

Prognostic Significance of the Ratio of Absolute Neutrophil Count to Absolute Lymphocyte Count in Classic Hodgkin Lymphoma

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Upon completion of this activity you will be able to:

- predict prognosis in patients with classic Hodgkin lymphoma using the ratio of absolute neutrophil count to absolute lymphocyte count (ANC/ALC).
- differentiate a high-risk group among patients who are considered to be at low risk on the basis of an International Prognostic Score <4 using the ANC/ALC ratio.
- recognize the effect of the ANC/ALC ratio in combination with the ALC to absolute monocyte count ratio for survival prediction of classic Hodgkin lymphoma.

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Abstract

The aim of this study was to determine the prognostic effect of the absolute neutrophil count/absolute lymphocyte count ratio (ANC/ALC ratio) in patients with classic Hodgkin lymphoma (cHL). We performed a retrospective analysis of 312 patients with cHL. Univariate analysis revealed that a high ANC/ALC ratio (≥ 4.3) correlated with poor overall survival (OS) ($P < .001$). Subgroup analysis of advanced-stage disease showed that the ANC/ALC ratio was significant for OS ($P = .032$). Multivariate analysis revealed the ANC/ALC ratio to be an independent prognostic factor for OS ($P = .048$). The ANC/ALC ratio allowed further risk stratification in patients who were considered to be at low risk on the basis of an International Prognostic Score less than 4 ($P = .002$). The ANC/ALC ratio is a simple, inexpensive, and independent prognostic factor for OS that may improve the ability to identify high-risk patients with cHL.

The current gold standard for risk stratification in Hodgkin lymphoma (HL) is the International Prognostic Score (IPS).¹ Although most cases of HL are indolent and can be cured, 34% to 37% of advanced-stage HLs recur or progress after first-line therapy.² On the other hand, approximately 20% of patients receive unnecessary treatment and thus are exposed to the risk of long-term complications.³ To address this issue and better stratify the risk, tests for various prognostic factors have been suggested, including gene expression profiling,^{4,5} immunohistochemical analysis of biomarkers,^{6,7} and positron emission tomography.^{8,9} However, these approaches are expensive, technically demanding, and not easily accessible. Therefore, prognostic models for classic HL (cHL) that are inexpensive, simple, and easy to perform and interpret are needed.

The histologic hallmark of cHL is the very small minority of Reed-Sternberg cells (usually <2%) in a background of bystander cells (usually >98%). This background, known as the tumor microenvironment, was recently shown to have not only pathogenetic but also prognostic significance.^{5,7,10,11} Tumor-infiltrating lymphocytes and tumor-infiltrating macrophages are reported to be prognostic factors for survival in patients with cHL.^{5,7,10,11} Because tissue inflammatory cells originate from the blood and are controlled by common cytokines, the peripheral blood (PB) count of these cells in patients with cHL may reflect the cytokine and immunologic profile of the tumor in these patients. It has long been known that the baseline lymphocyte count has a prognostic role in cHL—lymphopenia (<600 cells/ μ L or <8% of the WBC count) is related to adverse survival outcome.¹ Furthermore,

recent studies, including our previous report, reveal that the PB lymphocyte/monocyte ratio at diagnosis also has prognostic significance in cHL.^{12,13} In addition, PB neutrophil counts have been associated with survival in solid tumors.^{14,15} A recent study of the PB neutrophil/lymphocyte ratio in patients with diffuse large B-cell lymphoma showed that patients with a neutrophil/lymphocyte ratio less than 3.5 at diagnosis had a superior overall survival (OS) and progression-free survival.¹⁶ It has been suggested that a high number of neutrophils may actually promote tumor growth and metastasis¹⁷ and/or inhibit lymphocyte activity, thereby counteracting the antitumor immune response.¹⁸⁻²¹ These observations suggest that an imbalance in the ratio of neutrophil to lymphocyte in the PB of patients with cancer may be related to tumor development.

The prognostic significance of biomarkers that combine PB neutrophil and lymphocyte numbers in patients with cHL has not yet been studied. Thus, we investigated whether the ratio of absolute neutrophil count (ANC) to absolute lymphocyte count (ANC/ALC ratio) in PB at diagnosis is a predictor of survival in patients with cHL. Receiver operating characteristic (ROC) curve analysis was used to determine the optimal cutoff values. We then correlated the findings of this analysis with our previous data of the PB lymphocyte/monocyte ratio at diagnosis in the same cohort of patients with cHL.¹³

Materials and Methods

Patients

We carried out a retrospective study of 312 patients with cHL, including 254 patients from Asan Medical Center, Seoul, South Korea (1990-2011) and 58 patients from Seoul National University Boramae Medical Center (1996-2010), who met the following criteria: biopsy-proven cHL; no previous treatment or history of malignancy, transplantation, immunosuppression, or anti-HIV positivity. All patients received combination chemotherapy with or without radiation treatment.

The ANC, ALC, and absolute monocyte count (AMC) were obtained from the routine CBC with 4-part differential counts (lymphocytes, monocytes, eosinophils, and neutrophils) using the Sysmex automated hematology analyzers (models Sysmex E-4000, Sysmex SE-9000, and Sysmex XE-2100; Sysmex, Kobe, Japan) at the time of the diagnosis of cHL.²²

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.

In situ hybridization analysis for Epstein-Barr virus-encoded RNA-1 and RNA-2 was performed and scored as described elsewhere.²³

Statistical Analysis

OS was defined as the time between the first day of diagnosis and the date of death from any cause or the last follow-up. Event-free survival (EFS) was defined as the interval between the first day of diagnosis and the date of disease progression, relapse, death from any cause, or last follow-up. OS and EFS rates were estimated by using the Kaplan-Meier method and compared by using the log-rank test. Multivariate prognostic analyses were performed for OS and EFS by using the Cox proportional hazards regression model. The backward (conditional) method was used to determine the final Cox model for multivariate analysis. The optimal cutoff point was decided by using ROC curve analysis—the value with the maximum joint sensitivity and specificity was selected. The binary clinical outcome (death/survival) was determined at 5 years after diagnosis. Patients were categorized as “alive/censored” when the follow-up time was longer than 5 years and “dead” if the patient had died before this time point.²⁴ Categorical variables were compared by using χ^2 tests (2-sided Pearson or linear-by-linear association). Continuous variables were compared with the Mann-Whitney *U* Test. All statistical analyses were performed using the SPSS version 18.0 statistical software program (SPSS, Chicago, IL). The results were considered to be statistically significant when the *P* value was less than .05.

Results

Patient Characteristics

Patient age ranged from 4 to 77 years (median, 37 years). There were 188 (60.3%) male and 124 (39.7%) female patients. Nodular sclerosis was the most common subtype, noted in 177 (56.7%), followed by 90 (28.8%) cases of the mixed cellularity subtype, 12 (3.8%) cases of the lymphocyte-rich subtype, 9 (2.9%) cases of the lymphocyte-depleted subtype, and 24 (7.7%) cases that were “not otherwise specified.” Based on Ann Arbor staging, 42 patients were stage I, 112 were stage II, 73 were stage III, and 85 were stage IV. A minority of patients (36.2%) presented with B symptoms or high-risk IPS (19.2%). Increased levels of lactate dehydrogenase (LDH) were found in 177 (56.7%) patients. A majority of patients (210/312; 67.3%) received chemotherapy only and 102 (32.7%) patients were treated with chemoradiotherapy. Treatment regimens included doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) for 189 patients; mechlorethamine, vincristine, procarbazine, and prednisone (MOPP) for 13; ABVD/MOPP hybrid for 21; bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisolone for 10; and other forms of chemotherapy for 21 patients. The median follow-up after diagnosis was 64 months for the entire cohort (range, 0.1-245 mo) and 71 months for censored patients (range, 2-245 mo). We recorded relapse, disease progression,

or death in 100 patients. Median EFS was 36 months (95% confidence interval [CI], 28-40 mo). An estimated 5-year OS and EFS of 86.4% and 64.2%, respectively, was observed. At diagnosis, the median ANC was 5,569 cells/ μ L ($5.57 \times 10^9/L$; range, 336-30,056 cells/ μ L [0.34 - $30.06 \times 10^9/L$]), the median ALC was 1,561 cells/ μ L ($1.56 \times 10^9/L$; range, 56-7,276 cells/ μ L [0.06 - $7.28 \times 10^9/L$]), and the median ANC/ALC ratio was 3.8 (range, 0.09-29.67).

Determining the Cutoff Value of the ANC/ALC Ratio

An ROC curve of the ANC/ALC ratio according to survival was generated to determine the cutoff value. The area under the curve was recorded as 0.63 (95% CI, 0.536-0.724)

Figure 1. An ANC/ALC value of 4.3 corresponded to the maximum combined sensitivity and specificity on the ROC curve (66% sensitivity and 59% specificity).

Correlation of ANC/ALC Ratio With Clinicopathologic Variables

To evaluate the relevance of the ANC/ALC ratio of 4.3 or higher at diagnosis in patients with cHL, the patients were divided according to the ANC/ALC ratio at diagnosis: the group with an ANC/ALC ratio less than 4.3 contained 177 patients (56.7%), and the group with an ANC/ALC ratio of 4.3 or more contained 135 patients (43.3%). Patients with an ANC/ALC ratio of 4.3 or higher at diagnosis were more likely to present with presence of B symptoms ($P < .001$), advanced disease stage ($P < .001$), abnormal LDH ($P = .005$), lymphopenia ($P = .014$), hypoalbuminemia ($P = .01$), and anemia ($P < .001$). The groups did not differ in terms of age ($P = .337$),

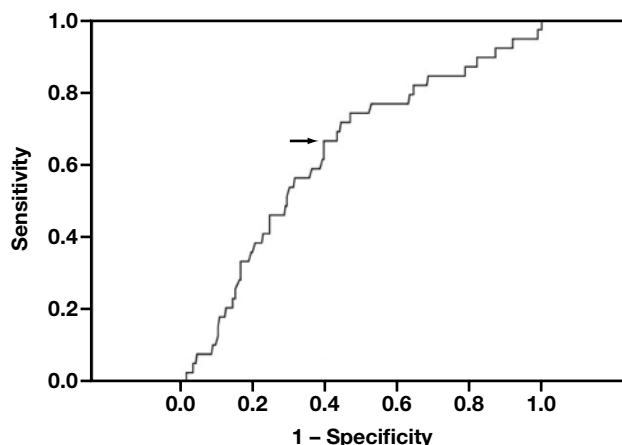


Figure 1 Receiver operating characteristic and area under the curve (AUC) analysis of the ratio of absolute neutrophil count to absolute lymphocyte count at the diagnosis of classic Hodgkin lymphoma. (AUC = 0.63 [arrow]; 95% confidence interval = 0.536-0.724.)

sex ($P = .641$), treatment method (chemotherapy vs chemoradiotherapy, $P = .543$), chemotherapeutic regimen (ABVD vs non-ABVD, $P = .876$), or Epstein-Barr virus status (positive vs negative, $P = .131$).

Prognostic Significance of the ANC/ALC Ratio

Patients with an ANC/ALC ratio of 4.3 or more had a lower OS than those with an ANC/ALC ratio less than 4.3 (5-year OS, 80.3% vs 91.9%; $P < .001$) **Figure 2A** but did not differ significantly in terms of EFS ($P = .168$) **Figure 2B**.

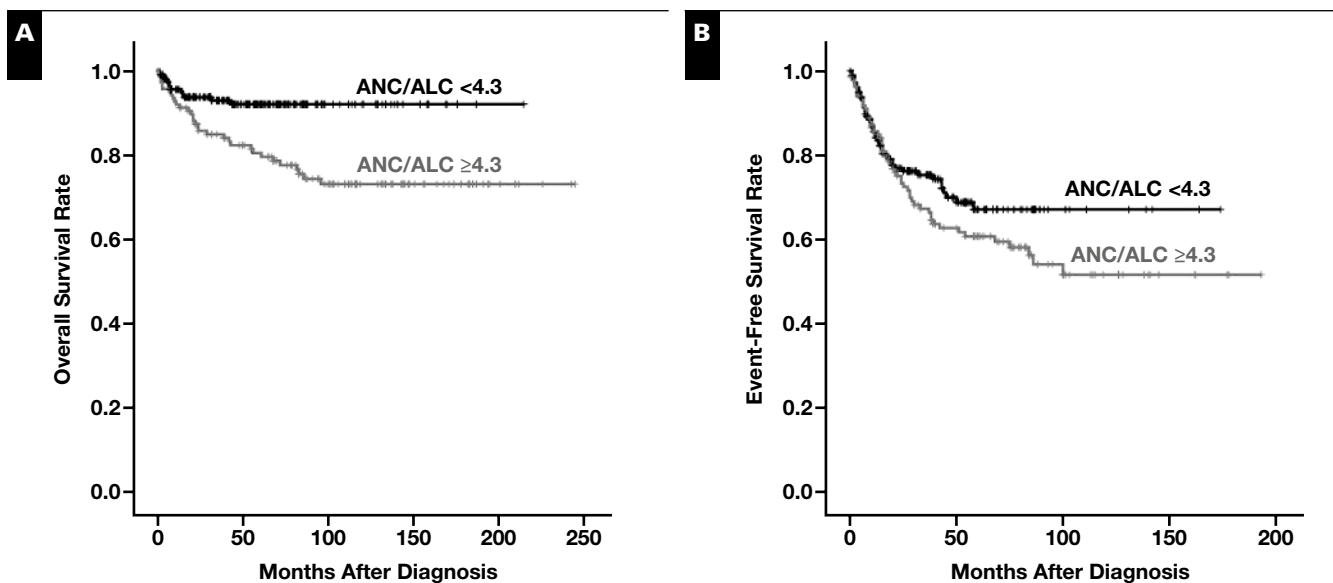


Figure 2 Overall survival (A) and event-free survival (B) rates, according to the ratio of absolute neutrophil count to absolute lymphocyte count (ANC/ALC) at diagnosis. (A, $P < .001$; B, $P = .168$).

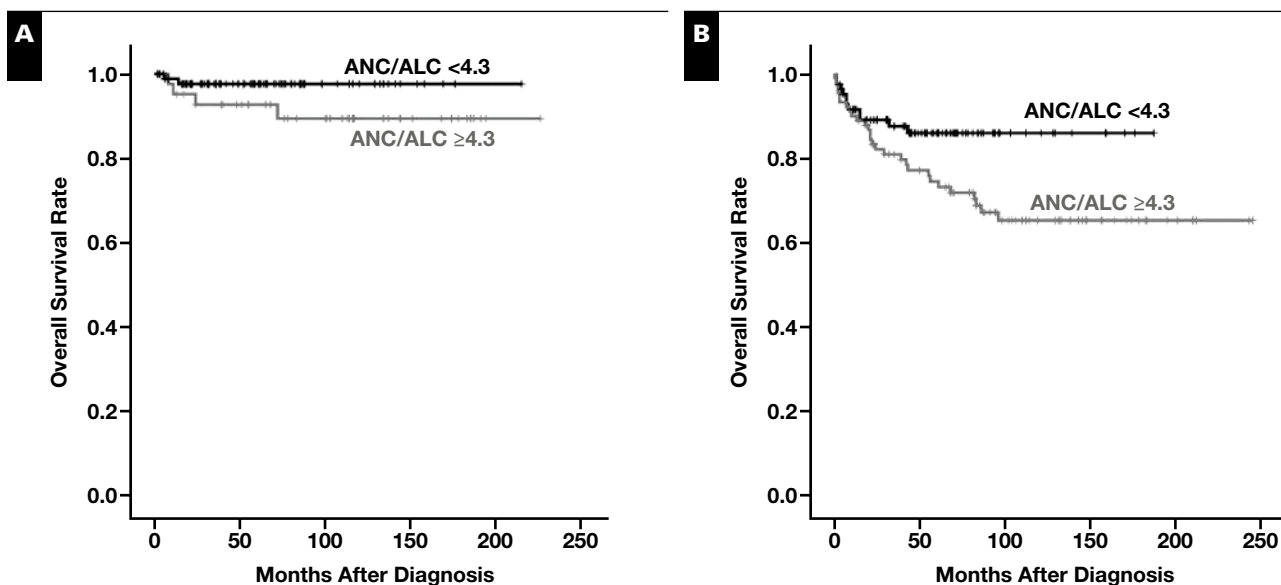


Figure 3 Overall survival in the subgroups with limited-stage (**A**) and advanced-stage (**B**) disease based on the ratio of absolute neutrophil count to absolute lymphocyte count (ANC/ALC). (**A**, $P = .108$; **B**, $P = .032$).

Subgroups were then analyzed based on stage (limited vs advanced) because some prognostic markers are restricted to certain stages. In the advanced-stage subgroup, patients with an ANC/ALC ratio of 4.3 or more had a lower OS rate than those with an ANC/ALC ratio less than 4.3 ($P = .032$) **Figure 3B**. In the limited-stage subgroup, patients with an ANC/ALC ratio of 4.3 or more and patients with an

ANC/ALC ratio less than 4.3 showed similar OS ($P = .108$)

Figure 3A.

Univariate analysis revealed that OS correlated significantly with older age (>45 years), B symptoms, abnormal LDH level, hypoalbuminemia, anemia, male sex, higher stage, high IPS (≥ 4), and ALC/AMC ratio less than 2.9 **Table 1**. Multivariate analysis adjusting for B symptoms, abnormal

Table 1
Univariate and Multivariate Analysis for OS and EFS

| Covariate | OS | | | EFS | | | |
|--|---|--------|-----------------|-------|--------|-----------------|-------|
| | HR | 95% CI | <i>P</i> Value* | HR | 95% CI | <i>P</i> Value* | |
| Univariate analysis | | | | | | | |
| Age, y | <45 vs ≥ 45 | 5.669 | 3.02-10.60 | <.001 | 2.564 | 1.72-3.80 | <.001 |
| B symptoms | (-) vs (+) | 3.145 | 1.70-5.79 | <.001 | 1.331 | 0.89-1.97 | .156 |
| LDH, U/L ($\mu\text{kat/L}$) | <250 (4.1) vs ≥ 250 | 2.863 | 1.375-5.962 | .005 | 1.571 | 1.034-2.388 | .034 |
| Albumin, g/dL (g/L) | ≥ 4 (40) vs <4 | 2.611 | 1.25-5.42 | .010 | 1.517 | 0.99-2.31 | .054 |
| Hemoglobin, g/dL (g/L) | ≥ 10.5 (105) vs <10.5 | 2.754 | 1.52-4.97 | .001 | 1.658 | 1.07-2.55 | .022 |
| Sex | F vs M | 2.180 | 1.10-4.29 | .024 | 1.540 | 1.00-2.35 | .046 |
| ALC count, cells/ μL ($\times 10^9/\text{L}$) | ≥ 600 (0.6) or $\geq 8\%$ of WBC vs <600 or <8% of WBC | 1.919 | 0.855-4.307 | .114 | 1.293 | 0.690-2.424 | .422 |
| WBC, $\times 10^3$ cells/ μL ($\times 10^9/\text{L}$) | ≤ 15 (15) vs >15 | 1.203 | 0.53-2.70 | .654 | 1.338 | 0.77-2.31 | .300 |
| Stage | 1-3 vs 4 | 3.096 | 1.72-5.55 | <.001 | 1.545 | 1.02-2.33 | .040 |
| IPS | <4 vs ≥ 4 | 2.710 | 1.48-4.95 | .001 | 1.406 | 0.88-2.22 | .146 |
| Treatment plan | Chemotherapy only vs chemoradiotherapy | 0.581 | 0.294-1.148 | .118 | 0.618 | 0.396-0.962 | .033 |
| Chemotherapeutic regimen | non-ABVD vs ABVD | 0.659 | 0.320-1.359 | .259 | 1.183 | 0.666-2.100 | .567 |
| ALC/AMC ratio | ≥ 2.9 vs <2.9 | 3.175 | 1.57-6.41 | .001 | 1.226 | 0.82-1.82 | .316 |
| ANC/ALC ratio | <4.3 vs ≥ 4.3 | 2.841 | 1.486-5.428 | .002 | 1.317 | 0.887-1.954 | .172 |
| Multivariate analysis | | | | | | | |
| ANC/ALC ratio | <4.3 vs ≥ 4.3 | 1.959 | 1.007-3.811 | .048 | 1.126 | 0.749-1.694 | .568 |
| B symptoms | (-) vs (+) | 2.345 | 1.200-4.582 | .013 | 1.204 | 0.785-1.848 | .394 |
| LDH, U/L ($\mu\text{kat/L}$) | <250 (4.1) vs ≥ 250 | 2.027 | 0.953-4.312 | .066 | 1.406 | 0.911-2.170 | .124 |
| IPS | <4 vs ≥ 4 | 1.532 | 0.796-2.947 | .201 | 1.139 | 0.690-1.882 | .610 |

ABVD, doxorubicin, bleomycin, vinblastine, and dacarbazine; ALC, absolute lymphocyte count; AMC, absolute monocyte count; ANC, absolute neutrophil count; CI, confidence interval; EFS, event-free survival; HR, hazard ratio; IPS, International Prognostic Score; LDH, lactate dehydrogenase; OS, overall survival; WBC, white blood cells.

* Cox univariate analysis.

level of LDH, high IPS (≥ 4), and ALC/AMC ratio less than 2.9 showed that an ANC/ALC ratio of 4.3 or more was an independent prognostic marker ($P = .048$).

Because both the ANC/ALC ratio and IPS remained independent prognostic markers, we next determined whether they yielded any additional prognostic value when combined. To evaluate the prognostic effect of IPS, the patients were divided into 2 groups: low-risk with IPS less than 4 and high-risk with IPS of 4 or more. IPS was previously shown to be a significant prognostic factor in advanced-stage disease only.¹

Similarly, in this study, the prognostic effect of IPS was limited to advanced-stage disease (Figure 4). For low-risk IPS cases, the ANC/ALC ratio enabled further stratification of the risks in these patients: the patients with an ANC/ALC ratio of 4.3 or more were found to have worse outcomes than those with an ANC/ALC ratio less than 4.3 (5-year OS of 83.1% vs 94%, $P = .002$) (Figure 5A). However, in high-risk IPS patients, those with an ANC/ALC ratio of 4.3 or more and those with an ANC/ALC ratio less than 4.3 showed similar OS rates ($P = .453$) (Figure 5B). Our previous study revealed

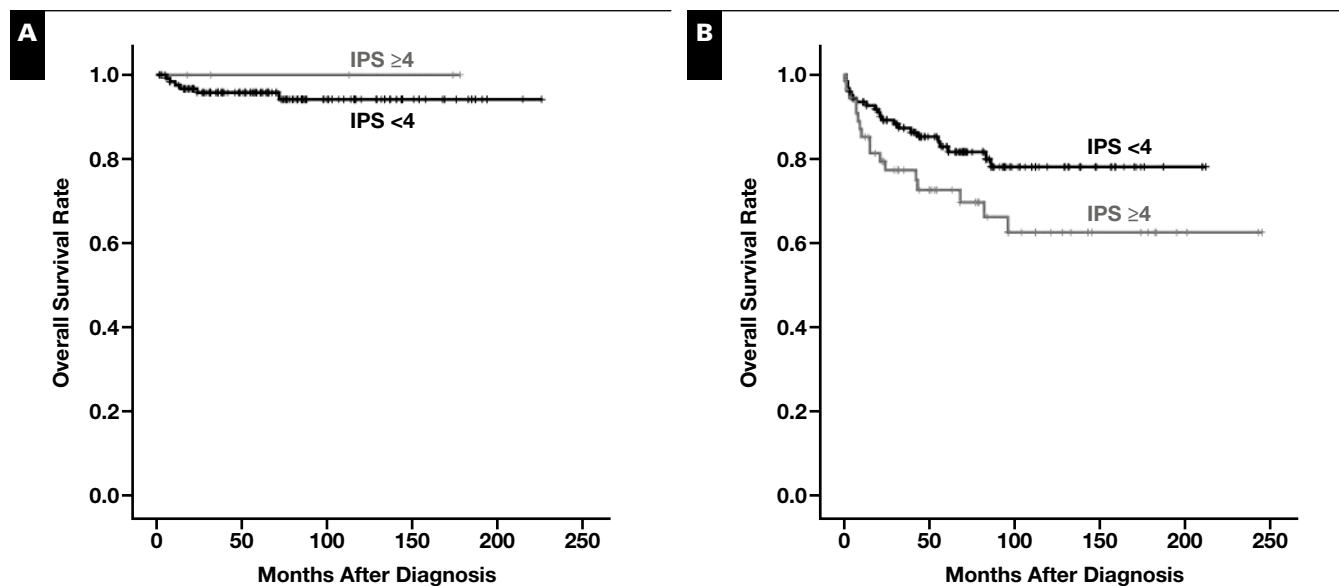


Figure 4 Overall survival in the limited stage (A) and advanced stage (B) subgroups according to the International Prognostic Score (IPS). (A, $P = .612$; B, $P = .048$).

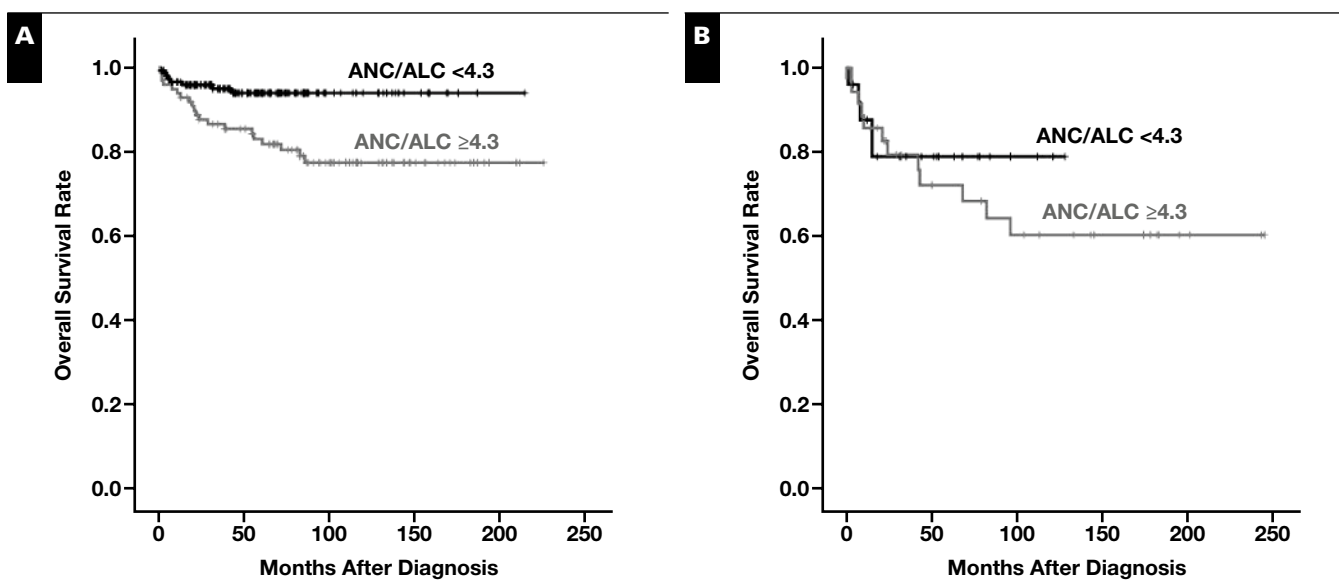


Figure 5 Patients identified by the International Prognostic Score as being either low risk (A) or high risk (B) were further stratified by the ratio of absolute neutrophil count to absolute lymphocyte count (ANC/ALC) prognostic score. (A, $P = .002$; B, $P = .453$).

that an ALC/AMC ratio less than 2.9 at diagnosis was significantly associated with poor OS in cHL.¹³ To evaluate the synergistic usefulness of ANC/ALC and ALC/AMC ratios, we combined the dichotomized ANC/ALC and ALC/AMC ratio and stratified patients into 4 groups (ANC/ALC ≥ 4.3 and ALC/AMC < 2.9 , ANC/ALC ≥ 4.3 and ALC/AMC ≥ 2.9 , ANC/ALC < 4.3 and ALC/AMC < 2.9 , ANC/ALC < 4.3 and ALC/AMC ≥ 2.9). Patients with an ANC/ALC ratio of 4.3 or more and ALC/AMC ratio less than 2.9 had a significantly

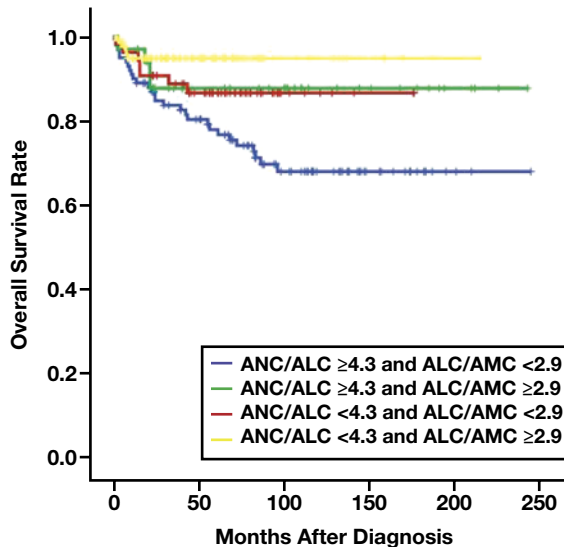


Figure 6 Comparison of the overall survival according to the patterns seen in the ratio of absolute neutrophil count to absolute lymphocyte count (ANC/ALC) and ratio of ALC to absolute monocyte count (AMC) ($P < .001$).

worse OS rate compared with the remaining groups, while patients with an ANC/ALC ratio less than 4.3 and ALC/AMC ratio of 2.9 or more had a significantly better OS rate than the remaining groups ($P < .001$) **Figure 6**.

When the nodular sclerosis subtype alone was analyzed, patients with an ANC/AMC ratio of 4.3 or more had a lower OS rate than patients with an ANC/AMC ratio less than 4.3 (5-year OS rate, 85.6% vs 96.9%, $P = .008$) **Figure 7A**. The 2 subgroups did not differ in terms of the EFS rate ($P = .362$) **Figure 7B**.

Discussion

This study has several novel and important aspects. First, it is the first study to evaluate the ANC/ALC ratio as a prognostic factor for survival in patients with cHL. Second, the ANC/ALC ratio provides additional prognostic information when superimposed on the IPS, enabling further stratification in patients with low-risk IPS. Third, the ANC/ALC ratio could predict survival for patients with the nodular sclerosis subtype of cHL. Fourth, the ANC/ALC and the ALC/AMC ratios are synergistic in the prediction of survival in cHL; by combining these 2 ratios, the authors were able to divide patients with cHL into groups with significantly different survival rates. Obtaining the ANC/ALC ratio prognostic score from a CBC at diagnosis is simple, widely available, and can be easily used in clinical practice, especially in resource-poor areas. The limitations of this study include the retrospective nature of the study design, the short follow-up period, and the relatively small sample size of the test participants.

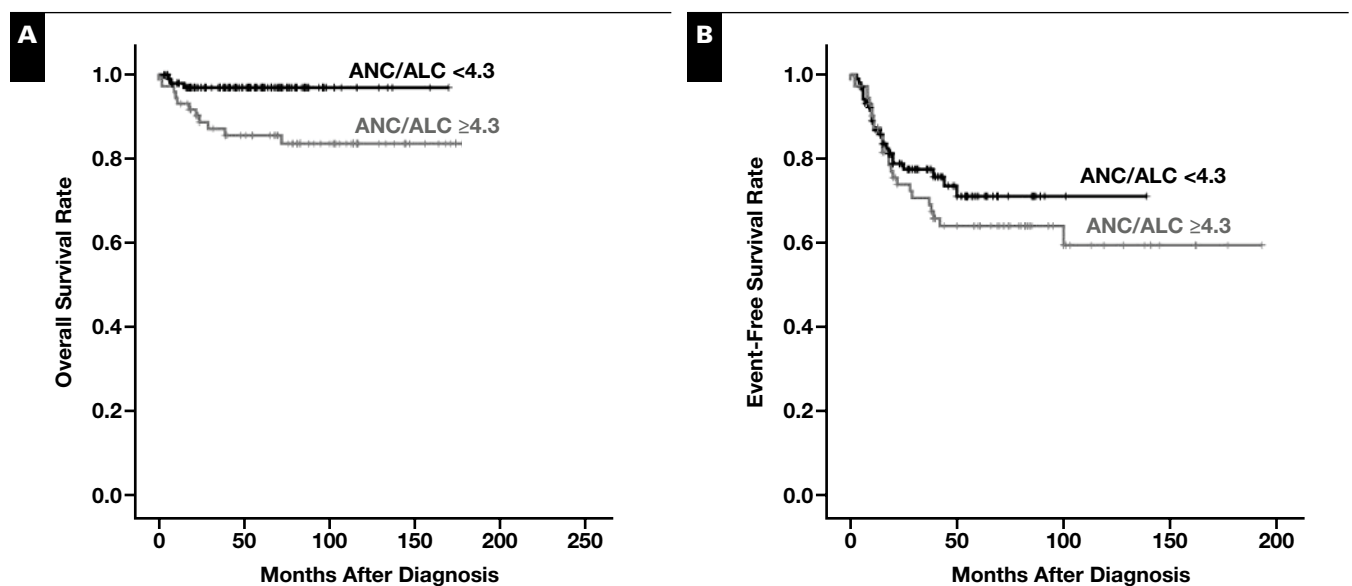


Figure 7 Overall survival (**A**) and event-free survival (**B**) according to the ratio of absolute neutrophil count to absolute lymphocyte count (ANC/ALC) in patients with the nodular sclerosis subtype ($n = 144$). (**A**, $P = .008$; **B**, $P = .362$.)

Many cytokines that may contribute to the accumulation of neutrophils, such as granulocyte macrophage–colony stimulating factor, transforming growth factor β , and interleukin (IL)-8, are expressed by Reed-Sternberg cell lines and primary cHL tissues.²⁵ In particular, IL-8 is a cytokine that strongly attracts and activates neutrophils.^{26,27} Previous studies indicate that IL-8 may play a role in the pathogenesis of HL. First, increased IL-8 levels have been detected in the serum samples of many patients with HL.²⁸⁻³⁰ Second, in situ hybridization detected IL-8 messenger RNA in 20 of 33 cHL cases, and its expression level correlated positively with the density of neutrophils in the tissue.³¹ IL-8 is also predominantly expressed by the reactive infiltrate cells; it was expressed by Reed-Sternberg cells in 3 cases only.³¹

Neutrophils are among the first inflammatory cells to respond to infectious agents. They are the most abundant type of WBCs, and an increase in neutrophil numbers is the most common cause of leukocytosis. In the present study, a high ANC/ALC ratio was associated with leukocytosis. However, leukocytosis per se did not correlate with survival outcome.

Previous research found that lymphopenia was related to adverse prognosis.¹ However, lymphopenia is a rather infrequent finding in patients with cHL and was seen in only 11% of patients in the original study¹ and in 8% of patients in the present study, which limits its usefulness. Moreover, in multivariate analysis that included lymphopenia criteria, the ANC/ALC ratio remained an independent prognostic factor along with IPS. These results suggest that the ANC/ALC ratio could provide additional prognostic information.

In the present study, a high ANC/ALC ratio was not significantly associated with EFS. The reason for this is largely unknown. A possible explanation could be that patients with an ANC/ALC ratio of 4.3 or more are more difficult to salvage after treatment failure or relapse: 13 (27.7%) of 47 patients with an ANC/ALC ratio less than 4.3 died after treatment failure or relapse, compared with 32 (60.4%) of 53 patients with an ANC/ALC ratio ≥ 4.3 . This indicates that cases with an ANC/ALC ratio of 4.3 or more may be more resistant to salvage treatment.

Myeloid lineage cells with immunosuppressive properties, recently categorized as myeloid-derived suppressor cells (MDSCs), were previously correlated with cellular immunosuppression in a number of disease states.³² In most cases, MDSCs can be subdivided into 2 phenotypic populations with either monocytic or neutrophilic characteristics.³³ The ability of normal neutrophils to suppress T-cell function was reported previously²¹ and was linked to immunosuppression in non–small cell lung, pancreatic, colon, renal, and breast cancer.³⁴⁻³⁶ Activated neutrophils have enhanced activity of signal transducer and activator of transcription 3 and nicotinamide adenine dinucleotide phosphate, resulting

in high levels of reactive oxygen species but low nitric oxide production. Finally, activated neutrophils have increased levels of arginase 1, which result in T-cell suppression.³² In addition to this immune suppression, neutrophils may have additional tumor-promoting ability. Increased neutrophil counts have been observed in patients with gastric, hepatic, cervical, and colorectal cancer.³⁷ Moreover, recruited neutrophils, along with tumor-associated macrophages, can induce the expression of matrix metalloproteinase 9 in various murine tumor models.³⁸ CXCL1/MIP-2, an angiogenic chemokine, is associated with neutrophil recruitment and induces vascular endothelial growth factor production in neutrophils, resulting in angiogenesis in vivo.³⁹ Finally, infiltration with large numbers of peritumoral neutrophils is associated with progression of angiogenesis at the edge of hepatocellular carcinoma.⁴⁰ These observations support the notion that neutrophils may participate in driving cancer invasion, angiogenesis, and metastasis.

Many investigators, including the present authors, validated the adverse prognostic effect of high tissue-associated macrophage contents using immunohistochemical staining for CD68 and/or CD163 in cHL.⁵⁻⁷ Furthermore, our previous study demonstrated that the ALC/AMC ratio correlated with tissue-associated macrophage contents and survival outcome. In this study, the ANC/ALC and ALC/AMC ratios showed a significant synergistic effect on the prediction of survival in cHL patients; patients with a high ANC/ALC ratio and a low ALC/AMC ratio had the worst survival, whereas patients with a low ANC/ALC ratio and a high ALC/AMC ratio had the best survival. The reason for this is still poorly understood but may involve the complex feedback loops of the MDSCs.

In conclusion, the present results suggest that the ANC/ALC ratio can be used to determine prognosis in cHL. This supports the possibility that host immunity (ie, lymphocytes) and inflammatory responses (ie, neutrophils) have prognostic relevance for clinical outcomes in cHL. Moreover, the ANC/ALC ratio may provide additional prognostic information independently from IPS and has a synergistic effect with the ALC/AMC ratio for survival prediction of cHL. Further prospective clinical trials are required to investigate the effect of the ANC/ALC ratio on clinical outcomes and to confirm the present findings.

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