Peripheral blood lymphocyte/monocyte ratio at diagnosis and survival in classical Hodgkin's lymphoma

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ABSTRACT

Background
Lymphopenia and tumor-associated macrophages are negative prognostic factors for survival in classical Hodgkin's lymphoma. We, therefore, studied whether the peripheral blood absolute lymphocyte count/absolute monocyte count ratio at diagnosis affects survival in classical Hodgkin's lymphoma.

Design and Methods
We studied 476 consecutive patients with classical Hodgkin's lymphoma followed at the Mayo Clinic from 1974 to 2010. Receiver operating characteristic curves and area under the curve were used to determine cut-off values for the absolute lymphocyte count/absolute monocyte count ratio at diagnosis, while proportional hazards models were used to compare survival based on the absolute lymphocyte count/absolute monocyte count ratio at diagnosis.

Results
The median follow-up period was 5.6 years (range, 0.1-33.7 years). An absolute lymphocyte count/absolute monocyte count ratio at diagnosis of 1.1 or more was the best cut-off value for survival with an area under the curve of 0.91 (95% confidence interval, 0.86 to 0.96), a sensitivity of 90% (95% confidence interval, 85% to 96%) and specificity of 79% (95% confidence interval, 73% to 88%). Absolute lymphocyte count/absolute monocyte count ratio at diagnosis was an independent prognostic factor for overall survival (hazard ratio, 0.18; 95% confidence interval, 0.08 to 0.38, $P<0.0001$); lymphoma-specific survival (hazard ratio, 0.10; 95% confidence interval, 0.04 to 0.25, $P<0.0001$); progression-free survival (hazard ratio, 0.35; 95% confidence interval, 0.18 to 0.66, $P<0.002$) and time to progression (hazard ratio, 0.27; 95% confidence interval, 0.17 to 0.57, $P<0.0006$).

Conclusions
The ratio of absolute lymphocyte count/absolute monocyte count at diagnosis is an independent prognostic factor for survival and provides a single biomarker to predict clinical outcomes in patients with classical Hodgkin's lymphoma.

Key words: prognosis, classical Hodgkin's lymphoma, biomarker, absolute lymphocyte count, absolute monocyte count, ratio.

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Introduction

The International Prognostic Score (IPS) uses seven prognostic factors to predict clinical outcomes in patients with newly diagnosed classical Hodgkin's lymphoma.[1] However, the IPS only applies to patients with advanced-stage disease and it does not offer risk stratification for classical Hodgkin's lymphoma patients diagnosed with limited disease [i.e., stages I and IIA, without constitutional symptoms and no bulky disease (i.e. not ≥ 10 cm in diameter)].

The pathological features of classical Hodgkin's lymphoma include the malignant Reed-Sternberg cell surrounded by an inflammatory infiltrate consisting of lymphocytes, neutrophils, eosinophils, plasma cells, macrophages, fibroblasts and collagen fibers.[2] Two components of the inflammatory background are associated with survival in classical Hodgkin's lymphoma: tumor-infiltrating lymphocytes[3,4] and a low lymphocyte count, which is defined by the IPS as less than 600 cells/μL or less than 8% of the white blood cell count and is a negative prognostic factor for survival in classical Hodgkin's lymphoma.[1] Recent gene-expression profiling studies have demonstrated that tumor-infiltrating myeloid-derived cells also predict clinical outcomes in classical Hodgkin's lymphoma.[5] These cells provide trophic factors (tumor microenvironment) which directly promote malignant lymphocyte growth and survival.[6-9] Tumor-associated macrophages are derived from circulating monocytes and are recruited to the tumor site by soluble tumor-derived chemotactic factors.[10-13]

We, therefore, studied the role of the peripheral blood absolute lymphocyte count/absolute monocyte count ratio at diagnosis (ALC/AMC-DX), as a simple biomarker combining an estimate of host immune homeostasis [i.e., absolute lymphocyte count (ALC)/tumor-infiltrating lymphocytes][14-16] and tumor microenvironment [i.e., absolute monocyte count (AMC)/tumor-associated macrophages], on clinical outcomes in patients with classical Hodgkin's lymphoma.

Design and Methods

Patients

In order to participate in the study, patients were required to have newly diagnosed classical Hodgkin's lymphoma and be followed at the Mayo Clinic, Rochester, Minnesota. Patients diagnosed with nodular lymphocyte predominant Hodgkin's lymphoma, treated only with radiation or palliative care, positive for human immunodeficiency virus and with concomitant autoimmune diseases receiving immunosuppressive therapy were excluded. From 1974 to 2010, 476 consecutive patients with classical Hodgkin's lymphoma qualified for the study. No patients refused authorization to use their medical records for research and none was lost to follow-up. Approval for the retrospective review of these patients' records was obtained from the Mayo Clinic Institutional Review Board and the research was conducted in accordance with USA federal regulations and the Declaration of Helsinki.

End-points

The primary end-point of the study was to assess the impact of ALC/AMC-DX on overall survival, lymphoma-specific survival, progression-free survival and time to progression from the

Table 1. Characteristics of the patients divided according to ALC/AMC-DX ratio ≥ 1.1 *versus* < 1.1.

Characteristics	ALC/AMC-DX ≥ 1.1 (N = 335)	ALC/AMC-DX < 1.1 (N = 141)	*P* value
At diagnosis			
Age, years, median (range)	35 (18-83)	44 (18-83)	<0.0008
Gender			<0.003
Male	162 (48%)	90 (64%)	
Female	173 (52%)	51 (36%)	
Histology			<0.05
Nodular sclerosis	280 (84%)	107 (76%)	
Mixed cellularity	45 (13%)	29 (20%)	
Lymphocyte-depleted	2 (0.6%)	4 (3%)	
Lymphocyte-rich	2 (0.6%)	0 (0%)	
Unclassified	6 (1.8%)	1 (1%)	
Stage			0.06
I	22 (6%)	8 (6%)	
II	129 (39%)	45 (32%)	
III	109 (33%)	39 (28%)	
IV	75 (22%)	49 (34%)	
Stage			0.07
Limited	140 (42%)	46 (33%)	
Advanced	195 (58%)	95 (67%)	
White blood cell count $\times 10^9$/L, median (range)	8.3 (1.1-53.9)	8.6 (1.7-24.3)	0.4
Albumin (g/dL), median (range) (N= 420)	3.9 (1.9-5.8)	3.5 (2.0-4.9)	<0.0001
Hemoglobin g/dL	12.7 (6.7-15.3)	12.4 (5.4-16.4)	0.2
Mediastinal bulky disease			0.08
≥10 cm	19 (6%)	15 (11%)	
< 10 cm	316 (94%)	126 (89%)	
Chemotherapy regimens			0.2
ABVD	233 (66.6%)	81 (57.4%	
BCVPP	21 (6.3%	17 (12.06%)	
ChLVPP	4 (1.2%)	4 (2.84%)	
MOPP	32 (9.5%)	18 (12.8%)	
MOPP-ABV	52 (15.5%)	20 (14.2%)	
Stanford V	3 (0.9%)	1 (0.7%)	
Treatment			<0.008
Chemotherapy	183 (55%)	96 (68%)	
Chemotherapy and radiation	152 (45%)	45 (32%)	
IPS risk factors			
Age in years			<0.006
> 45	231 (69%)	78 (55%)	
≤ 45	104 (31%)	63 (45%)	
Albumin (g/dL) (N = 420)	N = 293	N = 127	<0.0001
≥ 4	146 (50%)	28 (22%)	
< 4	147 (50%)	99 (78%)	
Hemoglobin (g/dL)			0.2
> 10.5	287 (86%)	114 (81%)	
≤ 10.5	48 (14%)	27 (19%)	
White blood cell count $\times 10^9$			0.7
> 15	34 (10%)	16 (11%)	
≤15	301 (90%)	125 (89%)	
Absolute lymphocyte count per μ/L			<0.0001
≥ 600	324 (97%)	66 (47%)	
< 600	11 (3%)	75 (53%)	
Male	162 (48%)	90 (64%)	<0.003
Stage 4	75 (22%)	49 (34%)	<0.0001

ALC/AMC-DX denotes absolute lymphocyte count/absolute monocyte count at diagnosis; ABVD: adriamycin, bleomycin, vinblastine, dacarbazine; BCVPP: BCNU, cyclophosphamide, vinblastine, procarbazine, prednisone; ChLVPP: chlorambucil, procarbazine, prednisone, vinblastine; MOPP: mechlorethamine, vincristine, procarbazine, prednisone; MOPP-ABV: mechlorethamine, vincristine, procarbazine, prednisone, adriamycin, bleomycin, vinblastine; Stanford V: adriamycin, vinblastine, vincristine, bleomycin, mechlorethamine, cyclophosphamide, etoposide, prednisone; IPS: International Prognostic Score.

moment that classical Hodgkin's lymphoma was diagnosed. The secondary end-point was to assess whether ALC/AMC-DX can further discriminate clinical outcomes in patients with limited or advanced stage at diagnosis; in patients with advanced stage with an IPS of 3 or more or in those with an IPS of less than 3; and in patients treated with chemotherapy plus radiation or chemotherapy alone. Limited-stage was defined as stage IA and IIA, without constitutional symptoms and absence of bulky disease defined as any mass of 10 cm or more in diameter. The absolute monocyte count at diagnosis (AMC-DX) and ALC/AMC-DX were calculated from the complete blood cell count obtained at the time the classical Hodgkin's lymphoma was diagnosed.[17] The ALC/AMC-DX ratio was obtained by dividing the ALC over the AMC from the complete blood count.

Prognostic factors

The prognostic factors evaluated in the study included: IPS[1] at diagnosis for advanced stage patients: [age >45 years, albumin <4 g/dL, ALC <600/μL or <8% of white cell count, hemoglobin <10.5 g/dL, male gender, stage IV, and white cell count ≥15,000/μL]; tumor size (≥10 cm); and treatment modality (combination chemotherapy plus radiation *versus* chemotherapy alone).

Response and survival

Definitions of response criteria, overall survival, lymphoma-specific survival, progression-free survival, and time to progression were based on the guidelines from the International Harmonization Project on Lymphoma.[18]

Statistical analysis

Overall survival, lymphoma-specific survival, progression-free survival and time to progression were analyzed using the approach of Kaplan and Meier.[19] Differences between survival curves were tested for statistical significance using the two-tailed log-rank test. The Cox proportional hazard model was used for the univariate and multivariate analyses to evaluate the variables under the prognostic factors' section to assess their impact on overall survival, lymphoma-specific survival, progression-free survival, and time to progression times.[20] The choice of the best cut-off values of AMC-DX and the ALC/AMC-DX ratio for assessing survival was based on their utility as a marker for the clinically relevant binary outcome of death/survival using the receiver operating characteristics curves (ROC) and area under the curve (AUC). The binary clinical outcome (death/survival) was established at 5 years after diagnosis. Patients were classified as "alive/censored" when the follow-up time was greater than 5 years and "death" for patients known to have died before this time point.[21] A k-fold cross-validation with k values of 10 was performed to validate the results of AMC-DX and the ALC/AMC-DX ratio.[22] Randomly chosen subsets containing 90% of the cohort were used for training, and the remaining 10% were left for testing. The cross-validation process was then repeated ten times. Based on this analysis, a cross-validation AUC by the ROC was produced, representing the discriminating accuracy of AMC-DX and ALC/AMC-DX ratio for the binary clinical outcome of death/survival.

Chi-square tests were used to determine relationships between categorical variables. The Wilcoxon-rank test was used to determine associations between continuous variables and categories, and Spearman's correlation coefficients were used to evaluate associations for continuous variables. All *P* values are two-sided and *P* values less than 0.05 are considered statistically significant.

Results

Patients' characteristics

The median age at diagnosis was 36 years (range, 18-83 years). The distribution of additional baseline characteristics is presented in Table 1 and summarized according to whether patients presented with an ALC/AMC-

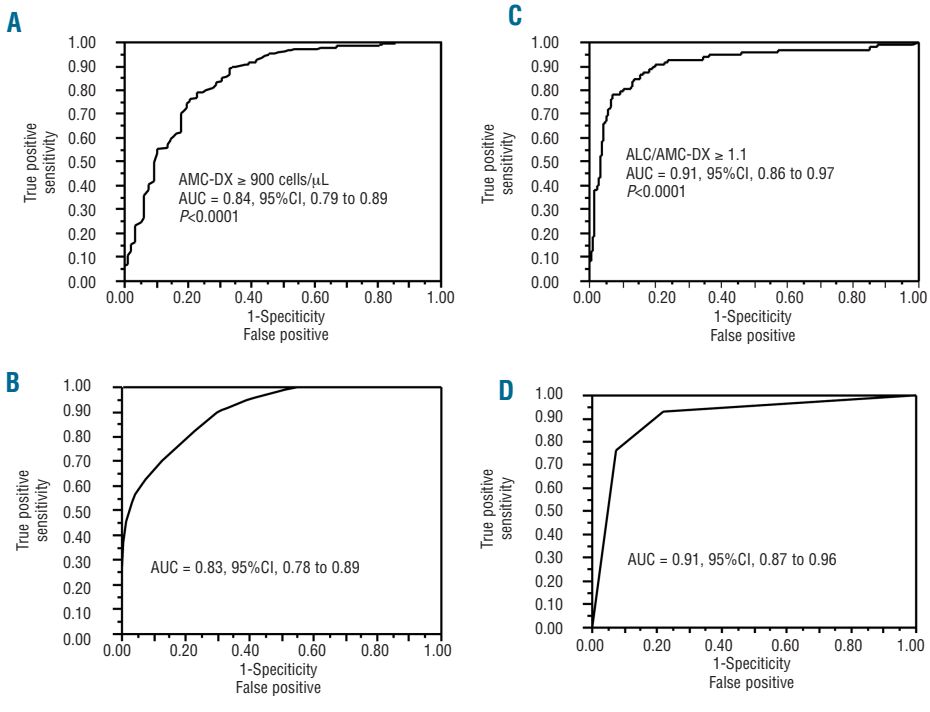

Figure 1. (A) Receiver operating-characteristic curve (ROC) and area under the curve (AUC) for absolute monocyte count at diagnosis (AMC-DX). (B) k-fold cross validation ROC and AUC for AMC-DX. (C) ROC and AUC for absolute lymphocyte count/absolute monocyte count at diagnosis (ALC/AMC-DX). (D) k-fold cross validation ROC and AUC for ALC/AMC-DX.

DX of 1.1 or more *versus* less than 1.1. The median follow-up period for the whole cohort was 5.6 years (range, 0.1-33.7 years) while that for living patients (n=299) was 6.4 years (range, 0.1-33.7 years). Forty-three patients died of causes unrelated to lymphoma and 134 patients died due to relapse/progression of lymphoma.

Higher numbers of patients in the group with ALC/AMC-DX greater or equal to 1.1 were younger (age ≤ 45 years, $P<0.0008$) and had an albumin concentration of 4 g/dL or more ($P<0.0001$). Fewer patients in the group with ALC/AMC-DX of 1.1 or more were male ($P<0.005$), presented with an ALC less than 600 cells/μL or less than 8% of the white blood cells ($P<0.0001$), and had stage 4 disease ($P<0.0001$). No difference between the groups was observed regarding bulky disease ($P=0.08$), chemotherapy regimens ($P=0.2$), hemoglobin concentration ($P=0.2$), limited *versus* advanced stage ($P=0.07$), and white blood cell count ($P=0.5$). Despite higher numbers of deaths unrelated to lymphoma observed in the group with ALC/AMC-DX of 1.1 or more [8.1% (27/335)] compared with the group with ALC/AMC-DX less than 1.1 [11.4% (16/141)], ($P=0.3$), this did not reach statistical significance.

Cut-off values for absolute monocyte count at diagnosis and ratio of absolute lymphocyte count to absolute monocyte count at diagnosis for survival analysis

An AMC-DX of 900 cells/μL or more had an AUC of 0.83 [95% confidence interval (CI), 0.78 to 0.88] with a sensitivity of 76% (95% CI, 66% to 84%) and specificity of 74% (95% CI, 65% to 78%) (Figure 1A). An ALC/AMC-DX ratio of 1.1 or more had an AUC of 0.91 (95% CI, 0.86 to 0.96) with a sensitivity of 90% (95% CI, 85% to 96%) and a specificity of 79% (95% CI, 73% to 88%) (Figure 1C). An internal validation of AMC-DX and ALC/AMC-DX ratio performances as markers for the clinical binary outcome of death/survival was performed using k-fold cross-validation with k =10. We obtained an average AUC of 0.84 (95% CI, 0.79 to 0.89) over the ten validation sets for AMC-DX, with a standard deviation of

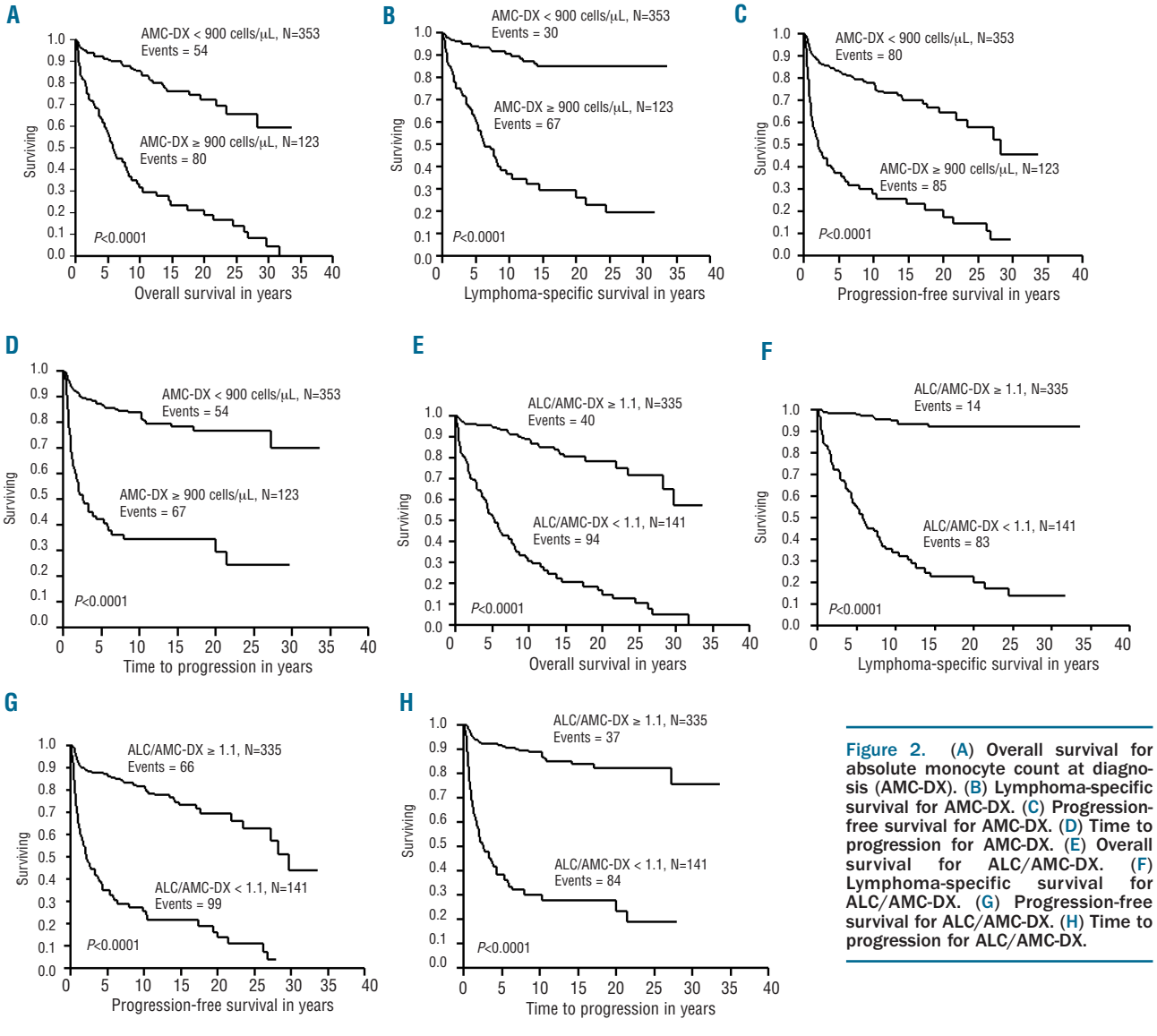

Figure 2. (A) Overall survival for absolute monocyte count at diagnosis (AMC-DX). (B) Lymphoma-specific survival for AMC-DX. (C) Progression-free survival for AMC-DX. (D) Time to progression for AMC-DX. (E) Overall survival for ALC/AMC-DX. (F) Lymphoma-specific survival for ALC/AMC-DX. (G) Progression-free survival for ALC/AMC-DX. (H) Time to progression for ALC/AMC-DX.

± 0.02. We also obtained an average AUC of 0.90 (95% CI, 0.86 to 0.96) over the ten validation sets for ALC/AMC-DX ratio, with a standard deviation of ± 0.02. We report the ROC for the complete dataset used in the 10-fold procedure, by collecting the AMC-DX and ALC/AMC-DX ratio obtained on each fold. For AMC-DX, the cross-validation ROC (Figure 1B) showed an AUC of 0.83 (95%CI, 0.78 to 0.89), and for ALC/AMC-DX ratio an AUC of 0.91 (95% CI, 0.87 to 0.96) (Figure 1D). The similar areas under the curves from the empirical ROC and the cross-validation ROC support the use of AMC-DX of 900 cells/μL or more and an ALC/AMC-DX ratio of 1.1 or more as the cut-off values as markers of the binary clinical outcome of death/survival.

Absolute monocyte count at diagnosis, ratio of absolute lymphocyte count to absolute monocyte count at diagnosis and survival

Patients with an AMC-DX of 900 cells/μL or more had inferior overall survival (Figure 2A), lymphoma-specific survival (Figure 2B), progression-free survival (Figure 2C), and time to progression (Figure 2D) compared with patients with an AMC-DX of less than 900 cells/μL [overall survival: median 5.8 years *versus* not reached, 5-year overall survival rates of 57% (95% CI, 45% to 62%) *versus* 91% (95% CI, 88% to 95%), *P*<0.0001; lymphoma-specific survival: median 6.3 years *versus* not reached, 5-year lymphoma-specific survival rates of 61% (95% CI, 47% to 65%) *versus* 94% (95% CI, 90% to 96%), *P*<0.000; progression-free survival: median 2.1 years *versus* 28.1 years, 5-year progression-free survival rates of 37% (95% CI, 29% to 49%) *versus* 82% (95% CI, 79% to 88%), *P*<0.000; and time to progression: median 2.6 years *versus* not reached, 5-year time to progression rates of 40% (95% CI, 31% to 48%) *versus* 87% (95% CI, 83% to 93%), *P*<0.0001, respectively]. Patients with an ALC/AMC-DX of 1.1 or more had superior overall survival (Figure 2E), lymphoma-specific survival (Figure 2F), progression-free survival (Figure 2G), and time to progression (Figure 2H) compared with patients with an ALC/AMC-DX less than 1.1 [overall survival: median not reached *versus* 5.2 years, 5-year overall survival rates of 95% (95% CI, 90% to 98%) *versus* 52% (95% CI, 35% to 58%), *P*<0.0001; lymphoma-specific survival: median not reached *versus* 5.8

Table 2. Univariate and multivariate analysis for overall survival (OS), lymphoma-specific survival (LSS), progression-free survival (PFS), and time to progression (TTP).

Univariate analysis												
Covariate	OS			LSS			PFS			TTP		
	HR	95%CI	*P* value	HR	95%CI	*P* value	HR	95%CI	*P* value	HR	95%CI	*P* value
Age (>45 years)	1.05	1.04-1.06	<0.0001	1.04	1.03-1.05	<0.0001	1.31	1.22-1.40	<0.0001	1.27	1.10-1.31	<0.0001
ALC-DX < 600 cells/μL	1.36	1.22-1.79	<0.0001	1.55	1.25-1.89	<0.0001	2.75	1.97-3.26	<0.0001	2.52	2.18-2.74	<0.0001
AMC-DX ≥ 900 cells/μL	1.79	1.55-2.05	<0.0001	2.00	1.71-2.29	<0.0001	1.77	1.54-2.01	<0.0001	1.89	1.69-2.17	<0.0001
ALC/AMC-DX ≥ 1.1	0.55	0.46-0.64	<0.0001	0.38	0.30-0.47	<0.0001	0.64	0.56-0.73	<0.0001	0.41	0.33-0.49	<0.0001
Albumin (≥ 4) g/dL	0.43	0.33-0.58	<0.0001	0.48	0.35-0.67	<0.0001	0.49	0.39-0.63	<0.0001	0.54	0.40-0.72	<0.0001
Bulky disease (≥ 10 cm)	1.09	0.92-2.36	0.8	1.19	0.84-1.66	0.6	1.11	0.90-1.75	0.7	1.48	0.87-1.98	0.2
Hemoglobin (< 10.5) g/dL	1.87	1.80-1.98	<0.0001	1.91	1.01-2.10	<0.05	1.89	1.82-1.96	<0.001	1.34	1.09-1.99	<0.04
Male	1.51	1.15-1.95	<0.02	1.50	1.02-1.86	<0.05	1.44	1.12-1.69	<0.02	1.55	1.27-1.64	<0.0003
WBC (> 15) ×10⁹/L	1.05	0.95-2.14	0.4	1.12	0.99-1.41	0.5	1.23	0.94-1.98	0.2	1.31	0.94-1.43	0.3
Stage 4	1.94	1.51-2.16	<0.0003	2.61	1.93-3.87	<0.0001	1.91	1.57-2.33	<0.0001	2.29	1.95-2.55	<0.0001
IPS ≥ 3	2.76	1.83-4.20	<0.0001	2.71	1.70-4.40	<0.0001	2.04	1.43-2.94	<0.0001	2.14	1.41-3.25	<0.0001
CT and RT *versus* CT alone	0.47	0.30-0.71	<0.0001	0.40	0.25-0.51	<0.0001	0.47	0.21-0.60	<0.0001	0.44	0.28-0.57	<0.0001

Multivariate Analysis												
Covariate	OS			LSS			PFS			TTP		
	HR	95%CI	*P* value	HR	95%CI	*P* value	HR	95%CI	*P* value	HR	95%CI	*P* value
Age (>45 years)	2.56	1.53-4.35	<0.0003	1.83	1.03-3.29	<0.04	1.64	1.03-2.60	<0.04	1.15	0.67-1.96	0.6
ALC-DX < 600 cells/μL	1.25	0.74-2.15	0.4	1.41	0.79-2.55	0.2	1.59	0.96-2.58	0.06	2.06	1.20-3.58	<0.008
AMC-DX ≥ 900 cells/μL	1.61	0.91-2.93	0.1	1.58	0.85-3.06	0.1	2.01	1.19-3.47	< 0.009	1.99	1.13-3.56	<0.02
ALC/AMC-DX ≥ 1.1	0.18	0.08-0.38	<0.0001	0.10	0.04-0.25	<0.0001	0.35	0.18-0.66	<0.002	0.27	0.13-0.57	<0.0006
Albumin (≥ 4) g/dL	0.71	0.35-1.43	0.3	0.92	0.40-2.09	0.8	0.48	0.26-0.86	<0.01	0.24	0.11-0.68	<0.02
Hemoglobin (< 10.5) g/dL	1.67	0.91-2.96	0.1	1.32	0.61-2.65	0.5	1.34	0.78-2.21	0.3	1.14	0.60-2.08	0.7
Male	1.43	0.87-2.40	0.1	1.33	0.75-2.42	0.3	1.49	0.95-2.36	0.08	1.68	0.98-2.92	0.06
Stage 4	1.43	0.86-2.40	0.2	2.11	1.18-3.90	<0.01	1.68	1.05-2.71	<0.03	2.14	1.24-3.78	<0.02
IPS ≥ 3	2.16	1.56-3.38	<0.001	2.29	1.58-3.90	<0.003	1.86	1.16-3.64	<0.01	1.83	1.26-3.97	<0.01
CT and RT *versus* CT alone	0.96	0.50-1.79	0.9	0.82	0.41-1.76	0.6	0.59	0.33-1.01	0.06	0.71	0.37-1.32	0.3

ALC-DX: absolute lymphocyte count at diagnosis; AMC-DX: absolute monocyte count at diagnosis; ALC/AMC-DX: absolute lymphocyte count/absolute monocyte count at diagnosis, CT: chemotherapy; IPS: International Prognostic Score for advanced stage patients; RT: radiation; WBC: white blood cell count.

years, 5-year lymphoma-specific survival rates of 98% (95% CI, 96% to 100%) *versus* 55% (95% CI, 42% to 60%), *P*<0.0001; progression-free survival: median 3.0 years *versus* 2.2 years, 5-year progression-free survival rates of 87% (95% CI, 81% to 92%) *versus* 34% (95% CI, 25% to 42%), *P*<0.0001; and time to progression: median not reached *versus* 2.5 years, and 5-year time to progression rates of 92% (95% CI, 87% to 96%) *versus* 37% (95% CI, 30% to 45%), *P*<0.0001].

Neither white cell count nor bulky disease was predictive of overall survival, lymphoma-specific survival, progression-free survival, or time to progression compared with the other prognostic factors studied (Table 2). ALC/AMC-DX remained an independent prognostic fac-

Table 3. Clinical outcomes based on the ALC/AMC-DX at diagnosis according to patients' treatment; International Prognostic Score and stage at diagnosis.

	Chemotherapy and radiation			Chemotherapy alone		
	Median Time (years)	5-year rate (95%CI)	*P* value	Median Time (years)	5-year rate (95%CI)	*P* value
Overall survival			<0.0001			<0.0001
ALC/AMC-DX ≥ 1.1	Not reached	97% (90%-100%)		28.2	93% (89%-97%)	
ALC/AMC-DX < 1.1	14.5	67% (50%-80%)		4.3	43% (35%-52%)	
Lymphoma-specific survival			<0.0001			<0.0001
ALC/AMC-DX ≥ 1.1	Not reached	100% (95%-100%)		Not reached	96% (92%-100%)	
ALC/AMC-DX < 1.1	14.5	69% (50%-82%)		4.4	46% (32%-58%)	
Progression-free survival			<0.0001			<0.0001
ALC/AMC-DX ≥ 1.1	Not reached	93% (85%-97%)		27.2	82% (75%-89%)	
ALC/AMC-DX < 1.1	10.3	53% (35%-67%)		1.7	24% (15%-35%)	
Time to progression			<0.0001			<0.0001
ALC/AMC-DX ≥ 1.1	Not reached	95% (89%-99%)		Not reached	88% (80%-96%)	
ALC/AMC-DX < 1.1	10.3	54% (37%-68%)		1.7	28% (20%-40%)	
	International Prognostic Score < 3			**International Prognostic Score ≥ 3**		
Overall survival			<0.0001			<0.0001
ALC/AMC-X ≥ 1.1	Not reached	98% (93%-100%)		28.2	89% (79%-98%)	
ALC/AMC-DX < 1.1	6.3	63% (50%-80%)		3.6	26% (15%-36%	
Lymphoma-specific survival			<0.0001			<0.0001
ALC/AMC-DX ≥ 1.1	Not reached	99% (92%-100%)		Not reached	98% (93%-100%)	
ALC/AMC-DX < 1.1	6.3	65% (50%-80%)		3.6	30% (19%-49%)	
Progression-free survival			<0.0001			<0.0001
ALC/AMC-DX ≥ 1.1	Not reached	86% (80%-95%)		17.7	76% (67%-89%)	
ALC/AMC-DX < 1.1	2.2	28% (17%-45%)		1.9	19% (10%-37%)	
Time to progression			<0.0001			<0.0001
ALC/AMC-DX ≥ 1.1	Not reached	91% (86%-97%)		27.2	84% (78%-97%)	
ALC/AMC-DX < 1.1	2.4	32% (21%-49%)		1.7	23% (11%-40%)	
	Limited stage			**Advanced stage**		
Overall survival			<0.0001			<0.0001
ALC/AMC-DX ≥ 1.1	26.9	94% (87%-99%)		Not reached	95% (90%-98%)	
ALC/AMC-DX < 1.1	8.2	62% (40%-75%)		4.3	45% (37%-58%)	
Lymphoma-specific survival			<0.0001			<0.0001
ALC/AMC-DX ≥ 1.1	Not reached	99% (90%-100%)		Not reached	97% (93% -100%)	
ALC/AMC-DX < 1.1	8.2	64% (50%-85%)		4.9	50% (39%-60%)	
Progression-free survival			<0.0001			<0.0001
ALC/AMC-DX ≥ 1.1	26.9	91% (86%-97%)		28.0	(83% (78%-89%)	
ALC/AMC-DX < 1.1	5.6	52% (40%-73%)		2.0	24% (18%-35%)	
Time to progression			<0.0001			<0.0001
ALC/AMC-DX ≥ 1.1	Not reached	95% (89%-98%		Not reached	89% (83%-95%)	
ALC/AMC-DX < 1.1	5.9	54% (42%-77%)		2.1	27% (20%-39%)	

ALC/AMC-DX: absolute lymphocyte count/absolute monocyte count at diagnosis; CI: confidence interval.

tor for overall survival, lymphoma-specific survival, progression-free survival, and time to progression (Table 2).

Survival based on the ratio of absolute lymphocyte count to absolute monocyte count at diagnosis by treatment, International Prognostic Score, and limited/advanced stage at diagnosis

We analyzed the ALC/AMC-DX in an attempt to further discriminate clinical outcomes in patients with classical Hodgkin's lymphoma divided according to treatment (chemotherapy plus radiation or chemotherapy alone); IPS score less than 3 (low risk) or IPS score of 3 or more (high risk) at diagnosis, and limited stage or advanced stage. Table 3 summarizes the clinical outcomes for overall survival, lymphoma-specific survival, progression-free survival, and time to progression based on ALC/AMC-DX in patients divided according to type of treatment, IPS score, and stage. Patients with an ALC/AMC-DX of 1.1 or more had superior clinical outcomes compared with patients with an ALC/AMC-DX less than 1.1 regardless of treatment (chemotherapy plus radiation or chemotherapy alone); low or high risk IPS score (< 3 or ≥ 3) at diagnosis, and limited stage or advanced stage. In comparison with the IPS study,[1] our 5-year time to progression rates were very similar to the freedom from progression (same definition as time to progression in our study): the 5-year time to progression rate for patients with an IPS score less than 3 was 81% (95% CI, 75% to 90%) in our study, while the 5-year freedom from progression in the IPS study was 76%;[1] the 5-year time to progression rate for patients with an IPS score of 3 or more was 60% (95% CI, 50% to 71%) in our study, while the 5-year freedom from progression rate in the IPS study was 55%.[1]

Discussion

The pathological biomarkers tumor-infiltrating lymphocytes and tumor-associated macrophages are associated with clinical outcomes in classical Hodgkin's lymphoma. We, therefore, combined the ALC and AMC at diagnosis, as representative biomarkers of tumor-infiltrating lymphocytes and tumor-associated macrophages, to study clinical outcomes in cHL.

To support the hypothesis that the biomarker ALC/AMC-DX ratio affects survival in classical Hodgkin's lymphoma, it was necessary to demonstrate that peripheral blood monocytes were associated with clinical outcomes in classical Hodgkin's lymphoma. We determined that patients presenting with an AMC-DX of 900 cells/μL or more had an inferior survival. By univariate analysis, the AMC-DX was a predictor of overall survival, lymphoma-specific survival, progression-free survival, and time to progression. To our knowledge, this is the first paper reporting the association between AMC-DX and survival in classical Hodgkin's lymphoma. The ALC in the IPS study was only evaluated in relation to overall survival and freedom from progression. The two end-points of overall survival and freedom from progression in the IPS study had the same definitions for overall survival and time to progression used in our study. Our study showed that ALC was not only a predictor for overall survival and time to progression, but also for lymphoma-specific survival and progression-free survival. Thus, we combined the prognostic factors for overall survival, lymphoma-specific survival, progression-free survival, and time to progression in classical Hodgkin's lymphoma, ALC and AMC, into a single prognostic factor: ALC/AMC-DX ratio. An ALC/AMC-DX ratio of 1.1 or more was associated with superior overall survival, lymphoma-specific survival, progression-free survival, and time to progression. By multivariate analysis, the ALC/AMC-DX ratio outperformed other prognostic factors, including the IPS score. Furthermore, patients in the group with an ALC/AMC-DX less than 1.1 tended to have adverse features, including advanced stage (i.e., tumor burden), suggesting an impact of host immunity (i.e., ALC) *versus* tumor microenvironment (i.e., AMC) on tumor growth control.

A limitation of the IPS scoring system is that it only applies to patients with advanced stage classical Hodgkin's lymphoma and not to those with limited stage disease.[1] We, therefore, investigated the prognostic ability of ALC/AMC-DX to assess survival in patients with limited and advanced stage disease. The ALC/AMC-DX was able to discriminate clinical outcomes not only in patients with limited or advanced stage disease, but also in those with an IPS score of less than 3 or of 3 or more at diagnosis and in patients receiving different treatments (chemotherapy plus radiation or chemotherapy alone).

To minimize the inherent biases of a retrospective study, the following steps were taken. With regards to selection bias, we included only patients with classical Hodgkin's lymphoma and excluded patients with nodular lymphocyte predominant Hodgkin's lymphoma who are considered to have a different disease entity. We excluded patients treated up-front with palliative care or radiation therapy alone, as chemotherapy and combination chemotherapy and radiation are considered the current standard of care for classical Hodgkin's lymphoma. Patients who were positive for human immunodeficiency virus and concomitant autoimmune disease treated with immunosuppressive therapies were also excluded as these diseases and treatment directly influence ALC and AMC values. Furthermore, the 5-year time to progression rates in this study for patients with low risk or high risk IPS score were similar to the 5-year freedom from progression rates in the IPS study.[1] The similarity between the clinical outcomes in our study and the IPS study argues that the selection and treatment of the classical Hodgkin's lymphoma patients in our study was in accordance with the changes in patterns of care of classical Hodgkin's lymphoma during the time period analyzed in the study. With regards to confounding factors, our study included currently known prognostic factors, such as tumor size, treatment modalities, and the IPS score. Due to the young population, the impact of age on residual survival, which gets shorter with age, was felt to be negligible. In the multivariate analysis, ALC/AMC-DX ratio remained an independent prognostic factor for survival when compared to these prognostic factors.

A strength of the study is the long-term follow-up of a well-defined group of patients with classical Hodgkin's lymphoma. The median follow-up period for the overall cohort of patients was 5.6 years and 6.4 years for living patients. Secondly, the ALC/AMC-DX ratio combines the clinical surrogate biomarkers for the inflammatory, pathological biomarkers – tumor-infiltrating lymphocytes and tumor-associated macrophages – which directly affect the biology of classical Hodgkin's lymphoma. Thirdly, the ALC/AMC-DX ratio is a simple, easily determined clinical biomarker that can be used to assess the clinical outcome

in limited and advanced stages of classical Hodgkin's lymphoma. Fourthly, we report the clinical value of a single biomarker (ALC/AMC-DX) to assess clinical outcomes in classical Hodgkin's lymphoma based on a worldwide, routine clinical test: the complete blood count.

A major limitation of this study is that formal investigations of the tumor microenvironment in this population were not performed. The tumor microenvironment is a complex evolving system with an array of different non-malignant cells and a variety of malignant clones. Future research should correlate the peripheral blood absolute lymphocyte count and monocyte count with microenvironmental data.

In conclusion, ALC/AMC-DX is a single, low cost, predictive biomarker for clinical outcomes in classical Hodgkin's lymphoma.

Authorship and Disclosures

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