CSF-1R Expression in Tumor-Associated Macrophages Is Associated With Worse Prognosis in Classical Hodgkin Lymphoma

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ABSTRACT

Objectives: *The aim of this study was to determine the prognostic relevance of colony-stimulating 1 receptor (CSF-1R) expression in both Hodgkin/Reed-Sternberg (HRS) cells and the surrounding cells (non-HRS cells) in patients with classical Hodgkin lymphoma (CHL) .*

Methods: *Diagnostic tissues from 112 patients with CHL treated with doxorubicin, bleomycin, vinblastine, and dacarbazine were evaluated retrospectively by immunohistochemical analysis for CSF-1R and CD68 and CD163 for tissue-associated macrophages.*

Results: *High numbers (≥30%) of non-HRS cells expressing CSF-1R conferred inferior event-free survival and overall survival in univariate and multivariate analysis. High numbers of non-HRS cells expressing CSF-1R were significantly associated with a high number of tumor-associated macrophages as detected by CD163 expression (*$P < .001$*). In particular, coexpression of CSF-1R and CD163 was associated with a worse survival outcome than either CSF-1R or CD163 expression alone or no expression.*

Conclusions: *Our data demonstrate that a high number of non-HRS cells expressing CSF-1R are correlated with an increased tumor macrophage content and worse survival.*

Although classical Hodgkin lymphoma (CHL) can be considered a successful paradigm of modern treatment strategies, 5% to 10% of patients are resistant to initial therapy, and 10% to 30% will relapse after initial remission.[1] To assist treatment decisions, clinicians commonly use in clinical practice the distinction between CHL and nodular lymphocyte-predominant Hodgkin lymphoma and the separation into limited- and advanced-stage disease. The International Prognostic Score (IPS) is the standard stratification system for survival in patients with CHL. However, it is less suitable for patients with limited-stage disease.[2] If new biomarkers that improve prediction of the primary treatment outcome across all clinical stages can be identified, a decrease in mortality and treatment-related late sequelae, including second solid tumors and end-organ dysfunction, may be achievable among patients with CHL.[3]

The histologic hallmark of CHL is the presence of the malignant Hodgkin/Reed-Sternberg (HRS) cells in the inflammatory background. These malignant cells are greatly outnumbered by a population of reactive cells composed of T and B lymphocytes and other cell types in the tumor microenvironment. The tumor microenvironment has a pivotal role in the progression of malignant tumors, including CHL. Steidl et al[4] recently reported a differentially expressed gene signature of tumor-associated macrophages (TAMs) and monocytes in patients with CHL that correlated with outcome and chemoresistant disease. They were able to validate this correlation in an independent patient cohort by immunohistochemical analysis of the macrophage/monocyte markers CD68 and CD163.[4-6] Furthermore, the peripheral blood lymphocyte/monocyte ratio at diagnosis in CHL is a prognostic indicator of clinical outcomes.[7-9]

Colony-stimulating factor 1 receptor (CSF-1R), which is encoded by the *C-FMS* proto-oncogene, is a transmembrane receptor tyrosine kinase and is the receptor for colony-stimulating factor 1 (CSF-1, also known as macrophage–colony-stimulating factor).[10] The CSF-1/CSF-1R pathway has essential physiologic functions in the generation of osteoclasts and macrophages.[11] In pathologic conditions, macrophages can be recruited by activation initiated by the binding of CSF-1 to CSF-1R. Macrophages secrete growth factors that are important for the formation of a premetastatic niche and helpful for tumor growth or metastasis, resulting in a higher rate of disease recurrence.[12-14] Expression of CSF-1R was identified in primitive multipotent hematopoietic cells[15] and mononuclear phagocytic lineage cells.[16] Expression of CSF-1R and/or CSF-1 has also been documented in HRS cells.[17] CSF-1R has prognostic significance in leiomyosarcoma[18] and hepatocellular,[19] breast,[20] and prostate[21] cancer.

A recent study found that CSF-1R expression, assessed by messenger RNA (mRNA) in situ hybridization, was significantly associated with progression-free and overall survival (OS) in patients with CHL.[22] CSF-1R antagonist BAY 43-9006 induces apoptosis in various CHL cell lines,[23] and PLX3397, an inhibitor of CSF-1R, has shown limited activity in a heavily pretreated patient cohort with CHL in a phase 2 clinical trial.[24] Although a previous study evaluated CSF-1R expression in HRS cells, CSF-1R expression was also observed in macrophages in CHL tissue. Furthermore, CSF-1R expression in the peritumoral area is associated with a poor prognosis in leiomyosarcoma and prostate cancer.[18,21] However, the possible prognostic impact of CSF-1R expression in non-HRS cells has not been investigated. In the present study, we examined the prognostic significance of CSF-1R expression in HRS and non-HRS cells, as well as its correlation with TAMs, in a retrospective analysis of 112 patients with CHL.

Materials and Methods

Patients

This retrospective study reviewed histologic and immunohistochemical data from 112 consecutive patients diagnosed with CHL at the Asan Medical Center, Seoul, South Korea, between 1990 and 2012. All patients had pathologically confirmed CHL, were 15 years or older at diagnosis, had received no previous treatment, had no history of malignancy, and had been treated with a doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) therapy regimen, with or without radiation. Paraffin-embedded tumor tissue and follow-up data were available for all patients.

The median follow-up time was 6.3 years (range, 0.2-17.3 years). Response criteria were based on standard guidelines. Routine follow-up imaging analyses were performed every 3 months for the first 2 years, every 6 months for the next 3 years, and then annually (or whenever clinically indicated) thereafter. This study was approved by the Asan Medical Center Institutional Review Board.

Histopathologic Analysis and Immunohistochemistry

All histologic and immunophenotypic data from the 112 patients with CHL were reviewed by two pathologists (J.H. and Y.W.K.). The CHL cases were subtyped according to the World Health Organization criteria as follows: nodular sclerosing, lymphocyte rich, mixed cellularity, lymphocyte depleted, or not otherwise specified (not classifiable). Tissue microarrays (TMAs) were constructed with three tumor cores 1 mm in diameter from selected areas of formalin-fixed, paraffin-embedded tumor samples. The TMA sections were stained using an automatic immunohistochemistry staining device (Benchmark XT, Ventana Medical Systems, Tucson, AZ). Briefly, 5-µm-thick sections were transferred onto poly-L-lysine–coated adhesive slides and dried at 62°C for 30 minutes. After standard heat-induced epitope retrieval for 30 minutes in EDTA (pH 8.0), the samples were incubated with antibodies against cleaved CD68 (dilution 1:2,000; DAKO, Glostrup, Denmark), CD163 (dilution 1:400; Novocastra, Newcastle, England), and CSF-1R (dilution 1:50; Santa Cruz Biotechnology, Santa Cruz, CA). The sections were then incubated with biotinylated anti–mouse immunoglobulins, peroxidase-conjugated streptavidin (LSAB kit, DAKO), and 3,3′-diaminobenzidine. Slides were counterstained with Harris hematoxylin.

Each case was represented on the TMA by three tissue cores, and at least 10 HRS cells in at least one of the three cores from each patient were analyzed. The expression of CD68 and CD163 was evaluated using the criteria described previously.[5,8] Samples were assigned to the high-CD68 or high-CD163 groups when 20% or more of the overall cells were positive **Image 1A** and **Image 1B**. The percentage of HRS and non-HRS cells expressing CSF-1R that showed the most significant difference with respect to OS was selected as the boundary value for defining high– and low–CSF-1R groups (≥10% HRS cells and ≥30% non-HRS cells **Table 1**, **Image 1C**, and **Image 1D**.

In situ hybridization analysis for Epstein-Barr virus–encoded RNA-1 and RNA-2 (EBER) was performed and scored as previously described.[25]

Statistical Analysis

OS was defined as the interval between the date of diagnosis and death from any cause. The follow-up of living patients (with or without events) was censored at their last

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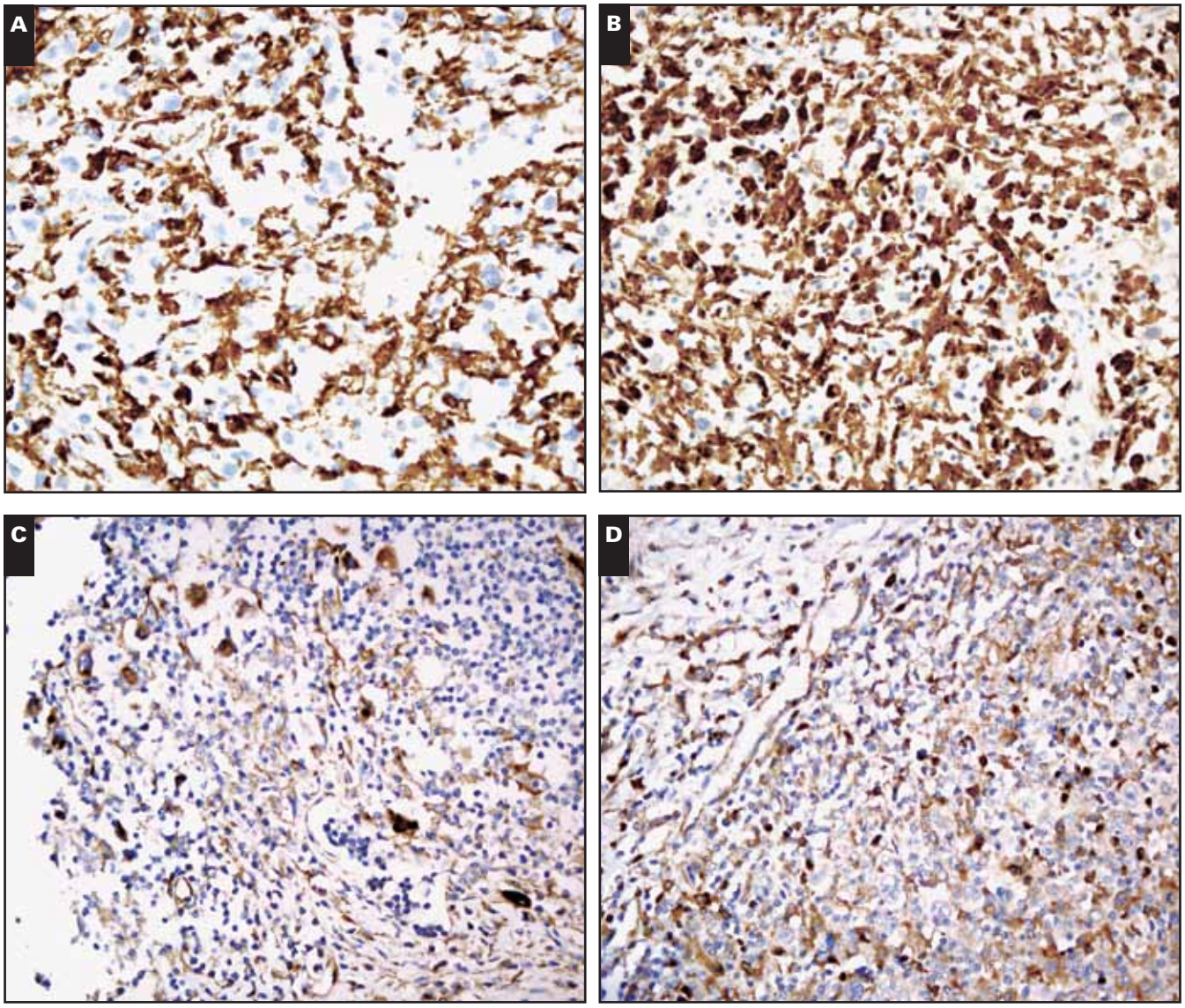

Image 1 CD68, CD163, and colony-stimulating factor 1 receptor (CSF-1R) expression in classic Hodgkin lymphoma (CHL) samples. **A**, High CD68 expression (×400). **B**, High CD163 expression (×400). **C**, CSF-1R expression in Hodgkin/Reed-Sternberg (HRS) cells (×400). **D**, CSF-1R expression in non-HRS cells (×400).

follow-up date. Event-free survival (EFS) was defined as the interval between the date of diagnosis and the date of disease progression, relapse, or death from any cause. Cumulative OS and EFS were analyzed by the Kaplan-Meier method, and comparisons were made by the log-rank test.

Multivariate prognostic analyses were performed on OS and EFS using the Cox proportional hazards regression model using the Enter method. Categorical variables were compared using the χ^2 test. All statistical analyses were performed with SPSS (version 18.0; SPSS, Chicago, IL) or R 2.15.2 (R Foundation for Statistical Computing, Vienna, Austria) statistical software programs. All *P* values are two-sided, with *P* less than .05 considered statistically significant.

Results

Patient Characteristics

The clinical characteristics of the 112 patients included in the study are summarized in **Table 2**. Patient age ranged from 15 to 77 years (median, 35.5 years). Forty-two patients experienced relapse, disease progression, or death; 20 patients died. Median OS and EFS were not reached. The estimated 5-year OS and EFS were 83.1% and 59.2%, respectively.

CD68, CD163, and CSF-1R Expression in CHL Tissue

Correlations between CD68, CD163, and CSF-1R expression and clinical variables are summarized in **Table 3**.

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Table 1
CSF-1R vs Overall Survival

	HRS Cells				
	Cutoff (%)	**Below Cutoff (n)**	**Above Cutoff (n)**	***P* Value for OS**	**χ^2-Log Rank for OS**
CSF-1R	10	14	98	.137	2.214
	20	32	82	.154	2.030
	30	35	77	.236	1.403
	40	38	74	.166	1.922
	Non-HRS Cells				
	Cutoff (%)	**Below Cutoff (n)**	**Above Cutoff (n)**	***P* Value for OS**	**χ^2-Log Rank for OS**
CSF-1R	10	36	76	.094	2.801
	20	46	66	.009	6.886
	30	62	50	<.001	12.090
	40	68	44	.003	8.653

CSF-1R, colony-stimulating factor 1 receptor; HRS, Hodgkin/Reed-Sternberg; OS, overall survival.

Table 2
Demographics and Clinical Characteristics of Patients[a]

Characteristic at Diagnosis	**Value**
Age, median (range), y	35.5 (15-77)
Male sex	66 (58.9)
Histologic subtype	
Nodular sclerosing	75 (67.0)
Mixed cellularity	22 (19.6)
Lymphocyte rich	5 (4.5)
Lymphocyte depleted	3 (2.7)
Not classifiable	7 (6.3)
Ann Arbor stage	
I	21 (18.8)
II	38 (33.9)
III	26 (23.2)
IV	27 (24.1)
Stage	
Limited	42 (37.5)
Advanced	70 (62.5)
B symptoms present	36 (32.1)
International Prognostic Score ≥4 (high risk)	21 (18.8)
EBER positivity	42 (37.5)
Primary treatment	
Chemotherapy	82 (73.2)
Chemoradiotherapy	30 (26.8)

EBER, Epstein-Barr virus–encoded RNA-1 and RNA-2 assessed by in situ hybridization.
[a] Values are presented as number (%) unless otherwise indicated.

Patients were divided into those with tumor samples expressing low numbers (<20%) of CD68-positive cells (low-CD68 group) and those with 20% or more cells expressing CD68 (high-CD68 group). Compared with the low-CD68 group (n = 50), the high-CD68 group (n = 62) included more patients with a lower level of lactate dehydrogenase (LDH) (53.2% vs 28%, *P* = .012) and EBER positivity (46.8% vs 26%, *P* = .031).

Patients were similarly divided according to CD163 expression. The high-CD163 group (n = 62) included more patients who were older (51.6% vs 28%, *P* = .013), were male (69.4% vs 46.0%, *P* = .02), had a higher IPS (25.8% vs 10.0%, *P* = .049), and had a lower level of LDH (53.2% vs 28.0%, *P* = .012) and EBER positivity (46.8% vs 26.0%, *P* = .031) than the low-CD163 group (n = 50).

The number of HRS cells expressing CSF-1R was evaluated in the patient tumor samples. Patients with samples with 10% or more CSF-1R–positive HRS cells (high–CSF-1R in HRS cells group) (n = 98) were older (≥45 years) (44.9% vs 14.3%, *P* = .041) than those in the low–CSF-1R in HRS cells group (n = 14). CSF-1R expression in non-HRS cells was not associated with any of the clinical variables tested.

A statistically significant correlation was observed between a high incidence of CSF-1R expression in non-HRS cells and CD163 expression (*P* < .001) **Table 4**. There was no correlation between CSF-1R expression in non-HRS cells and CD68 expression (*P* = .056). There was no correlation between CSF-1R expression in HRS cells and CD68 expression (*P* = .572) or between CSF-1R expression in HRS cells and CD163 expression (*P* = .153).

Prognostic Significance of CD68, CD163, and CSF-1R Expression

Patients in the high-CD68 group had lower 5-year EFS rates (43.5% vs 76.5%, *P* = .003) **Figure 1A** and lower but not significantly different 5-year OS rates (75.3% vs 91.1%, *P* = .081) **Figure 1B** than those in the low-CD68 group. Patients in the high-CD163 group had lower 5-year EFS rates (47.3% vs 72.2%, *P* = .015) **Figure 1C** and lower but not significantly different 5-year OS rates (78.1% vs 88.5%, *P* = .117) **Figure 1D** than those in the low-CD163 group. High CSF-1R in HRS cells was not significantly associated with either EFS or OS (*P* = .917 and *P* = .137, respectively) **Figure 1E** and **Figure 1F**. Patients in the high–CSF-1R in non-HRS cells group had lower 5-year EFS rates (47.5% vs 69.6%, *P* = .028) **Figure 1G** and lower 5-year OS rates (68.3% vs 95.7%, *P* < .001) **Figure 1H** than those in the low–CSF-1R in non-HRS cells group.

To further examine the prognostic significance of CSF-1R expression in non-HRS cells, we performed subgroup analyses according to Ann Arbor stage. In advanced-stage disease, the high–CSF-1R in non-HRS cells group showed inferior EFS or OS rates compared with the low–CSF-1R in non-HRS cells group (*P* = .037 and *P* = .009, respectively) **Figure 2A** and **Figure 2B**. Among patients with limited-stage disease, those in the high–CSF-1R in non-HRS cells group had inferior OS rates (*P* = .021) **Figure 2D** and inferior but not significantly different EFS rates (*P* = .380) **Figure 2C** compared with those in the low–CSF-1R in non-HRS cells group.

Since a correlation had been found between CD163 and CSF-1R expression in non-HRS cells (Table 4), we next

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Table 3
Correlation Between CD68, CD163, and CSF-1R and Clinical Variables[a]

Characteristic	CD68 Expression, No. (%)			CD163 Expression, No. (%)			CSF-1R (HRS Cells), No. (%)			CSF-1R (Non-HRS Cells), No. (%)		
	Low (<20%) (n = 50)	High (≥20%) (n = 62)	*P* Value	Low (<20%) (n = 50)	High (≥20%) (n = 62)	*P* Value	Low (<10%) (n = 14)	High (≥10%) (n = 98)	*P* Value	Low (<30%) (n = 62)	High (≥30%) (n = 50)	*P* Value
Age, y			.342[b]			.013[b]			.041[b]			.122[b]
<45	32 (64.0)	34 (54.8)		36 (72.0)	30 (48.4)		12 (85.7)	54 (55.1)		41 (66.1)	25 (50.0)	
≥45	18 (36.0)	28 (45.2)		14 (28.0)	32 (51.6)		2 (14.3)	44 (44.9)		21 (33.9)	25 (50.0)	
Sex			.699[b]			.02[b]			.776[b]			>.999[b]
Male	28 (56.0)	38 (61.3)		23 (46.0)	43 (69.4)		5 (35.7)	41 (41.8)		37 (59.7)	29 (58.0)	
Female	22 (44.0)	24 (38.7)		27 (54.0)	19 (30.6)		9 (64.3)	57 (58.2)		25 (40.3)	21 (42.0)	
Disease subtype			. 507[c]			.227[c]			.437[c]			.289[c]
Nodular sclerosing	34 (68.0)	41 (66.1)		34 (68.0)	41 (66.1)		8 (57.1)	67 (68.4)		38 (61.3)	37 (74.0)	
Mixed cellularity	11 (22.0)	11 (17.7)		8 (16.0)	14 (22.6)		3 (21.4)	19 (19.4)		13 (21.0)	9 (18.0)	
Lymphocyte rich	3 (6.0)	2 (3.2)		4 (8.0)	1 (1.6)		1 (7.1)	4 (4.1)		4 (6.5)	1 (2.0)	
Lymphocyte depleted	0	3 (4.8)		0	3 (4.8)		0	3 (3.1)		1 (1.6)	2 (4.0)	
Not classifiable	2 (4.0)	5 (8.1)		4 (8.0)	3 (4.8)		2 (14.3)	5 (5.1)		6 (9.7)	1 (2.0)	
B symptoms			>.999[b]			.423[b]			.542[c]			.309[b]
Absent	34 (68.0)	42 (67.7)		36 (72.0)	40 (64.5)		11 (78.6)	65 (66.3)		45 (72.6)	31 (62.0)	
Present	16 (32.0)	20 (32.3)		14 (28.0)	22 (35.5)		3 (21.4)	33 (33.7)		17 (27.4)	19 (38.0)	
Ann Arbor stage			>.999[b]			>.999[b]			.379[b]			>.999[b]
Limited	19 (38.0)	23 (37.1)		19 (38.0)	23 (37.1)		7 (50.0)	35 (35.7)		23 (37.1)	19 (38.0)	
Advanced	31 (62.0)	39 (62.9)		31 (62.0)	39 (62.9)		7 (50.0)	63 (64.3)		39 (62.9)	31 (62.0)	
IPS			.628[b]			.049[b]			.462[c]			.811[b]
<4	42 (84.0)	49 (79.0)		45 (90.0)	46 (74.2)		13 (92.9)	78 (79.6)		51 (82.3)	40 (80.0)	
≥4	8 (16.0)	13 (21.0)		5 (10.0)	16 (25.8)		1 (7.1)	20 (20.4)		11 (17.7)	10 (20.0)	
LDH, U/L			.012[b]			.012[b]			>.999[b]			.847[b]
<250	14 (28.0)	33 (53.2)		14 (28.0)	33 (53.2)		6 (42.9)	41 (41.8)		27 (43.5)	20 (40.0)	
≥250	36 (72.0)	29 (46.8)		36 (72.0)	29 (46.8)		8 (57.1)	57 (58.2)		35 (56.5)	30 (60.0)	
EBER			.031[b]			.031[b]			.244[b]			.241[b]
Negative	37 (74.0)	33 (53.2)		37 (74.0)	33 (53.2)		11 (78.6)	59 (60.2)		42 (67.7)	28 (56.0)	
Positive	13 (26.0)	29 (46.8)		13 (26.0)	29 (46.8)		3 (21.4)	39 (39.8)		20 (32.3)	22 (44.0)	
Primary treatment			.095 [b]			.056[b]			.346[c]			.525[b]
Chemotherapy	33 (66.0)	49 (79.0)		32 (64.0)	50 (80.6)		12 (85.7)	70 (71.4)		47 (75.8)	35 (70.0)	
Chemoradiotherapy	17 (34.0)	13 (21.0)		18 (36.0)	12 (19.4)		2 (14.3)	28 (28.6)		15 (24.2)	15 (30.0)	

CSF-1R, colony-stimulating factor 1 receptor; EBER, Epstein-Barr virus–encoded RNA-1 and RNA-2 assessed by in situ hybridization; HRS, Hodgkin/Reed-Sternberg; IPS, International Prognostic Score; LDH, lactate dehydrogenase.
[a] Laboratory values are given in conventional units; conversions to Système International units are as follows: LDH (μkat/L), multiply by 0.0167.
[b] χ^2 test by two-sided Pearson test.
[c] χ^2 test by two-sided Fisher test.

Table 4
Correlation Between CD68, CD163, and CSF-1R Expression

Characteristic	CSF-1R Expression (HRS Cells), No. (%)			CSF-1R Expression (Non-HRS Cells), No. (%)		
	Low (n = 14)	High (n = 98)	*P* Value	Low (n = 62)	High (n = 50)	*P* Value
CD68 expression			.572[a]			.056[a]
Low (n = 50)	5 (35.7)	45 (45.9)		33 (53.2)	17 (34.0)	
High (n = 62)	9 (64.3)	53 (54.1)		29 (46.8)	33 (66.0)	
CD163 expression			.153[a]			<.001[a]
Low (n = 50)	9 (64.3)	41 (41.8)		37 (59.7)	13 (26.0)	
High (n = 62)	5 (35.7)	57 (58.2)		25 (40.3)	37 (74.0)	

CSF-1R, colony-stimulating factor 1 receptor; HRS, Hodgkin/Reed-Sternberg.
[a] χ^2 test by two-sided Pearson test.

determined whether a combination of CD163 and CSF-1R scores provided additional prognostic information. Patients were stratified into three groups (CD163 <20% and CSF-1R in non-HRS cells <30%, CD163 ≥20% and CSF-1R in non-HRS cells ≥30%, and discordant cases). Patients with a low incidence of CD163-positive cells and of CSF-1R–expressing non-HRS cells (33%) had significantly better EFS rates (P = .019) **Figure 3A** than patients with the other expression patterns. Patients with a higher incidence of CD163 and CSF-1R–expressing non-HRS cells (33%) had significantly worse OS (P = .009) **Figure 3B** than patients with the other expression patterns.

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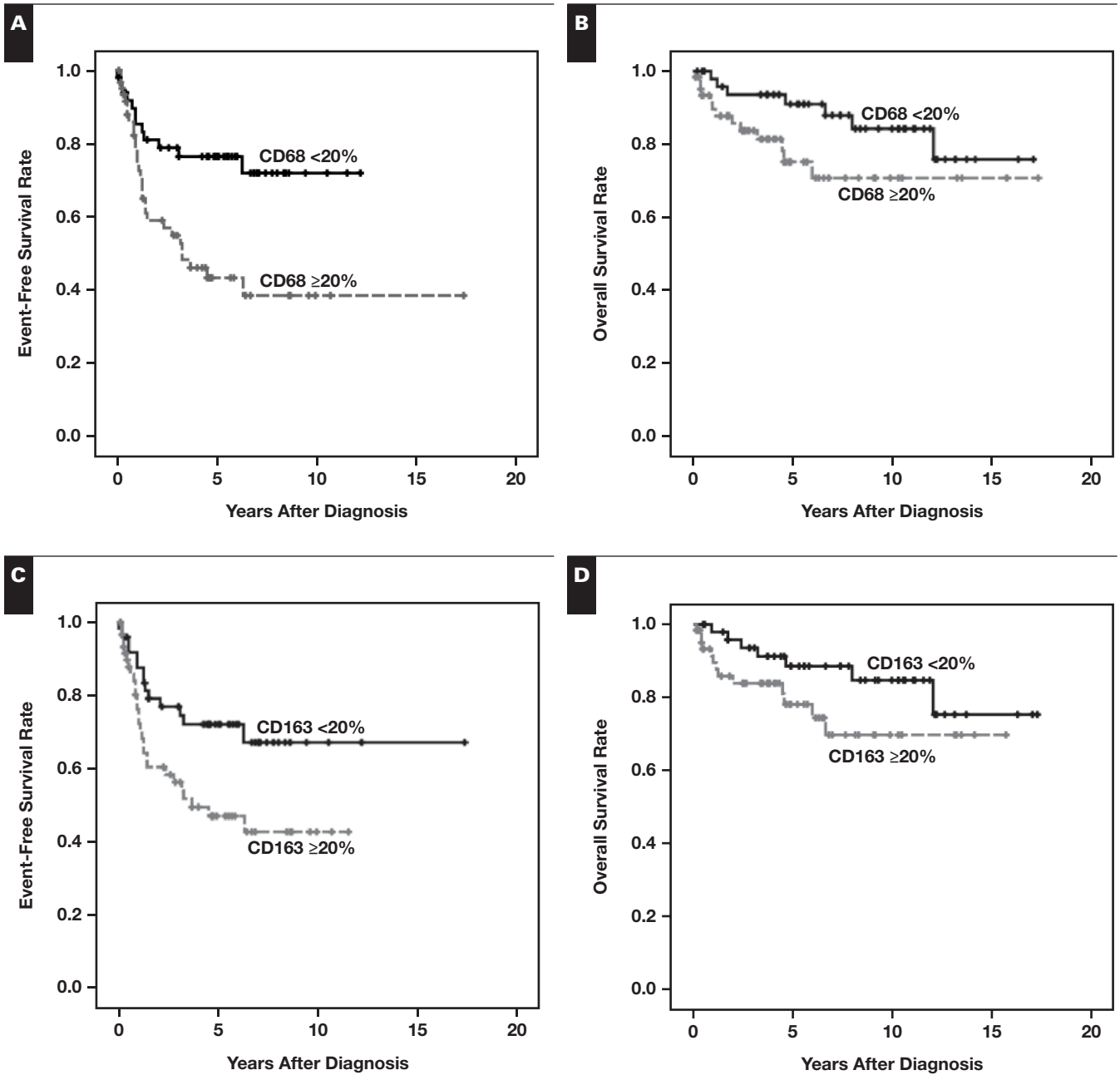

Figure 1 Comparison of survival rates according to CD68, CD163, and colony-stimulating factor 1 receptor (CSF-1R) expression. **A**, Event-free survival (EFS) was significantly worse in the high-CD68 group ($P = .003$). **B**, The difference in overall survival (OS) between high-CD68 vs low-CD68 groups was not statistically significant ($P = .081$). **C**, EFS was significantly worse in the high-CD163 group ($P = .015$). **D**, The difference in OS between high-CD163 vs low-CD163 groups was not statistically significant ($P = .117$).

By univariate analysis, both OS and EFS were associated with IPS (<4 vs ≥4) **Table 5**. By multivariate analysis, along with high-risk IPS (≥4), CSF-1R expression in non-HRS cells was also an independent prognostic marker for EFS and OS ($P < .001$ and $P = .025$, respectively; Table 5).

Discussion

To our knowledge, this study is the first to evaluate the prognostic significance of CSF-1R in HRS and non-HRS cells in patients with CHL receiving ABVD therapy. We found that CSF-1R expression in non-HRS cells was associated with clinical outcomes, while CSF-1R expression in HRS cells

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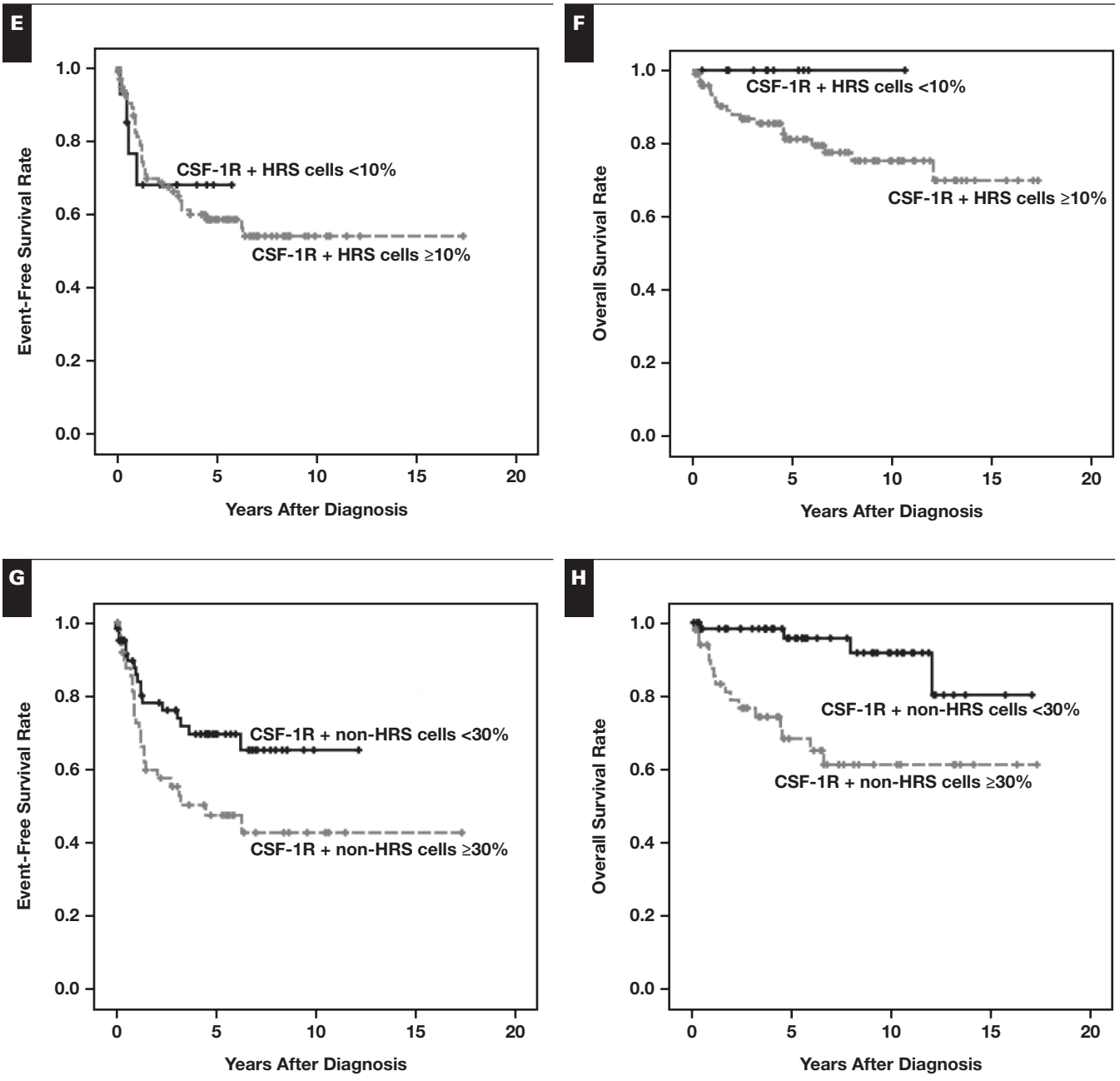

Figure 1 (cont) CSF-1R expression in Hodgkin/Reed-Sternberg (HRS) cells was not significantly associated with (**E**) EFS ($P = .917$) or (**F**) OS ($P = .137$). CSF-1R expression in non-HRS cells was significantly associated with worse (**G**) EFS ($P = .028$) and (**H**) OS ($P < .001$).

was not. A positive correlation was found between CSF-1R in non-HRS cells and CD163 expression, and furthermore, a combination of the CSF-1R and CD163 expression scores was predictive of survival. CSF-1R expression in non-HRS cells has prognostic value in CHL, particularly within limited and advanced Ann Arbor stage subgroups.

In the present study, CSF-1R expression in non-HRS cells was significantly associated with clinical outcomes; however, CSF-1R expression in HRS cells was not. By contrast, Steidl et al[22] found that elevated CSF-1R expression in HRS cells was associated with poor treatment outcome. The reason for this difference is unclear but could be related to differences in the patient demographics or in the study design. Steidl et al examined CSF-1R mRNA expression, whereas we used CSF-1R immunohistochemical staining. Protein levels are affected by various factors, including the expression level and stability of the mRNA; the translational activity, which may be regulated by exogenous and endogenous microRNAs;

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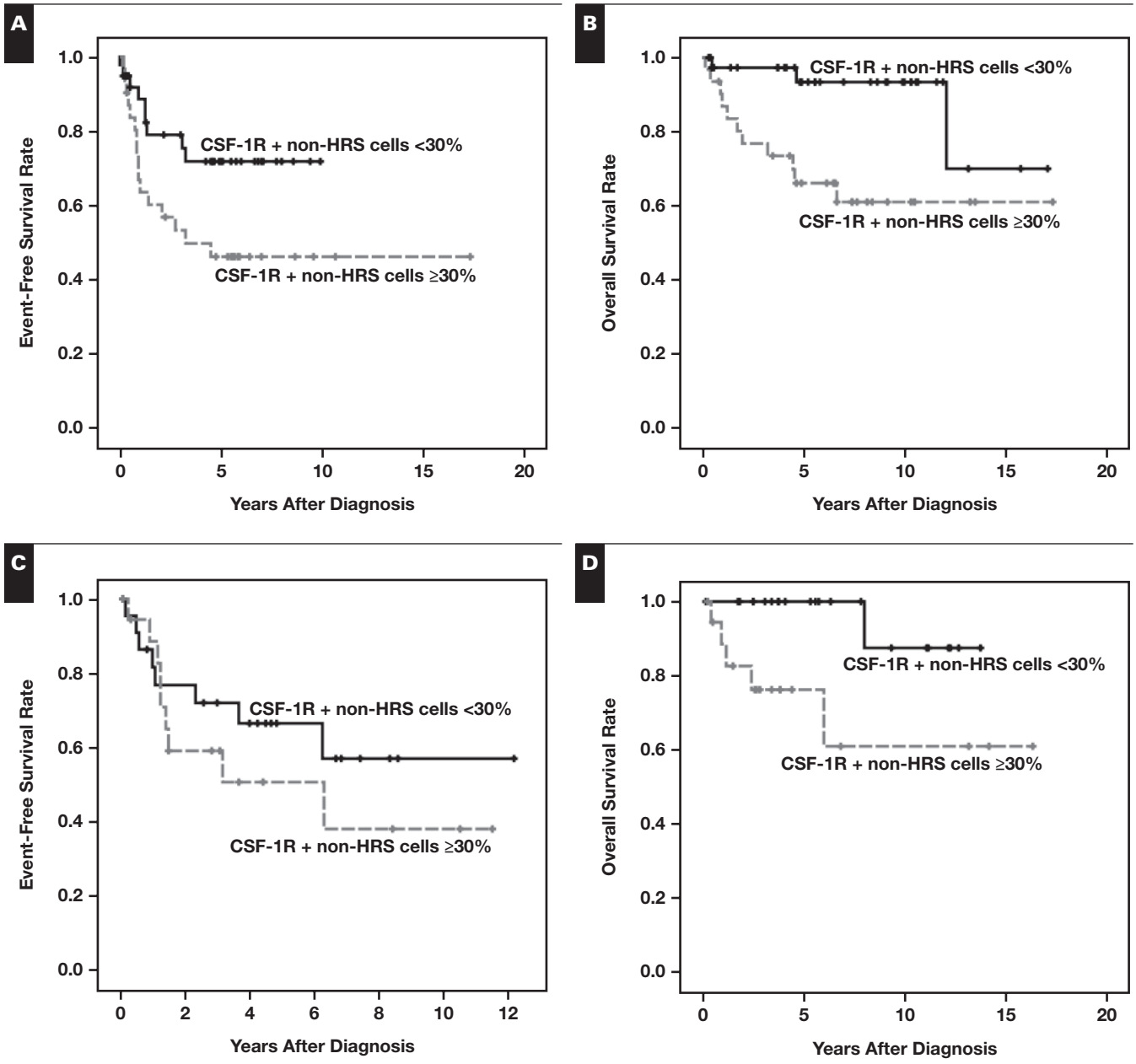

Figure 2 Comparison of survival rates according to Ann Arbor stage. Colony-stimulating factor 1 receptor (CSF-1R) expression in non–Hodgkin/Reed-Sternberg (HRS) cells was significantly associated with worse (**A**) event-free survival (EFS) ($P = .037$) and (**B**) overall survival (OS) ($P = .009$) in advanced-stage disease. In limited-stage disease, CSF-1R expression in non-HRS cells was significantly associated with worse (**D**) OS ($P = .021$) but not (**C**) EFS ($P = .380$).

and proteasomal degradation. Therefore, further external validation of our results is required.

CSF-1 is the most pleiotropic of the macrophage growth factors; it stimulates the survival, proliferation, and differentiation of mononuclear phagocytes and also promotes the spreading and motility of macrophages.[11] Binding of CSF-1 to the extracellular domain of CSF-1R leads to the activation of the receptor through trans-autophosphorylation; a series of membrane-proximal tyrosine phosphorylation cascades are initiated, leading to rapid stimulation of cytoskeletal remodeling, gene transcription, and protein translation.[11,26] Activation of CSF-1R rapidly induces morphologic changes in quiescent macrophages, stimulating lamellipodial protrusions and dorsal ruffling, followed by polarization, increased motility, and chemotaxis toward the source of CSF-1.[11] In our study, CSF-1R–positive non-HRS cells were directly correlated with the presence of CD163-positive macrophages. Therefore, most of the CSF-1R–positive non-HRS

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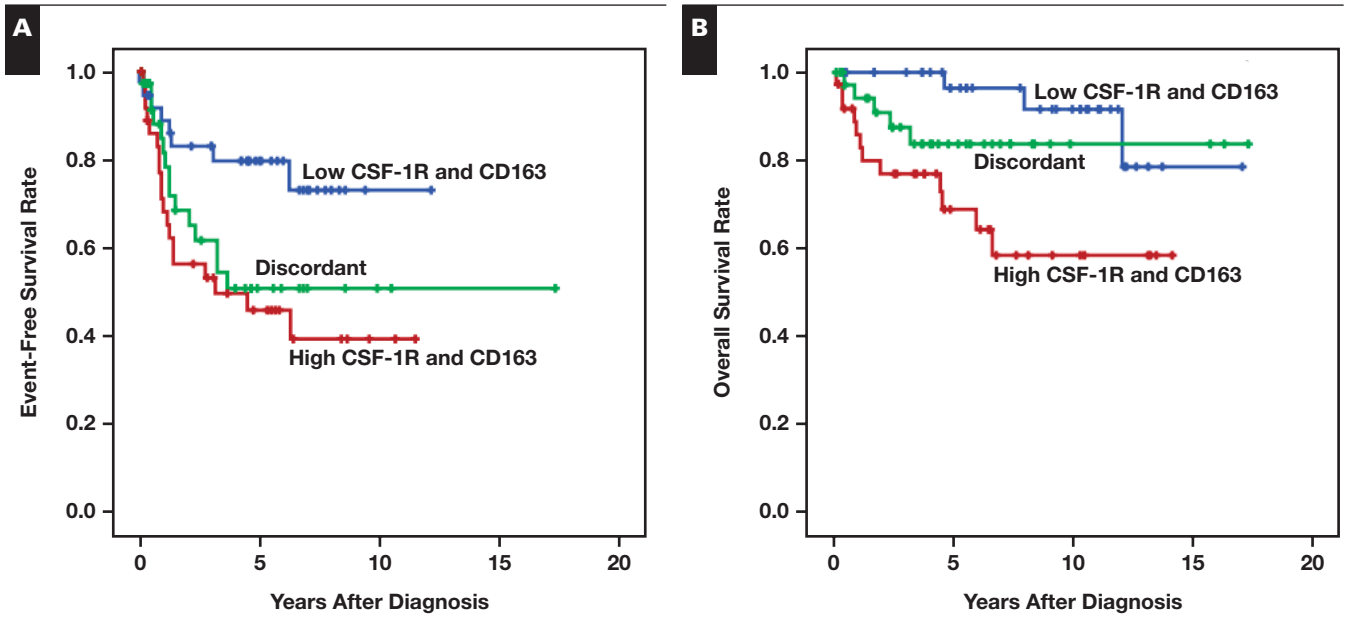

Figure 3 Comparison of survival rates according to colony-stimulating factor 1 receptor (CSF-1R)/CD163 protein expression pattern. Patients with a low incidence of both CD163-positive cells and CSF-1R–positive non–Hodgkin/Reed-Sternberg (HRS) cells had significantly better event-free survival (**A**) than patients with the other expression patterns ($P = .019$). Patients with a high incidence of both CD163-positive cells and CSF-1R–positive non-HRS cells had significantly worse overall survival (**B**) than patients with the other expression patterns ($P = .009$).

Table 5
Univariate and Multivariate Analysis for Overall Survival and Event-Free Survival

	OS		EFS	
Covariate	**HR (95% CI)**	***P* Value**	**HR (95% CI)**	***P* Value**
Univariate analysis				
B symptoms, negative vs positive	1.775 (0.73-4.30)	.204	1.239 (0.66-2.33)	.506
IPS, <4 vs ≥4	4.556 (1.84-11.20)	<.001	2.704 (1.37-5.31)	.004
LDH (U/L), normal vs abnormal	1.421 (0.50-4.01)	.506	1.082 (0.57-2.04)	.807
EBER, negative vs positive	1.601 (0.66-3.88)	.298	1.591 (0.86-2.92)	.135
Treatment, chemotherapy vs chemoradiotherapy	1.997 (0.66-5.99)	.218	1.619 (0.79-3.29)	.185
CD68 expression, low vs high	2.248 (0.88-5.71)	.089	2.660 (1.35-5.21)	.004
CD163 expression, low vs high	2.063 (0.81-5.20)	.125	2.160 (1.13-4.11)	.019
CSF-1R (HRS cells), low vs high	24.42 (0.03-170.00)	.339	1.056 (0.37-2.97)	.918
CSF-1R (non-HRS cells), low vs high	5.621 (1.87-16.80)	.002	1.960 (1.05-3.63)	.032
Multivariate analysis				
IPS, <4 vs ≥4	4.992 (1.99-12.40)	<.001	2.819 (1.42-5.56)	.003
CSF-1R (non-HRS cells), low vs high	6.042 (2.00-18.20)	<.001	2.034 (1.09-3.77)	.025

CI, confidence interval; CSF-1R, colony-stimulating factor 1 receptor; EBER: Epstein-Barr virus–encoded RNA-1 and RNA-2 assessed by in situ hybridization; EFS, event-free survival; HR, hazard ratio; HRS, Hodgkin/Reed-Sternberg; IPS, International Prognostic Score; LDH, lactate dehydrogenase; OS, overall survival.

cells could be activated TAMs, which are associated with poor clinical outcome.

The importance of CSF-1 in cancer was first reported in breast cancer; invading breast carcinoma cells express high levels of CSF-1, and the invaded regions are rich in TAMs.[27] A paracrine loop between CSF-1–secreting malignant cells and CSF-1R–positive TAMs underlies the promotion of tumor spread by CSF-1.[28,29] Other studies have also demonstrated CSF-1 expression in tumor cells and shown that CSF-1R expression is restricted to TAMs.[28,29] Therefore, a synergistic interaction between CSF-1–secreting tumor cells and CSF-1R–positive TAMs could induce tumor progression by promoting local invasion and distant metastasis. In our study, CSF-1R–positive non-HRS cells, but not HRS cells, were correlated with CD163-positive TAMs. Furthermore, cases with coexpression of CSF-1R and CD163 in the tumor microenvironment showed more aggressive clinical behavior than those with other expression patterns. Cases

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with coexpression of CSF-1R and CD163 may associate with TAMs showing high malignant potential. These results provide further evidence of the existence of a paracrine loop between malignant cells and CSF-1R–positive TAMs.

Our study found that CSF-1R–positive non-HRS cells more closely correlated with the incidence of cells expressing CD163 than those expressing CD68. CD163 is more specifically expressed by the monocyte/macrophage lineage than CD68[30] and may therefore be a superior marker of TAMs. M2 macrophages are involved in tumor angiogenesis and progression,[31] and CD163 is believed to be a better marker of M2 macrophages than CD68.[32] CSF-1 induces TAMs to polarize toward the M2 phenotype and promote tumor progression.[33]

In a phase 2 clinical trial, although CSF-1R inhibitor PLX3397 showed limited activity in a heavily pretreated CHL patient cohort, targeted inhibition of CSF-1R was clearly demonstrated.[24] Therefore, further prospective clinical trials are necessary to determine the effects of therapies targeting CSF-1R, particularly in patients with CHL who have CSF-1R positivity in non-HRS cells.

The limitations of the present study include its retrospective design, short follow-up period, relatively small sample size, and the use of TMAs, which, because of regional variation, may not reflect the true distribution of TAMs in the tissue.

In conclusion, our results show that CSF-1R expression in non-HRS cells is associated with a worse prognosis in uniformly treated patients with CHL. Coexpression of CSF-1R and CD163 can be used to identify a subgroup of patients with CHL at high risk of recurrence or progression who may benefit from aggressive chemotherapy. The ability to disrupt paracrine-based interaction between tumor cells and CSF-1R–positive TAMs raises the possibility for new therapeutic targets to specifically inhibit invasion and metastasis.

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