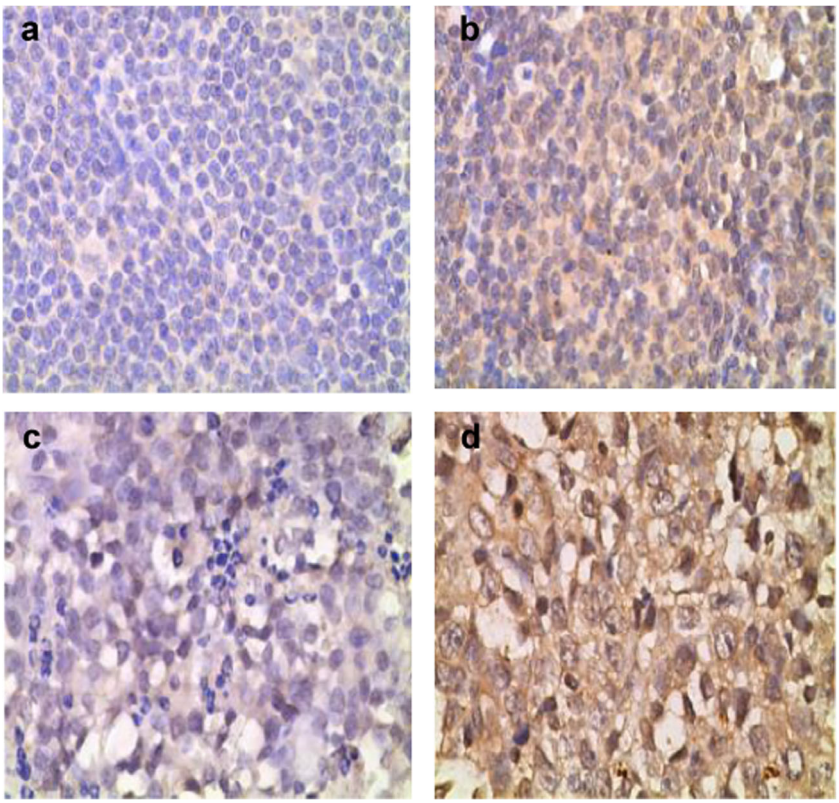


Fig. 1 MCP-1 and CCR2 expression in DLBCL (× 400). Low cytoplasmic staining of MCP-1 (**a**), high cytoplasmic staining of MCP-1 (**b**), low cytoplasmic or membrane staining of CCR2 (**c**), high cytoplasmic or membrane staining of CCR2 (**d**)

Prognostic value of MCP-1 and CCR2 intensity for clinical outcome of DLBCL patients

After first-line chemotherapy, the overall response rate, including CR and PR, was 69% in our study. MCP-1 or CCR2 expression significantly affecting the overall response rate is shown in Table 3 ($P < 0.001$ and $P < 0.001$, respectively). We applied Kaplan-Meier survival analysis to compare OS and PFS according to the MCP-1 or CCR2 expression. Patients with high MCP-1 ($n = 161$) or high CCR2 ($n = 171$) expression had significantly poorer OS and PFS than those with low MCP-1 ($n = 60$) or low CCR2 ($n = 50$) expression, respectively (OS: $P < 0.001$ and $P < 0.001$, respectively; Figs. 3 a and 4a; PFS: $P < 0.001$ and $P < 0.001$, respectively; Supplementary Figs. 1a and 2a). We further examined whether MCP-1 and CCR2 expression could stratify patients with low-risk ($n = 113$) (IPI score = 0–1), intermediate-risk ($n = 77$) (high-intermediate and low-intermediate were combined, IPI score = 2–3), and high-risk ($n = 31$) (IPI score = 4–5) diseases. When the analysis was restricted to low-risk and intermediate-risk, patients could be significantly stratified by MCP-1 expression (OS: $P < 0.001$ and $P < 0.001$, respectively; Fig. 3b, c; PFS: $P < 0.001$ and $P < 0.001$, respectively; Supplementary Fig. 1b, c). Although high-risk DLBCL patients with high

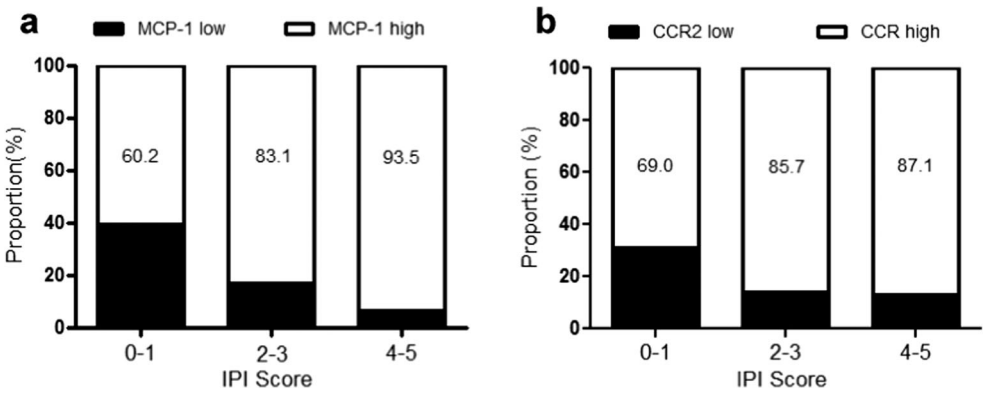


Fig. 2 Frequencies of MCP-1 expression level in IPI score = 0–1, 2–3, and 4–5 (**a**), CCR2 expression level in IPI score = 0–1, 2–3, and 4–5 (**b**)

Table 2 Univariate and multivariate Cox regression analyses of potential prognostic factors for overall survival

Variables	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P*	HR (95%CI)	*P*
Age				
< 60 years/≥ 60 years	1.454(1.041–2.030)	0.028	0.956(0.657–1.392)	0.816
ECOG-PS				
≤ 1/> 1	2.126(1.519–2.976)	< 0.001	1.054(0.687–1.619)	0.808
Extranodal sites				
≤ 1/> 1	1.998(1.365–2.924)	< 0.001	0.839(0.499–1.410)	0.508
Ann Arbor stage				
I or II/III or IV	2.177(1.557–3.046)	< 0.001	1.359(0.860–2.147)	0.189
LDH				
≤normal/>normal	3.560(2.483–5.105)	< 0.001	1.903(1.235–2.932)	0.004
IPI score				
0–1/3–5	3.194(2.255–4.523)	< 0.001	1.464(0.752–2.850)	0.262
AMC				
< 460/μl/≥ 460/μl	2.915(2.059–4.125)	< 0.001	1.544(1.060–2.248)	0.024
Treatment				
R-CHOP/CHOP	2.009(1.322–3.053)	0.001	2.283(1.430–3.646)	0.001
MCP-1				
Low/high	19.709(9.389–41.375)	< 0.001	10.983(4.936–24.436)	< 0.001
CCR2				
Low/high	6.796(3.680–12.550)	< 0.001	2.713(1.401–5.256)	0.003

MCP-1 expression ($n = 29$) had significantly poorer OS than those with low MCP-1 expression ($n = 2$) (OS: $P = 0.029$, Fig. 3d), in these group, MCP-1 expression had no association with PFS (PFS: $P = 0.067$, Supplementary Fig. 1d). The low-risk and intermediate-risk identified by the IPI were also subsequently risk stratified using the CCR2 expression (OS: $P < 0.001$ and $P < 0.001$, respectively; Fig. 4b, c; PFS: $P < 0.001$ and $P < 0.001$, respectively; Supplementary Fig. 2b, c). However, the CCR2 expression was not predictive in high-risk DLBCL in our study (OS: $P = 0.313$, Fig. 4d; PFS: $P = 0.142$, Supplementary Fig. 2d).

Table 3 Response rate according to MCP-1 and CCR2 expression

Characteristic	Response		Response rate	
	CR/PR	Progression	%	*P*
MCP-1				
Low	59	1	98	< 0.001
High	94	67	58	
CCR2				
Low	43	7	86	< 0.001
High	110	61	64	

In addition, received R-CHOP patients with high MCP-1 ($n = 34$) or high CCR2 ($n = 42$) expression had significantly poorer OS and PFS than those with low MCP-1 ($n = 25$) or low CCR2 ($n = 17$) expression, respectively (OS: $P < 0.001$ and $P = 0.001$, respectively; Fig. 5a, c; PFS: $P < 0.001$ and $P = 0.001$, respectively; Supplementary Fig. 3a, c). Similar results were obtained when intermediate-risk ($n = 19$) (IPI = 2–3) patients treated with R-CHOP were risk-stratified by the MCP-1 or CCR2 expression (OS: $P = 0.024$ and $P = 0.015$, respectively; Fig. 5b, d; PFS: $P = 0.014$ and $P = 0.010$, respectively; Supplementary Fig. 3b, d).

Extension of the IPI score prognostic model with MCP-1 and CCR2 intensity for DLBCL patients

On the basis of above-mentioned findings, we investigated whether incorporation of the MCP-1 and CCR2 expression into the IPI score would improve its predictive accuracy (Fig. 6). The combination of MCP-1 expression and IPI (AUC 0.853, $P < 0.001$) showed a better prognostic value than IPI score alone (AUC 0.717, $P < 0.001$) or MCP-1 expression alone (AUC 0.788, $P < 0.001$). The similar results found in the combination of CCR2 expression and IPI (AUC 0.811, $P < 0.001$).

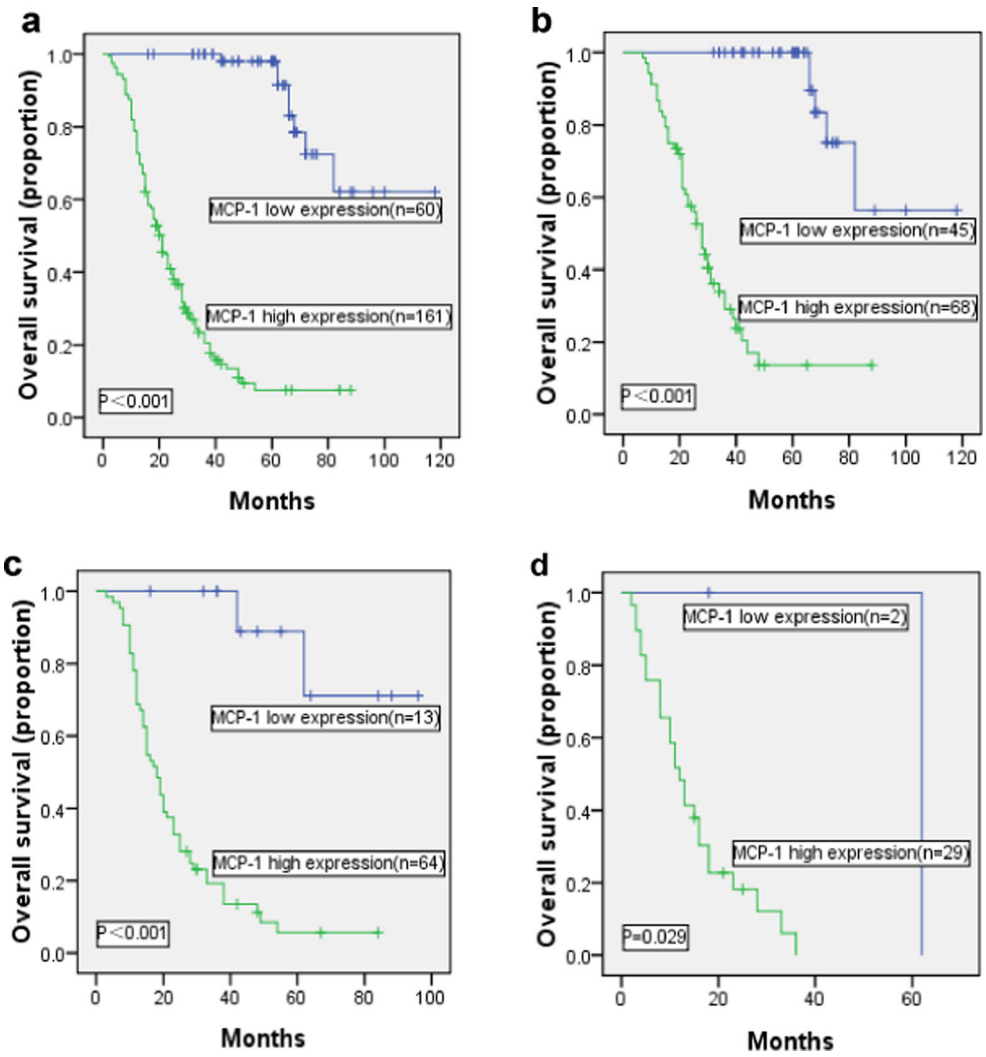


Fig. 3 Kaplan-Meier analysis of OS according to the expression of MCP-1 in patients with DLBCL. Kaplan-Meier analysis of OS of all patients (**a**), patients with IPI score = 0–1 DLBCL (**b**), patients with IPI score = 2–3 DLBCL (**c**), patients with IPI score = 4–5 DLBCL (**d**). *P* value was calculated by log-rank test

Discussion

In the present study, we found striking correlations between MCP-1 and CCR2 immunostaining in DLBCL and clinical pathological characteristics at initial diagnosis and subsequent overall response rate. We showed that MCP-1 or CCR2 expression is an independent prognostic factor for OS and PFS in patients with DLBCL, further identified high-risk patients otherwise classified as low (IPI = 0–1) or intermediate (IPI = 2–3) risk, and provided additional prognostic information when superimposed on the IPI, even in the rituximab era. Furthermore, incorporation of MCP-1 or CCR2 expression into the IPI score could improve prognostic value for OS, and these data suggested that the MCP-1 or CCR2 expression might increase prognostic information for patients with DLBCL and lead to a more accurate classification under the IPI score.

MCP-1, also known as CC ligand 2 (CCL2), is the most representative member of the CC chemokine subfamily. In addition to its well-characterized role as a chemoattractant for monocytes in the immune response [30], there is evidence suggesting that an autocrine MCP-1-dependent signaling pathway has been suggested to promote the survival and mobility of tumor cells [14–16], while tumor cell-derived MCP-1 has been shown to stimulate metastasis [18–22] and angiogenesis [13, 17]. Some studies reflected the unfavorable prognostic influence of MCP-1 on certain types of cancer. Koide et al. observed that MCP-1 expression in esophageal carcinoma cells was correlated with venous invasion [31]. However, other studies demonstrated that MCP-1 was a favorable prognostic marker. Zhang et al. observed that MCP-1 is overexpressed in non-small cell lung cancer (NSCLC) cells, and its expression in cancer cells is associated with better survival in NSCLC patients [9]. In our study, MCP-1 immunostaining was exclusively observed in cytoplasm of DLBCL cancer cells. MCP-1 expression was significantly associated with clinicopathological characteristics and overall response rate, high MCP-1 expression is an adverse prognostic factor for OS

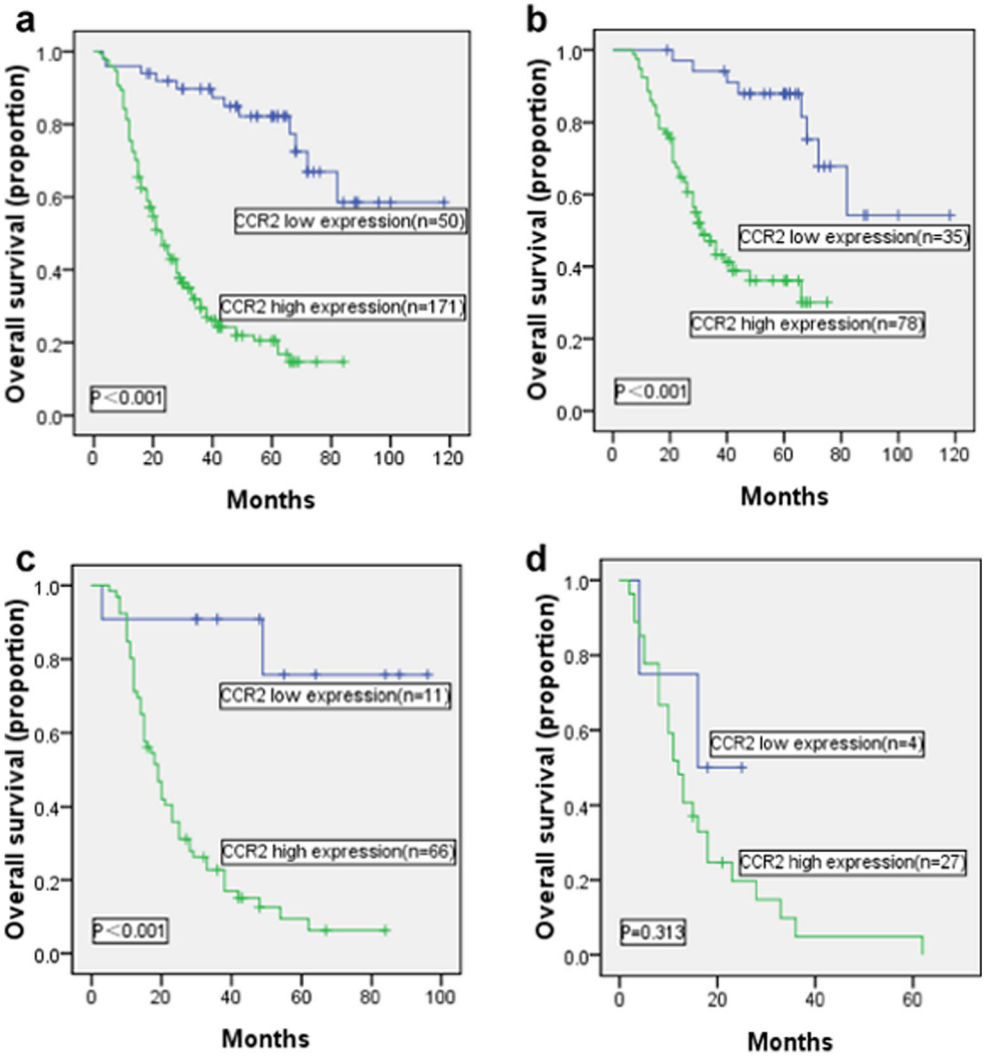


Fig. 4 Kaplan-Meier analysis of OS according to the expression of CCR2 in patients with DLBCL. Kaplan-Meier analysis of OS of all patients (**a**), patients with IPI score = 0–1 DLBCL (**b**), patients with IPI score = 2–3 DLBCL (**c**), patients with IPI score = 4–5 DLBCL (**d**). *P* value was calculated by log-rank test

and PFS of DLBCL patients, and patients with high MCP-1 expression had significantly poorer OS and PFS than those with low MCP-1 expression, even in the rituximab era. To our knowledge, there has been no report involving the biologic and prognostic significance of MCP-1 for patients with DLBCL. Our data suggest that the expression level of MCP-1 has a crucial role in the development and carcinogenesis of DLBCL tumor cells, and it may be an important clinical marker of therapy for patients with DLBCL.

As a high affinity receptor of MCP-1, CCR2 expression was considered relatively restricted to certain types of cells. Several recent studies have detected CCR2 expression in cancer cells, including multiple myeloma [32], prostate cancer [33], lung cancer [9], and renal cancer cells [34]. Expression of CCR2 in multiple myeloma predicted improved survival [32], while positive CCR2 presence in prostate and renal cancer cells was associated with tumor progression [33, 34], and there was no significant relationship between CCR2 expression in lung cancer cells and clinical pathological characteristics [9]. In our study, however, not only clinicopathological characteristics but also prognosis was correlated with expression of CCR2 in DLBCL tumor cells.

As MCP-1 and CCR2 had such crucial roles in tumor growth, invasion, migration, and survival, optimal use of anti-MCP-1 antibody or CCR2 inhibition (CCR2i) might be a part of potential targeted therapy. A human anti-MCP-1 antibody, designated CNTO888, the first agent evaluated in the clinic to target MCP-1/CCR2 signaling for the treatment of solid tumors, was generated in partnership with Morphosys (Germany) using the Human Combinatorial Antibody Library (HuCAL GOLD) [35], and PF-04136309 (Pfizer) is a CCR2 kinase antagonist and the details have been published previously [36]. Previous study has demonstrated that anti-MCP-1 antibody may be useful for impeding metastatic seeding, reducing tumor burden, enhancing the cytotoxic effects of chemotherapy, and improved survival in tumor xenograft models [18, 20, 22, 37, 38]. Current results demonstrated that inhibiting CCR2 signaling (CCR2 inhibitor PF-04136309)

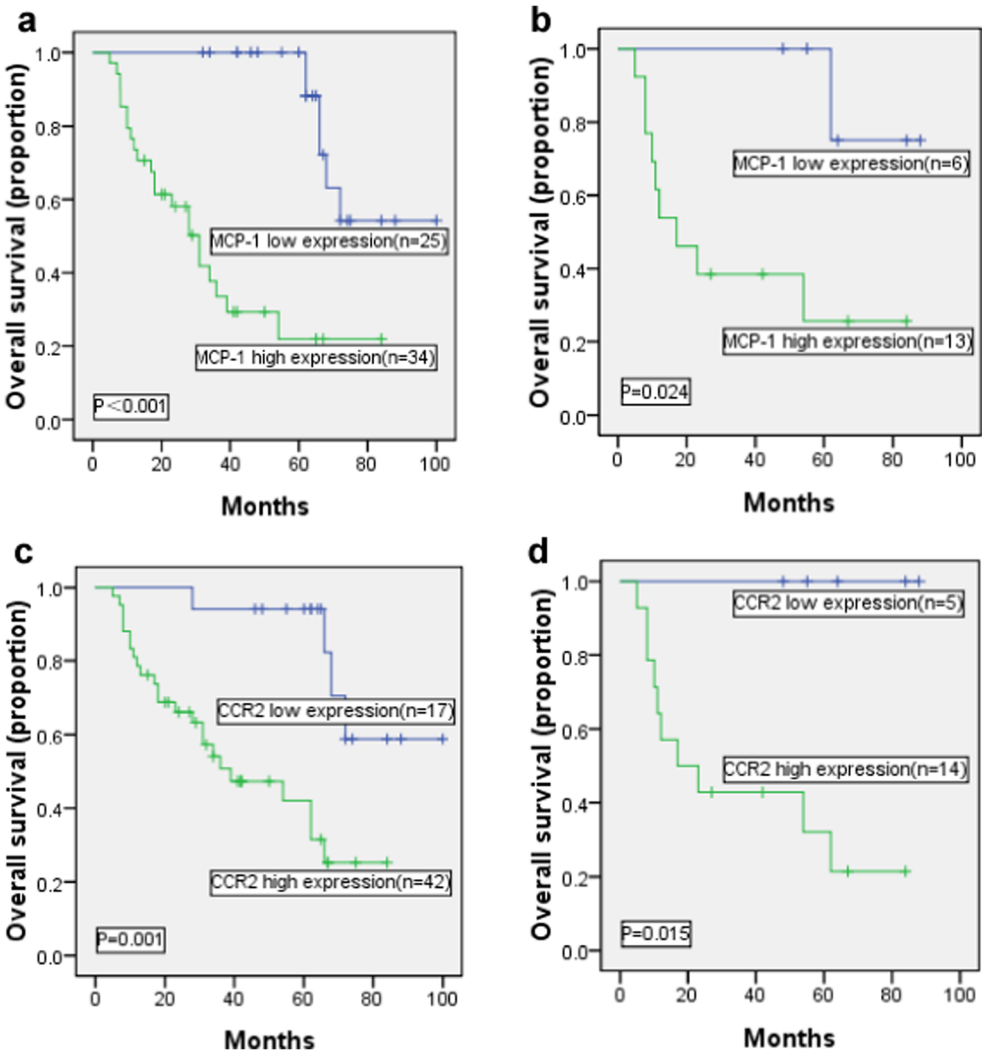
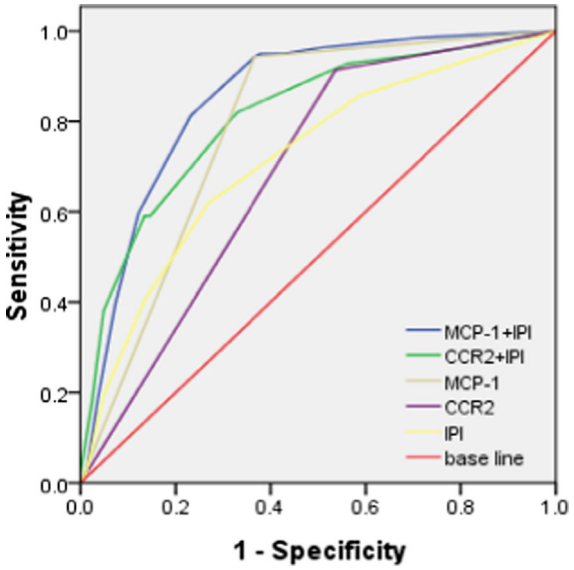


Fig. 5 Kaplan-Meier analysis of OS according to the expression of MCP-1 or CCR2 in DLBCL patients with R-CHOP therapy. Kaplan-Meier analysis of OS of all patients according to MCP-1 expression (**a**), patients with IPI score = 2–3 according to MCP-1 expression (**b**), all patients according to CCR2 expression (**c**), patients with IPI score = 2–3 according to CCR2 expression (**d**). *P* value was calculated by log-rank test

can reduce the viability of cells, block metastasis, increase chemotherapeutic efficacy, and overcome macrophage-induced suppression [39–41]. These data suggested that on the one hand, DLBCL tumor cell-derived MCP-1 might promote the survival and invasion through the combination of CCR2, on the other hand that targeting MCP-1/CCR2 signaling with MCP-1 or CCR2 targeting agents might be a novel and efficient strategy for the treatment of DLBCL. The profound molecular roles of MCP-1 and CCR2 and their antagonists in DLBCL remain far from being fully elucidated and need further investigation.

Lymphoma microenvironment is a complex system composed of stromal cells, blood vessels, immune cells as well as extracellular matrix, cytokines, exosomes, and chemokines [42]. Tumor-associated macrophages (TAMs), as intrinsic cellular components of the essential tumor microenvironment (TME), are macrophages (MPs) with defined specific M2 phenotypes now known to play central roles in the pathophysiology of a wide spectrum of malignant neoplasms such as lymphoma, but relatively, little is known about the increasingly important interactions between MPs and B lymphoid cells, particularly in the TME in patients with aggressive B-NHL such as DLBCL [42]. We have previously reported that peripheral blood AMC at the time of diagnosis predicts poor outcome for patients with DLBCL after standard first-line regimens [28]. In the present study, we also observed that MCP-1 expression was significantly associated with AMC at the time of diagnosis ($r = 0.305$, $P < 0.001$; Supplementary Table 3), and then, elevation in the CD14+/CCR2+ monocytes is an adverse prognostic factor in DLBCL (Supplementary Figs. 4 and 5). So, we deduced that MCP-1/CCR2 axis might mediate recruitment of CD14+/CCR2+ monocytes, aggregation of MPs, and polarization of an alternatively activated M2-phenotype (M2 TAMs) thereby leading to the progression of the disease. In other words, MCP-1/CCR2 axis may be involved in the interactions between MPs and neoplastic B cells in the TME in patients with DLBCL and promote the development of the disease.



	AUC	95%CI	P
MCP-1+IPI	0.853	0.797-0.909	<0.001
CCR2+IPI	0.811	0.753-0.870	<0.001
MCP-1	0.788	0.720-0.857	<0.001
CCR2	0.689	0.612-0.765	<0.001
IPI	0.717	0.647-0.786	<0.001

Fig. 6 Receiver operating characteristic (ROC) analysis for the prediction of OS in patients with DLBCL. *P* values show statistical significance of the AUC of the MCP-1 expression and IPI combined model, the CCR2 expression and IPI combined model, the MCP-1 expression model, the CCR2 expression model, and the IPI model

In conclusion, our study clearly demonstrated that the high expression of MCP-1 or CCR2 can serve as an independent prognostic factor for predicting worse outcome after first-line treatment and may have an important role in the metastasis, cell proliferation, angiogenesis, and invasiveness of DLBCL. MCP-1 and CCR2 can further stratify low IPI score DLBCL patients with significantly different OS and PFS, even in the rituximab era. The inhibition of MCP-1 or CCR2 may, therefore, be a potential target for anticancer therapy in DLBCL.

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Compliance with ethical standards

This study was approved by the hospital's ethics committee and informed consent was obtained from each patient. This study was performed in accordance with the principles expressed in the Declaration of Helsinki.

Conflicts of interest The authors declare that they have no conflict of interest.

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