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Absolute Monocyte Count and Lymphocyte-Monocyte Ratio Predict Outcome in Nodular Sclerosis Hodgkin Lymphoma: Evaluation Based on Data From 1450 Patients

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Abstract

Objective: To verify whether absolute monocyte count (AMC) and lymphocyte- monocyte ratio (LMR) at diagnosis are valid prognostic parameters in classical Hodgkin lymphoma (cHL).

Patients and Methods: Data were collected from 1450 patients with cHL treated in Israel and Italy from January 1, 1988, through December 31, 2007.

Results: The median age of the patients was 33 years (range, 17-72 years), and 70% (1017) of the patients had nodular sclerosis (NS); the median follow-up duration was 87 months. The best cutoff value for AMC was 750 cells/mm^3, and the best ratio for LMR was 2.1. The adverse prognostic impact of an AMC of more than 750 cells/mm^3 was confirmed for the entire cohort, and its clinical significance was particularly evident in patients with NS histology. The progression-free survival (PFS) at 10 years for an AMC of more than 750 cells/mm^3 was 65% (56%-72%), and the PFS at 10 years for an AMC of 750 cells/mm^3 or less was 81% (76%-84%; $P<.001$). The overall survival (OS) at 10 years for an AMC of more than 750 cells/mm^3 was 78% (70%-85%), and the OS at 10 years for an AMC of 750 cells/mm^3 or less was 88% (84%-90%; $P=.01$). In multivariate analysis, both AMC and LMR maintained prognostic significance for PFS (hazard ratio [HR], 1.54, $P=.006$, and HR, 1.50, $P=.006$) after adjusting for the international prognostic score, whereas the impact on OS was confirmed (HR, 1.56; $P=.04$) only in patients with NS and an AMC of more than 750 cells/mm^3.

Conclusion: This study confirms that AMC has prognostic value in cHL that is particularly significant in patients with NS subtype histology. This finding links the known impact of macrophages and monocytes in Hodgkin lymphoma with routine clinical practice.

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Current therapy for classical Hodgkin lymphoma (cHL) cures about 80% of the patients with this disease, whereas the rest relapse or are refractory to therapy. Major efforts have been made to avoid possible overtreatment and potential long-term toxicity in younger patients and to identify patients requiring more aggressive therapy in order to avoid the development of refractory disease. In this regard, to define a scoring system that could stratify patients, and possibly even predict outcome, would be both helpful and practical to apply in daily practice. The international prognostic score (IPS) proposed in 1998[1] uses a model that incorporates 7 prognostic factors at initial diagnosis to predict outcome in patients with cHL. The major limitation of the IPS relates to the fact that it was first proposed for advanced cHL in an attempt to avoid overtreatment in some patients and identify others in whom standard therapy would be inadequate. However, its role in both favorable and unfavorable early stage disease is limited.

TABLE 1. Clinical and Laboratory Characteristics of the 1450 Patients Included in the Study

Variable	Median (2.5th-97.5th percentile)
Age (y)	33 (17-72)
Hemoglobin (g/dL)	12.4 (7.9-16.1)
WBC (cells/mm^3)	8600 (3200-20,000)
AMC (cells/mm^3)	550 (82-1527)
ALC (cells/mm^3)	1543 (334-3981)
LMR	2.8 (0.7-17)
Albumin (g/dL)	4.0 (2.4-5.0)
Variable	**n (%)**
Age >45 y	402 (28)
Sex: male	728 (50)
Stage IV disease	234 (16)
Hemoglobin <10.5 g/dL	253 (17)
Albumin <4 g/dL	846 (58)
WBC >15,000 cells/mm^3	157 (11)
ALC <600 cells/mm^3	90 (6)
Histology, NS	1017 (70)
Systemic symptoms	640 (44)
IPS	
0-2	1054 (73)
3-7	396 (27)

ALC = absolute lymphocyte count; AMC = absolute monocyte count; IPS = international prognostic score; LMR = lymphocyte-monocyte ratio; NS = nodular sclerosis; WBC = white blood cell.

An absolute lymphocyte count (ALC) of less than 600 cells/mm^3 or less than 8% of the total white blood cell (WBC) count is one of the factors included in the IPS and is regarded as an important prognostic parameter influencing the interval of freedom from progression.[1] Indeed, ALC is considered a surrogate biomarker of tumor-infiltrating lymphocytes and reflects the general status of host immunity. Recently, however, several studies have reported that absolute monocyte count (AMC) at diagnosis also has prognostic value in lymphomas.[2-7] The rationale for using AMC as a prognostic parameter in cHL is even more relevant than in other malignancies because of the immunohistochemical and molecular data, including gene expression profile, which identify a key role for monocytes and macrophages in the biology of cHL, particularly in patients with nodular sclerosis (NS) histology.[8-12]

Combining AMC and ALC as a lymphocyte-monocyte ratio (LMR) has been proposed and shown to have prognostic potential in both non-Hodgkin lymphoma (NHL) and cHL.[13,14] Each of the above studies has used different cutoff values for AMC and LMR, which are easily accessible and simple to apply, but an agreed standard value has as yet not been defined.

The aim of the present study was to verify, using a large cohort of patients from 2 countries and continents, whether AMC and LMR represent valid prognostic parameters in cHL and at the same time identify the best cutoff value for AMC and LMR.

PATIENTS AND METHODS

This study is a retrospective analysis of previously untreated patients with cHL. We reviewed clinical and laboratory data of "therapy-naive" patients, treated in different centers from January 1, 1988, to December 31, 2007, in Israel and Italy. Italian cases were retrieved from 38 centers belonging to the Gruppo Italiano Studio Linfomi archive. Data from Israeli patients were collected from 2 medical centers after approval by local institutional review boards. All studies were performed in accordance with the principles of the Declaration of Helsinki.

Patients were accepted into this study if the following criteria were fulfilled: histopathological diagnosis of cHL, no previous therapy, age more than 18 years, no human immunodeficiency virus infection, availability of data on all clinical and laboratory features and treatments given, as well as outcome, and follow-up. The database contained a total of 1848 patients who had received combination chemotherapy with or without radiotherapy. Analysis was performed on a final cohort of 1450 patients after the exclusion of those with missing data relating to IPS (n=166), or monocyte count (n=137), or missing reports (n=95). Definition of response was based on guidelines revised by Cheson et al.[15]

Primary end points of the study were to assess the impact of AMC, ALC, and LMR on progression-free survival (PFS) and overall survival (OS). The secondary end point was to establish the best cutoff value for AMC and LMR on the basis of different values reported in the literature.

AMC and ALC Adjusted to the IPS

Absolute monocyte count and ALC were adjusted to the IPS[1] used to predict survival in patients with advanced cHL. The IPS is based on 7 adverse clinical and laboratory parameters: age more than 45 years, albumin less than 4 g/dL, ALC of less than 600 cells/mm^3 or

TABLE 2. Treatment According to the Chemotherapy Regimens Used With or Without Radiotherapy and Response Rates[a,b]

Treatment	n (%) RT, no	RT, yes	Total
Chemotherapy, n (%)			
ABVD	268 (40)	394 (60)	662 (46)
MEC/MAC	219 (62)	134 (38)	353 (24)
VBM	54 (20)	213 (80)	267 (18)
BEACOPP	53 (60)	65 (40)	88 (6)
Stanford V	16 (36)	29 (64)	45 (3)
EVE	4 (11)	31 (89)	35 (2)
RT			836 (58)
Response CHT ± RT			n (%)
CR			1298 (90)
PR			48 (3)
SD			34 (2)
PD			50 (4)
Early failure/withdrawal			20 (1)

[a]ABVD = adriamycin, bleomycin, vinblastine, and dacarbazine; BEACOPP = bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazine, and prednisone; CR = complete response; EVE = epirubicin, vinblastine, and etoposide; MAC = mechlorethamine, vincristine, procarbazine, prednisone, epidoxorubicin, bleomycin, vinblastine, lomustine, doxorubicin, and vindesine; MEC = mechlorethamine, lomustine, vindesine, melphalan, prednisone, epidoxorubicin, vincristine, procarbazine, vinblastine and bleomycin; PD = progression disease; PR = partial response; RT = radiotherapy; SD = stable disease; Stanford V = doxorubicin, vinblastine, mechlorethamine, vincristine, bleomycin, etoposide, and prednisone; VBM = vinblastine, bleomycin, and methotrexate.
[b]Percentage of RT expressed by row.

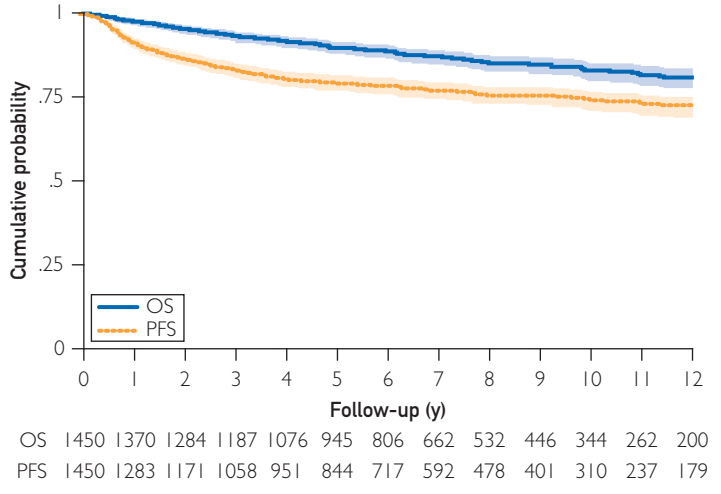

FIGURE 1. Kaplan-Meier curves for OS and PFS for the cohort of 1450 patients entered in the study. OS = overall survival; PFS = progression-free survival.

less than 8% of the WBC count, hemoglobin level of less than 10.5 g/dL, male sex, and leukocytosis (WBC count>15,000 cells/mm^3).

Statistical Analyses

Progression-free survival was measured from the date of study entry to either the last follow-up or the occurrence of one of the following events: disease progression, relapse, or death from any cause. Continuous variables were reported as the median and 2.5th to 97.5th percentile. Comparisons were performed with the Mann-Whitney test or the Kruskall-Wallis test. Categorical variables were reported as proportions and compared with the chi-square test or the exact Fisher test.

Survival was assessed by the Kaplan-Meier estimates[16] and compared by risk groups using the log-rank test and the Cox proportional hazards model.[17] The proportional hazard assumption was verified graphically by means of scaled Schoenfeld residuals.[18] The effect size was reported as hazard ratio (HR) with the associated 95% CI.

We assessed the optimal cutoff for AMC and LMR using the maximum log-rank statistic and by means of receiver operating characteristic (ROC) curve analysis at 5-year follow-up.

We evaluated the following cutoff values for monocytes in cHL: 500, 630,[14,19] 750,[20] 800, and 900 cells/mm^3.[21] For LMR, we also studied several cutoff values based on data in the literature and our own experience with statistical analysis including 1.1,[14,19] 1.5,[20] 2.1,[6] 2.8, and 3.5.[2]

Thereafter, we chose cutoff levels that had the best discriminating power to distinguish patients with good outcome from those with worse outcome, after adjusting for IPS values. Attempts to define the different cutoffs were checked using HRs and the respective z score (from the Wald test) and by comparing the ability to enforce the discriminating power (ROC curve analysis at 5-year follow-up), adding the AMC or the LMR to the IPS score (delta [difference] of area under the curve between ROC curve analysis at 5-year follow-up with IPS and IPS + AMC or LMR [dAUC]).

Given the broad agreement in the literature regarding the definition of lymphopenia as an ALC of 600 cells/mm^3, we decided to use this level as our optimal lymphocyte cutoff, whereas analysis of different cutoff points was done only

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for AMC and LMR. All statistical tests were 2-sided.

RESULTS

Patients' Characteristics

The median age at diagnosis for the entire cohort of 1450 patients was 33 years (range, 17-72 years); 50% (728) were males, and 44% (640) presented with systemic symptoms. In terms of cHL subtype, NS was diagnosed in 70% of the patients (1017). Other clinical characteristics and those included in the IPS are presented in Table 1.

Details of therapy are summarized in Table 2. All patients were treated with combination chemotherapy and considering the reference treatment (adriamycin, bleomycin, vinblastine, and dacarbazine), other types of regimens given had no impact on the PFS (score test from Cox regression model, P=.78) and the OS (score test, P=.37). A total of 836 (58%) patients received radiotherapy in addition to chemotherapy, and of the entire cohort, 90% (1298 patients) of the patients achieved complete remission.

The median follow-up for the entire cohort of 1450 patients was 87 months (range, 1-243 months). The estimated 5- and 10-year OS was 90% (95% CI, 88%-91%) and 83% (95% CI, 80%-85%), respectively. A total of 201 deaths were recorded; of these, 67% (135) were from progressive disease and 15% (30) due to second cancers. The PFS at 5 and 10 years was 79% (95% CI, 77%-81%) and 74% (95% CI, 71%-76%), respectively (Figure 1).

Considering the period of diagnosis 1988 to 1999 as reference, the periods 2000 to 2003 and 2004 to 2007 did not show an effect on the PFS (score test from Cox regression model, P=.37) and the OS (score test, P=.41).

Cutoff Value for AMC

Absolute monocyte count was taken from the pretreatment complete blood cell counts recorded at diagnosis of cHL. The median AMC for all patients was 550 cells/mm^3 (2.5th-97.5th percentile, 82-1527 cells/mm^3). Absolute monocyte count was higher in patients older than 45 years (P=.007) and in patients with a WBC count of more than 15,000 cells/mm^3 (P<.001), NS histology (P<.001), and an IPS of 3 to 7 (P=.009). In the reported literature, the most common AMC cutoff levels evaluated were more than 500,[2] 630,[19,22,23] 750,[20] 800, and 900 cells/mm^3.[21] Our optimal cutoff level on the PFS taken from the ROC curve analysis at 5-year follow-up was 749 cells/mm^3 (ROC curve=0.56) and with maximal log-rank test was 748 cells/mm^3 (χ^2=13.8). Thus, all these proposed thresholds were analyzed for PFS by means of the estimated HR (in the univariate Cox model) and the dAUC after adding AMC to IPS 3 to 7. The best cutoff value was seen with an AMC of more than 750 cells/mm^3 (HR, 1.47; 95% CI, 1.17-1.86) and dAUC of 1.7 (P=.05) (Table 3).

Three hundred fifty-nine patients (25%) were enrolled with an AMC of more than 750 cells/mm^3, compared with 1091 patients (75%) with an AMC of 750 cells/mm^3 or less at diagnosis. The 5-year PFS was 81% (95% CI, 79%-83%) for an AMC of 750 cells/mm^3 or less and 74% (95% CI, 69%-78%) for an

TABLE 3. Multiple Cox Regression Analysis for AMC > 750 Cells/mm^3 Adjusted by IPS Parameters for Patients with Classical Hodgkin Lymphoma and Nodular Sclerosis Histology: Progression-Free Survival and Overall Survival[a]

AMC	Cutoff	n (%)	HR (95% CI)	*P*	dAUC	*P*[b]
	≤500	636 (44)	1.00			
	>500	814 (56)	1.20 (0.96-1.49)	.104	+0.4	.70
	≤630	908 (63)	1.00			
	>630	542 (37)	1.38 (1.11-1.71)	.004	+1.5	.11
	≤750	1091 (75)	1.00			
	>750	359 (25)	1.47 (1.17-1.86)	.001	+1.7	.05
	≤800	1140 (79)	1.00			
	>800	310 (21)	1.38 (1.08-1.76)	.010	+1.1	.19
	≤900	1232 (85)	1.00			
	>900	218 (15)	1.29 (0.97-1.71)	.075	+0.4	.53
LMR	**Cutoff**	**n (%)**	**HR (95% CI)**	***P***	**dAUC**	***P*[b]**
	>1.1	1328 (92)	1.00			
	≤1.1	122 (8)	1.35 (0.95-1.92)	.099	+0.4	.46
	>1.5	1180 (81)	1.00			
	≤1.5	270 (19)	1.55 (1.21-2.00)	.001	+1.2	.11
	>2.1	957 (66)	1.00			
	≤2.1	493 (34)	1.49 (1.20-1.86)	<.001	+2.2	.02
	>2.8	739 (51)	1.00			
	≤2.8	711 (49)	1.30 (1.05-1.61)	.017	+1.1	.28
	>3.0	656 (45)	1.00			
	≤3.0	794 (55)	1.45 (1.16-1.80)	.001	+1.6	.10

[a]AMC = absolute monocyte count; AUC = area under the curve; dAUC = delta (difference) of AUC between ROC curve analysis at 5-y follow-up with IPS and IPS + AMC or LMR; HR = hazard ratio; IPS = international prognostic score; LMR = lymphocyte-monocyte ratio; ROC = receiver operating characteristic.

[b]*P* value of the delta over 1000 bootstrap resamples.

AMC of more than 750/mm^3 (log-rank test, P=.001).

The OS for the entire cohort, according to an AMC of 750 cells/mm^3 or less and more than 750 cells/mm^3, was 91% (95% CI, 89%-92%) and 86% (95% CI, 82%-90%), with an HR of 1.35 (95% CI, 0.99-1.84; P=.06), respectively.

In patients with NS cHL, the 5- and 10-year PFS was 81% (95% CI, 78%-84%) and 77% (95% CI, 74%-80%), respectively, whereas the OS was 91% (95% CI, 89%-93%) after 5-year follow-up and 85% (95% CI, 82%-88%) after 10-year follow-up (Figure 2).

Thus, by using the peripheral blood AMC, we were able to discriminate patients with less favorable outcome, with a PFS of 81% (95% CI, 76%-84%) for those with an AMC of 750 cells/mm^3 or less compared with 65% (95% CI, 56%-72%) for those with an AMC of more than 750 cells/mm^3 at 10 years (P=.001) (Figure 3). The OS was 88% (95% CI, 84%-90%) in those with an AMC of 750 cells/mm^3 or less compared with 78% (95% CI, 70%-85%) in those with an AMC of more than 750 cells/mm^3 at 10 years (P=.01) (Figure 3). After adjusting for the IPS, the AMC had clinical prognostic significance in patients with NS (HR, 1.54, 95% CI, 1.23-2.10, P=.006 for PFS; HR, 1.56, 95% CI, 1.02-2.40, P=.04 for OS) as reported in Table 4.

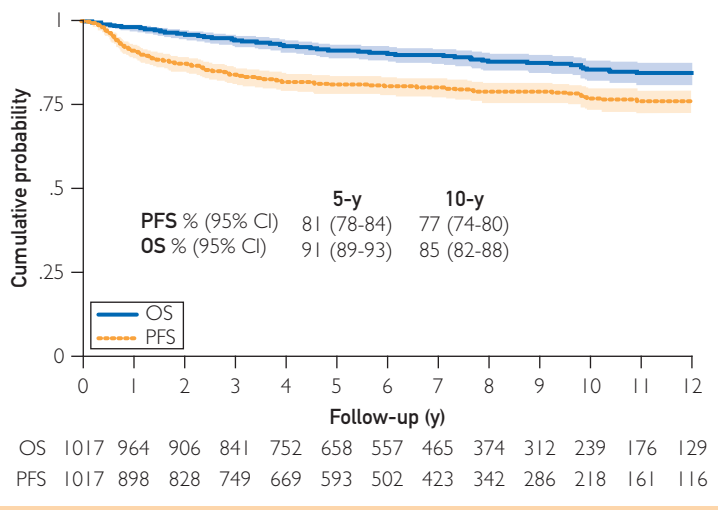

FIGURE 2. Kaplan-Meier curves for OS and PFS for the cohort of 1017 patients with nodular sclerosis Hodgkin lymphoma histology. OS = overall survival; PFS = progression-free survival.

Cutoff Value for ALC

The ALC was derived from pretreatment complete blood cell count at diagnosis, and for all patients the median ALC was 1543 cells/mm^3 (2.5th-97.5th percentile, 334-3981). The accepted IPS cutoff value of 600 cells/mm^3 for ALC and lymphopenia was defined as in the formal scoring system. Of the entire cohort, only 92 patients (6%) presented with lymphopenia at the time of diagnosis.

Cutoff Value for LMR

The LMR was obtained by dividing the ALC by the AMC taken from the complete peripheral blood count at diagnosis. The median LMR for all patients was 2.8 (2.5th-97.5th percentile, 0.7-17). The LMR was lower in patients with stage IV disease (P<.001), a hemoglobin level of less than 10.5 g/dL (P<.001), a WBC count of 15,000 cells/mm^3 (P<.001), an albumin level of 4 g/dL (P<.001), with NS histology (P<.001), and an IPS of 3 to 7 (P=.009). For LMR, we also tested several cutoff values based on the literature data including 1.1,[14,19] 1.5 ,[20] 2.1,[6,22] 2.8, and 3.5.[2] Our optimal cutoff on PFS from the ROC curve analysis at 5-year follow-up was 2.1 (ROC curve=0.56) and with maximally log-rank test was 1.5/mm^3 (χ^2=16.7). All these proposed thresholds were analyzed for PFS using the estimated HR (in the univariate Cox model) and the dAUC after adding LMR to IPS 3 to 7. The most promising cutoff value for LMR was 2.1, with an HR of 1.49 (95% CI, 1.20-1.86) and dAUC of 2.2 (P=.02) (Table 3).

Four hundred ninety-three patients (34%) were enrolled with an LMR of 2.1 or less compared with 957 patients (66%) with an LMR of more than 2.1 at diagnosis, with a 5-year PFS of 74% (95% CI, 70%-78%) and 82% (95% CI, 80%-85%), respectively (P=.001).

The OS for those with an LMR of more than 2.1 and 2.1 or less was 91% (95% CI, 89%-92%) and 88% (95% CI, 84%-91%), respectively, with an HR of 1.29 (95% CI, 0.97-1.72, P=.08).

An LMR of less than 2.1 was shown to have a higher risk in terms of PFS (HR, 1.69; 95% CI, 1.28-2.22; P=.001) and OS (HR, 1.53; 95% CI, 1.05-2.22; P=.03) for patients with histological subtype. After adjusting for IPS and NS histology, the HR was 1.50 (95% CI, 1.12-2.00;

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P=.006) for PFS, whereas for OS it was less significant with an HR of 1.27 (95% CI, 0.85-1.88; *P*=.239) (Figure 4).

DISCUSSION

Recently, the AMC and its ratio with the ALC at diagnosis (LMR) have been used as prognostic parameters to identify high-risk patients with lymphoma.[2-4,6] Although cutoff values reported in the various studies differed, similar results were obtained by different working groups, indicating that monocytosis or lymphopenia at diagnosis has an adverse impact on survival in patients with HL and NHL.

Lymphopenia is a well-recognized prognostic marker in advanced cHL, and it is included as one of the criteria of the IPS.[1] In NHL, ALCs have been evaluated already and decreased levels have been shown to be associated with worse OS.[22] In cHL, *lymphopenia* is formally defined as an ALC of 600 cells/mm^3 or less, whereas in some of the studies on lymphoma this value was defined differently and the cutoff generally used was 1000 cells/mm^3 or less lymphocytes.[4] In our study, only 6% of the entire cohort had an ALC of less than 600 cells/mm^3 and because of this, we did not relate to this parameter. The biology underlying the adverse significance of lymphopenia has not been extensively investigated; however, this finding probably relates to host characteristics and is regarded as an indicator of impaired immunity, which may contribute to tumorigenesis and subsequent tumor growth.

In the present study of a very large cohort of patients with cHL, we demonstrate that AMC (cutoff value of 750 cells/mm^3) has prognostic significance in terms of PFS and OS in patients with cHL with NS histology. Our results are in keeping with those of other recent studies, evaluating the convenient clinical application of AMC as a simple prognostic parameter in cHL.[13,21]

Here, we confirm our earlier results[2,20] obtained in a smaller patient cohort, and also verify those reported by other study groups.[7,13,21] In our view, this study has several advantages over other earlier reports. First, it was performed in a very large cohort of patients with cHL; second, results were obtained on both binational and multicenter levels, not from a single center in one country; third, we performed a comprehensive analysis to select the best cutoff for AMC in cHL, testing a wide range of all previously proposed values. Furthermore, we chose cutoff values that showed the best discriminating power for distinguishing patient outcome, after adjusting for IPS and histological subtype. In our large patient cohort, an AMC of more than 750 cells/mm^3 had a statistically significant adverse impact on outcome after both univariate (*P*<.001) and multivariate analyses (*P*=.006) in patients with NS. These results validate our earlier observations showing that this parameter can be used routinely to evaluate newly diagnosed patients with cHL

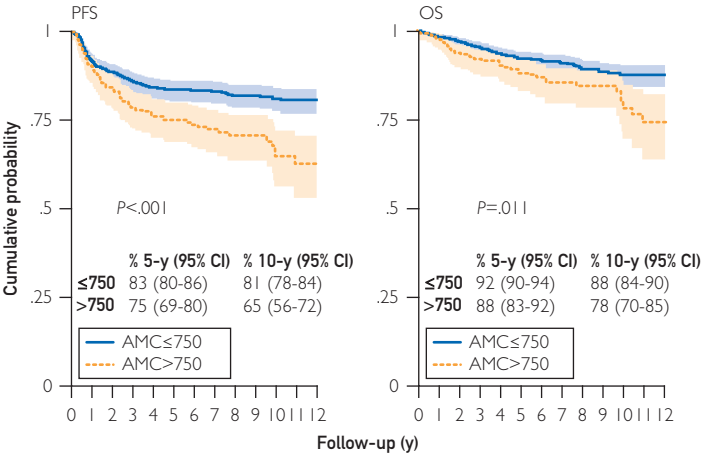

FIGURE 3. Kaplan-Meier curves for OS and PFS for the cohort of 1017 patients with nodular sclerosis Hodgkin lymphoma histology based on an AMC of ≤ or >750 cells/mm^3. AMC = absolute monocyte count; OS = overall survival; PFS = progression-free survival.

TABLE 4. Multiple Cox Regression Analysis for AMC > 750 Cells/mm^3 and Adjusted by IPS Parameters for Patients With Classical Hodgkin's Lymphoma and Nodular Sclerosis Histology for PFS and OS

	PFS, HR (95% CI)		OS, HR (95% CI)	
Factor	Univariate	*P*	Univariate	*P*
AMC >750 cells/mm^3	1.68 (1.26-2.24)	<.001	1.66 (1.12-2.45)	.01
	Multivariate		Multivariate	
AMC >750 cells/mm^3	1.54 (1.23-2.10)	.006	1.56 (1.02-2.40)	.04
Age > 45 y	1.63 (1.20-2.22)	.002	2.59 (1.76-3.81)	<.001
Sex: male	1.09 (0.83-1.44)	.52	1.01 (0.69-1.47)	.98
Stage IV disease	1.24 (0.87-1.76)	.24	1.29 (0.81-2.06)	.28
Albumin <4 g/dL	1.20 (0.88-1.63)	.26	1.38 (0.89-2.14)	.15
Hemoglobin <10.5 g/dL	1.67 (1.19-2.32)	.003	1.49 (0.95-2.34)	.08
WBC >15,000 cells/mm^3	1.39 (0.94-2.06)	.10	1.53 (0.91-2.57)	.11
ALC <600 cells/mm^3	1.20 (0.65-2.10)	.60	1.55 (0.78-3.08)	.22

ALC = absolute lymphocyte count; AMC = absolute monocyte count; HR = hazard ratio; IPS = international prognostic score; OS = overall survival; PFS = progression-free survival; WBC = white blood cell.

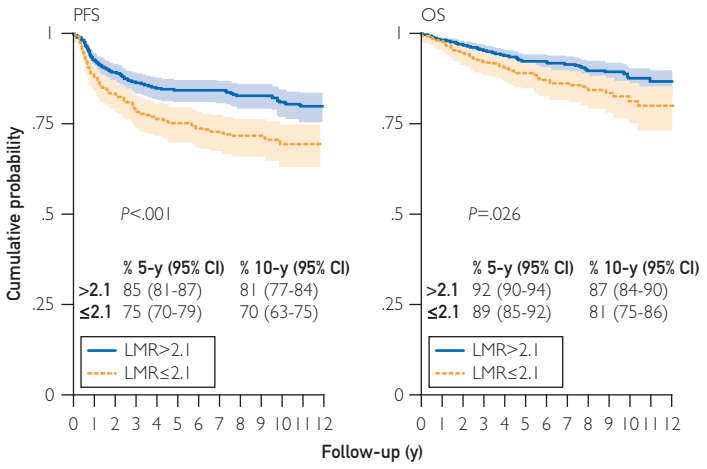

FIGURE 4. Kaplan-Meier curves for OS and PFS for the cohort of 1017 patients with nodular sclerosis Hodgkin lymphoma histology based on an LMR of ≤ or >2.1. LMR = lymphocyte to monocyte ratio; OS = overall survival; PFS = progression-free survival.

and identify higher-risk patients with a worse outcome. This AMC value can be combined meaningfully with our best cutoff for LMR (>2.1) and can also be applied to daily clinical practice. In addition, our results demonstrated that a lower LMR was associated with worst prognostic factors such as stage IV disease, a hemoglobin level of less than 10.5, an IPS of 3 to 7, a WBC count of more than 15,000 cells/mm^3, and an albumin level of less than 4 g/dL, indicating that this parameter not only correlates with host immunity (ALC) and tumor microenvironment (AMC) but also may indirectly serve as a measurement of tumor growth and tumor "mass."

In 1978, Schechter and Soehnlen[24] described the role of monocytes in the pathogenesis of cHL, but investigations of the clinical association with macrophages and monocytes have been reported only more recently.[8,11,12] Using gene expression profiling, Steidl et al[8] identified a gene signature of tumor-associated macrophages (TAMs) that is significantly associated with primary treatment failure. In this important study, now a landmark in understanding of the biology of cHL, 80% of the samples used were of NS subtype, implying that in addition to being the most frequent histological subtype encountered it may have the best association with macrophage/monocyte biology.[12] Others have also shown that the presence of TAMs within the tumor itself predicted an inferior outcome in cHL.[11,25,26] In our study, we also identified that AMC had the greatest significance in terms of PFS and OS in this subgroup of patients with cHL with NS histopathology. In this respect, monocytes and their total number, as reflected by AMC in the peripheral blood count, may indeed be regarded as a surrogate biomarker of TAMs within the tumor microenvironment.

Epstein-Barr virus is also known to play a role in the development of HL, and Kamper et al[9] demonstrated that TAMs correlate with not only adverse prognosis but also Epstein-Barr virus status in patients with cHL. This observation lends further support for the suggestion that the microenvironment plays an important role in lymphomagenesis and prognosis of lymphoma.

The most sensitive imaging modality to define the site of involvement in HL and to assess response to therapy is positron emission tomography–computed tomography (PET-CT) scan. This imaging modality has become the criterion standard guide in the management of cHL, and is usually performed at diagnosis (for baseline information on sites of disease and initial tumor volume), and after 2 cycles as an interim analysis and a guide for continuing or changing therapy. Very recently it has been reported to have predictive value, even if performed after 1 cycle of chemotherapy,[27,28] and methods for assessing and measuring total tumor volume are currently improving significantly.[29] It seems most appropriate that combined analysis of the total PET-CT uptake and quantitation of the AMC in patients with cHL will serve as a combination of valuable surrogate markers for detecting disease activity. It is of interest to note that Touati et al[10] have recently reported pioneering studies demonstrating that increases in CD68-positive TAMs predict an unfavorable outcome in cHL, when correlated with interim fluorodeoxyglucose-positron emission tomography (PET) assessment. In this respect, analysis regarding the role of AMC in combination with PET-CT data has already been reported to be considerable in NHL.[30]

Several markers other than AMC, ALC, and LMR have been examined and reported to have prognostic significance in cHL.[31] These include the expression of COX-2 on Reed-Sternberg cells,[31] insulin-like growth factor 1 receptor expression,[32] and levels of IL-10[33] and/or other cytokines.[34] The main limitation of recording these important markers

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routinely relates to the fact that results are not readily reproducible in all laboratories and appear to be very variable, particularly for levels of relevant cytokines. In contrast to this, one of the obvious advantages favoring the routine use of AMC is its simplicity and that it can be calculated easily and routinely in all laboratories.

Our study has some obvious limitations, including the fact that it is retrospective and that patients were not treated uniformly, but with different chemotherapy combinations. However, all regimens used appeared to induce similar overall response rates, and as a result we are of the opinion that this variable has a negligible influence on our results.

CONCLUSION

In this study of cHL we confirm that AMC at diagnosis has prognostic value and can identify about 25% of the cases with adverse outcome. It has a major impact on survival, particularly in patients with NS histology. Its significance is also maintained when examined in correlation with IPS, and appears to be independent of the clinical stage of the disease.

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Abbreviations and Acronyms: **ALC** = absolute lymphocyte count; **AMC** = absolute monocyte count; **cHL** = classical Hodgkin lymphoma; **dAUC** = delta (difference) of AUC between ROC curve analysis at 5-year follow-up with IPS and IPS + AMC or LMR; **HR** = hazard ratio; **IPS** = international prognostic score; **LMR** = lymphocyte- monocyte ratio; **NHL** = non-Hodgkin lymphoma; **NS** = nodular sclerosis; **OS** = overall survival; **PET-CT** = positron emission tomography—computed tomography; **PFS** = progression-free survival; **ROC** = receiver operating characteristic; **TAM** = tumor-associated macrophage; **WBC** = white blood cell

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