Peripheral blood absolute lymphocyte/monocyte ratio recovery during ABVD treatment cycles predicts clinical outcomes in classical Hodgkin lymphoma

LF Porrata1, KM Ristow1, TM Habermann1, WR Macon2, TE Witzig1, JP Colgan1, DJ Inwards1, SM Ansell1, IN Micaleff1, PB Johnston1, G Nowakowski1, CA Thompson1 and SN Markovic1

The peripheral blood absolute lymphocyte/monocyte count ratio at diagnosis (ALC/AMC-DX) predicts survival in classical Hodgkin lymphoma (cHL). However, a limitation of the ALC/AMC-DX is the inability to assess sequentially the host/tumor interaction during treatment. Therefore, we retrospectively examined the ALC/AMC ratio, as a surrogate marker of host immunity (ALC) and tumor microenvironment (AMC), at each treatment cycle as a predictor for clinical outcomes during treatment cycles is a predictor for survival and provides a platform to develop therapeutic modalities to manipulate the ALC/AMC ratio during chemotherapy to improve clinical outcomes in cHL.

Keywords: classical Hodgkin lymphoma; survival ALC/AMC ratio; ABVD chemotherapy
int-PET-scan and the ALC/AMC ratio recovery during each cycle phase of ABVD chemotherapy.

Response and survival

Definitions of response criteria, OS and PFS were based on the guidelines from the International Harmonization Project Lymphoma. OS was defined as the time from cHL diagnosis to death as a result of any cause or last follow-up. PFS was defined as the time from cHL diagnosis to the time of progression, relapse from complete response, death as a result of any cause or last follow-up. Patients without an event or death were censored at the time of last known follow-up.

Statistical analysis

OS and PFS were analyzed using the approach of Kaplan and Meier. 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 OS and PFS. Chi-squared tests were used to determine relationships between categorical variables. The Wilcoxon’s rank-sum 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 < 0.5 are considered statistically significant.

RESULTS

Patients’ characteristics

The median age at diagnosis for this cohort of 190 cHL patients was 36 years (range: 18–83 years). The distribution of additional baseline characteristics for these patients is presented in Table 1. The median follow-up for the cohort was 3.7 years (range: 0.2–20 years) and for the living patients ($N = 163$) it was 4.6 years (range: 0.5–22 years). Thirty-four patients died of causes not related to lymphoma or the treatment of lymphoma, and 14 patients died secondary to relapse/progression of cHL. Forty-one percent (77/190) of patients, and their ALC/AMC ratio at diagnosis was secondary to relapse/progression of cHL. Forty-one percent ($45/109$) of patients, and their ALC/AMC ratio at diagnosis was recovery at each treatment cycle and survival Kaplan–Meier analysis was used to study OS and PFS based on ALC/AMC ratio at each treatment cycle phase of ABVD. Patients with an ALC/AMC ratio $\geq 1.1$ experienced superior OS compared with patients with an ALC/AMC ratio < 1.1 at each treatment cycle phase of ABVD (Figure 1 and see Supplementary File). Similarly, patients with an ALC/AMC ratio $\geq 1.1$ at each treatment cycle experienced superior PFS (see Supplementary Figure 1).

Number of cycles with an ALC/AMC ratio $\geq 1.1$ and survival

To further understand the significance of the ALC/AMC ratio $\geq 1.1$ recovery during each treatment cycle phase, we categorized patients according to how many cycles the ALC/AMC ratio $\geq 1.1$ was observed. We observed a progressive worsening of OS, lymphoma-specific survival, PFS and time to progression the more treatment cycles patients did not achieve an ALC/AMC ratio $\geq 1.1$ (Figure 2). Specifically, patients with an ALC/AMC ratio < 1.1 during all treatment cycle phases experienced the worst OS and PFS.

ALC/AMC ratio $\geq 1.1$ during any treatment cycle versus ALC/AMC ratio < 1.1 during all treatment cycles

From the Kaplan–Meier curves in Figure 2, patients with an ALC/AMC ratio < 1.1 in all treatment cycles separated from patients with an ALC/AMC ratio $\geq 1.1$ in any treatment cycle with regard to OS and PFS. Thus, patients were divided into two groups: ALC/AMC ratio $\geq 1.1$ in any treatment cycle versus ALC/AMC ratio < 1.1 in all treatment cycles (Table 2). The

Table 1. Baseline patients’ characteristics

| Characteristics          | N (%) | Median | Range          |
|--------------------------|-------|--------|----------------|
| Age (years)              | 190 (100) | 36     | 18–83          |
| Gender                   |       |        |                |
| Male                     | 95 (50) |        |                |
| Female                   | 95 (50) |        |                |
| WBC $\times 10^9$/l at diagnosis | 190 (100) | 8.7     | 1.8–53.9       |
| ALC $\times 10^9$/l at diagnosis | 190 (100) | 1.27    | 0.15–9.1       |
| Hgb (g/dl)               | 190 (100) | 12.9   | 8.3–17.2       |
| Albumin (g/dl)           | 162 (85) | 4      | 1.9–5.8        |
| Stage                    |       |        |                |
| I                        | 12 (6) |        |                |
| II                       | 80 (42) |        |                |
| III                      | 57 (37) |        |                |
| IV                       | 41 (22) |        |                |
| Initial treatment        |       |        |                |
| CT + RT                  | 84 (44) |        |                |
| CT                       | 106 (56) |        |                |
| IPS                      |       |        |                |
| Age (years)              |       |        |                |
| > 45                     | 66 (33) |        |                |
| $\leq 45$                | 124 (65) |        |                |
| Albumin (g/dl)           |       |        |                |
| $\geq 4$                 | 88 (54) |        |                |
| < 4                      | 74 (46) |        |                |
| Hgb (g/dl)               |       |        |                |
| $\geq 10.5$              | 161 (85) |        |                |
| < 10.5                   | 29 (15) |        |                |
| WBC $\times 10^9$/l      |       |        |                |
| $\geq 15$                | 22 (12) |        |                |
| < 15                     | 168 (88) |        |                |
| ALC $\times 10^9$/l      |       |        |                |
| $\geq 0.6$               | 19 (10) |        |                |
| $\leq 0.6$               | 171 (90) |        |                |
| Male                     | 95 (50) |        |                |
| Stage                    |       |        |                |
| 1                        | 171 (90) |        |                |
| 2                        | 17 (9)  |        |                |
| 3                        | 5 (3)   |        |                |
| 4                        | 51 (26) |        |                |
| 5                        | 5 (3)   |        |                |
| 6                        | 112 (29) |        |                |
| Cycle 1A                 | 190 (100) | 2.01 | 0.15–85.5     |
| ALC/AMC ratio            |       |        |                |
| Cycle 1B                 | 190 (100) | 2.24 | 0.22–37.5     |
| ALC/AMC ratio            |       |        |                |
| Cycle 2A                 | 190 (100) | 1.98 | 0.22–60.2     |
| ALC/AMC ratio            |       |        |                |
| Cycle 2B                 | 190 (100) | 1.87 | 0.10–26.5     |
| ALC/AMC ratio            |       |        |                |
| Cycle 3A                 | 173 (91) | 1.91 | 0.15–20.8     |
| ALC/AMC ratio            |       |        |                |
| Cycle 3B                 | 173 (91) | 1.67 | 0.15–9.8      |
| ALC/AMC ratio            |       |        |                |
| Cycle 4A                 | 168 (88) | 1.71 | 0.20–19.4     |
| ALC/AMC ratio            |       |        |                |
| Cycle 4B                 | 167 (88) | 1.80 | 0.24–5.8      |
| ALC/AMC ratio            |       |        |                |
| Cycle 5A                 | 118 (62) | 1.60 | 0.24–9.3      |
| ALC/AMC ratio            |       |        |                |
| Cycle 5B                 | 118 (62) | 1.74 | 0.25–14.2     |
| ALC/AMC ratio            |       |        |                |
| Cycle 6A                 | 115 (61) | 1.66 | 0.30–5.0      |
| ALC/AMC ratio            |       |        |                |
| Cycle 6B                 | 114 (60) | 1.61 | 0.36–9.8      |

Abbreviations: ALC, absolute lymphocyte count; AMC, absolute monocyte count; CT, chemotherapy; Hgb, hemoglobin; IPS, International Prognostic Score; PET, positron emission tomography; RT, radiation therapy; WBC, white blood cell count.
differences between the groups were ALC at diagnosis, albumin, IPS risk factors and int-PET-scan. Both groups were balanced with regard to how many treatment cycles were given. In the ALC/AMC ratio $\geq 1.1$ in any cycle, 67% (8/12) patients experienced an unrelated lymphoma death versus 33% (5/15) in the ALC/AMC ratio $<1.1$ in all cycles ($P=0.1$).

Patients with an ALC/AMC ratio $\geq 1.1$ during any treatment cycle experienced superior OS and PFS (Figure 3) compared with patients with an ALC/AMC ratio $<1.1$ during all treatment cycles (OS: median was not reached versus 2.3 years, the 5-year OS rates were 93% (95% confidence interval (CI): 89–98%) versus 27% (95% CI: 10–52%) ($P<0.0001$), respectively; and PFS: median was not reached versus 0.8 years, the 5-year PFS rates were 88% (95% CI: 79–95%) versus 8% (95% CI: 5–39%) ($P<0.0001$), respectively).

Univariate and multivariate analyses
In the univariate analysis by the Cox model, the ALC/AMC ratio $\geq 1.1$ at each treatment cycles as well as ALC/AMC ratio $\geq 1.1$ during any treatment cycle were predictors for OS and PFS (Table 3). In the multivariate analysis by the Cox model, the ALC/AMC ratio $\geq 1.1$ during any treatment cycle remained an independent predictor for OS and PFS (Table 4).

**DISCUSSION**
The ALC/AMC ratio, as a surrogate marker of host immunity (that is, ALC) and tumor microenvironment (that is, AMC), is a predictive biomarker for clinical outcomes in cHL. However, a limitation of
Table 2. Baseline patients’ characteristics based on any cycle with an ALC/AMC ratio $\geq 1.1$ versus all cycles with an ALC/AMC ratio $< 1.1$

| Variables | Any cycle with an ALC/AMC ratio $\geq 1.1$ | All cycles with an ALC/AMC ratio $< 1.1$ | P-value |
|-----------|---------------------------------------------|-------------------------------------------|---------|
| Age (years), median (range) | 36 (18–79) | 49 (18–83) | 0.1 |
| Gender | | | 0.2 |
| Female | 87 (52.1%) | 8 (34.8%) | |
| Male | 80 (47.9%) | 15 (65.2%) | |
| ALC $< 10^9/l$ at diagnosis, median (range) | 1.35 (0.15–3.63) | 0.69 (0.27–9.1) | $< 0.0002$ |
| Albumin (g/dl), median (range) | 4.05 (1.9–5.8) | 3.8 (2.3–4.1) | $< 0.009$ |
| Hgb (g/dl), median (range) | 12.9 (8.3–17.2) | 12.4 (8.8–14.3) | 0.2 |
| AMC at diagnosis $< 10^9/l$ | 0.65 (0.14–1.63) | 1.11 (0.21–2.61) | $< 0.0001$ |
| Stage | | | 0.3 |
| Limited | 81 (48.5%) | 8 (34.8%) | |
| Advanced | 86 (51.5%) | 15 (65.2%) | |
| WBC $< 10^9/l$ | 8.7 (1.8–53.9) | 9.7 (4.4–18.2) | 0.4 |
| Initial treatment | CT | 59 (35.3%) | 5 (21.7%) |
| CT + RT | 108 (64.7%) | 18 (78.3%) | |
| IPS risk factors | | | |
| Age (years) | | | 0.1 |
| $> 45$ | 113 (67.7%) | 12 (52.2%) | |
| Albumin (g/dl) ($N = 162$) | | | $< 0.03$ |
| $\geq 4$ | 82 (57.8%) | 6 (30%) | |
| $< 4$ | 60 (42.3%) | 14 (70%) | $< 0.0001$ |
| ALC per $\mu l$ | | | 0.8 |
| $> 600$ | 157 (94%) | 14 (60.9%) | |
| $\leq 600$ | 10 (6%) | 9 (39.1%) | |
| Hgb (g/dl) | | | 0.5 |
| $> 10.5$ | 142 (85%) | 19 (82.6%) | |
| $\leq 10.5$ | 25 (15%) | 4 (17.4%) | |
| WBC $< 10^9/l$ | | | 0.6 |
| $> 15$ | 21 (12.6%) | 1 (11.6%) | |
| $\leq 15$ | 146 (87.4%) | 22 (88.4%) | |
| Stage 4 | | | 0.6 |
| Yes | 35 (21%) | 6 (26.1%) | |
| No | 132 (79%) | 17 (73.9%) | |
| Number of IPS risk factors | | | $< 0.03$ |
| 0 | 21 (12.5%) | 2 (8.7%) | |
| 1 | 61 (36.5%) | 4 (17.4%) | |
| 2 | 50 (30.0%) | 4 (17.4%) | |
| 3 | 18 (10.8%) | 8 (34.8%) | |
| 4 | 13 (7.8%) | 3 (13.0%) | |
| 5 | 3 (1.8%) | 2 (8.7%) | |
| 6 | 1 (0.6%) | 0 (0.0%) | |
| IPS factors index | | | $< 0.0006$ |
| $\geq 3$ | 35 (21%) | 13 (56.5%) | |
| $< 3$ | 132 (79%) | 10 (43.5%) | |
| Radiation | | | 0.2 |
| Yes | 5 (35.3%) | 5 (21.7%) | |
| No | 108 (64.7%) | 18 (78.3%) | $< 0.0003$ |
| PET-scan | | | 0.6 |
| Positive | 7 (7%) | 6 (54.6%) | |
| Negative | 93 (93%) | 5 (45.4%) | |
| Number of cycles given | | | |
| 0 | | | |
| 1 | 15 (9%) | 2 (9%) | |
| 2 | 5 (3%) | 0 (0%) | |
| 3 | 15 (27%) | 6 (20%) | |
| 4 | 5 (9%) | 0 (0%) | |
| 5 | 57 (58%) | 15 (65%) | |
| Abbreviations: ALC, absolute lymphocyte count; AMC, absolute monocyte count; CT, chemotherapy; Hgb, hemoglobin; IPS, International Prognostic Score; PET, positron emission tomography; RT, radiation therapy; WBC, white blood cell count.

Figure 2. OS, lymphoma-specific survival, PFS and time to progression based on the number of treatment cycles phases that achieved an ALC/AMC ratio $\geq 1.1$. Worst clinical outcome observed in patients were the ALC/AMC ratio $< 1.1$ in all treatment cycles phases. 0 = all cycles with an ALC/AMC ratio $\geq 1.1$, $N = 96$, events = 4; 1 = 1 cycle with an ALC/AMC ratio $< 1.1$, $N = 34$, events = 3; 2 = 2 cycles with an ALC/AMC ratio $< 1.1$, $N = 10$, events = 1; 3 = 3 cycles with an ALC/AMC ratio $< 1.1$, $N = 13$, events = 2; 4 = 4 cycles with an ALC/AMC ratio $< 1.1$, $N = 12$, events = 2; 5 = 5 cycles with an ALC/AMC ratio $< 1.1$, $N = 2$, events = 0; and 6 = all cycles with an ALC/AMC ratio $< 1.1$, $N = 23$, events = 15.
the ALC/AMC-DX is its inability to assess the host/tumor interaction during treatment as it is performed at one point in time. Therefore, we analyzed the ALC/AMC ratio during treatment to assess its role on clinical outcomes in cHL.

To support the hypothesis that the biomarker ALC/AMC ratio affects survival in cHL during treatment, we evaluated the OS and PFS based on the ALC/AMC ratio ≥ 1.1 during all treatment cycles separated dramatically with regard to clinical outcomes compared with other prognostic factors. An ALC/AMC ratio ≥ 1.1 observed during each treatment cycle phase of ABVD chemotherapy. An ALC/AMC ratio affects survival in cHL during treatment, we evaluated the interaction during treatment as it is performed at one point in time. Therefore, we analyzed the ALC/AMC ratio during treatment to assess its role on clinical outcomes in cHL.

Table 3. Univariate analysis for OS, lymphoma-specific survival, PFS and time to progression

| Variables | OS | PFS |
|-----------|----|-----|
| Age > 45 years | 5.72, 2.58–13.90, <0.0001 | 2.61, 1.41–4.89, <0.002 |
| Albumin < 4 g/dl | 1.23, 0.60–2.75, 0.6 | 1.69, 0.88–3.34, 0.1 |
| ALC < 600 cells per μl | 7.43, 3.22–16.41, <0.0001 | 3.95, 1.83–7.83, <0.001 |
| ALC/AMC ≥ 1.1 at diagnosis | 0.09, 0.04–0.20, <0.0001 | 0.26, 0.14–0.48, <0.0001 |
| Hgb < 10.5 g/dl | 1.18, 0.39–2.87, 0.7 | 1.10, 0.45–2.33, 0.8 |
| IPS factors ≥ 3 | 3.30, 1.54–7.11, <0.002 | 2.86, 1.53–5.28, <0.001 |
| Limited disease | 0.48, 0.20–1.05, 0.07 | 0.34, 0.16–0.67, <0.001 |
| Male | 2.26, 1.04–5.28 | 1.11, 0.97–3.46, 0.06 |
| PET-scan negative | 0.33, 0.09–1.54, 0.1 | 0.13, 0.05–0.37, <0.0003 |
| CT + RT versus CT alone | 5.72, 2.58–13.90, <0.0001 | 5.72, 2.58–13.90, <0.0001 |
| Stage 4 | 1.31, 0.51–2.96, 0.6 | 1.75, 0.88–3.52, 0.1 |
| WBC ≥ 15 cells per μl | 1.88, 0.56–11.68, 0.3 | 1.84, 0.66–7.60, 0.3 |
| ALC/AMC cycle 1A ≥ 1.1 | 0.09, 0.04–0.20, <0.0001 | 0.15, 0.08–0.29, <0.0001 |
| ALC/AMC cycle 1B ≥ 1.1 | 0.05, 0.02–0.12, <0.0001 | 0.10, 0.5–0.20, <0.0001 |
| ALC/AMC cycle 2A ≥ 1.1 | 0.09, 0.04–0.19, <0.0001 | 0.13, 0.07–0.25, <0.0001 |
| ALC/AMC cycle 2B ≥ 1.1 | 0.10, 0.04–0.21, <0.0001 | 0.15, 0.07–0.28, <0.0001 |
| ALC/AMC cycle 3A ≥ 1.1 | 0.07, 0.02–0.18, <0.0001 | 0.21, 0.06–0.24, <0.0001 |
| ALC/AMC cycle 3B ≥ 1.1 | 0.13, 0.05–0.33, <0.0001 | 0.15, 0.07–0.29, <0.0001 |
| ALC/AMC cycle 4A ≥ 1.1 | 0.15, 0.06–0.38, <0.0001 | 0.22, 0.11–0.43, <0.0001 |
| ALC/AMC cycle 4B ≥ 1.1 | 0.12, 0.04–0.30, <0.0001 | 0.24, 0.12–0.47, <0.0001 |
| ALC/AMC cycle 5A ≥ 1.1 | 0.08, 0.02–0.23, <0.0001 | 0.08, 0.03–0.18, <0.0001 |
| ALC/AMC cycle 5B ≥ 1.1 | 0.13, 0.04–0.36, <0.0001 | 0.19, 0.08–0.40, <0.0001 |
| ALC/AMC cycle 6A ≥ 1.1 | 0.13, 0.03–0.39, <0.0001 | 0.25, 0.12–0.55, <0.0006 |
| ALC/AMC cycle 6B ≥ 1.1 | 0.09, 0.02–0.28, <0.0001 | 0.18, 0.08–0.39, <0.0001 |
| Any cycles ≥ 1.1 versus all cycles < 1.1 (ALC/AMC ratio) | 0.09, 0.04–0.19, <0.0001 | 0.08, 0.04–0.14, <0.0001 |

Abbreviations: ALC, absolute lymphocyte count; AMC, absolute monocyte count; CI, confidence interval; CT, chemotherapy; Hgb, hemoglobin; HR, hazard ratio; IPS, International Prognostic Score; OS, overall survival; PET, positron emission tomography; PFS, progression-free survival; RT, radiation therapy; WBC, white blood cell count.
infiltrating myeloid-derived cells predict clinical outcomes in cHL. In cHL, gene-expression profiling studies have reported that tumor-associated factors such as the IPS and int-PET-scan. In the multivariate analysis for OS, lymphoma-specific survival, PFS and time to progression

| Variables | OS |  | PFS |  |
|-----------|----|---|---|---|
| Age > 45 years | 5.69 | 2.29–15.58 | <0.0002 | 4.42 | 1.20–18.07 | <0.03 |
| Hgb < 100 cells per µl | 3.64 | 1.16–11.99 | <0.03 | 1.38 | 0.26–8.77 | 0.7 |
| ALC/AMC > 1.1 at diagnosis | 0.24 | 0.07–0.84 | <0.03 | 0.04 | 0.02–0.97 | <0.05 |
| Hgb < 10.5 g/dl | 1.41 | 0.39–4.39 | 0.6 | 1.30 | 0.31–5.20 | 0.7 |
| IPS factors > 3 | 2.14 | 0.44–10.29 | 0.3 | 1.42 | 0.02–6.65 | <0.01 |
| Male | 1.24 | 0.33–4.21 | 0.7 | 0.18 | 0.09–1.79 | 0.1 |
| Radiation (yes) | 0.27 | 0.05–0.96 | 0.06 | 0.19 | 0.05–0.98 | <0.03 |
| Any cycles > 1.1 versus all cycles < 1.1 (ALC/AMC ratio) | 0.14 | 0.04–0.40 | <0.0002 | 0.19 | 0.05–0.82 | <0.03 |

Abbreviations: ALC, absolute lymphocyte count; AMC, absolute monocyte count; CI, confidence interval; CT, chemotherapy; Hgb, hemoglobin; HR, hazard ratio; IPS, International Prognostic Score; OS, overall survival; PET, positron emission tomography; PFS, progression-free survival; RT, radiation therapy; WBC, white blood cell count.

Several studies have reported ALC recovery during the initial standard therapy in patients with acute lymphoblastic leukemia, acute myelogenous leukemia, and non-HL, suggesting that the host immune status during treatment might have a direct impact of the patient prognosis and survival. Furthermore, in diffuse large B-cell lymphoma (DLBCL) the published absolute monocyte/lymphocyte prognostic score was found to be independent prognostic factor of survival when compared with the cell of origin in DLBCL and the absolute monocyte/lymphocyte prognostic score was able to further discriminate clinical outcomes in patients with either activated B-cell or germinal center DLBCL. In cHL, gene-expression profiling studies have reported that tumor-infiltrating myeloid-derived cells predict clinical outcomes in CHL. However, a practical clinical limitation of gene-expression profiling is fresh frozen tissue samples to analyze. In patients who achieve a complete response during treatment, no tumor samples are available to biopsy to provide a dynamic real-time interaction between host response and tumor. Tumor-associated macrophages are derived from circulating monocytes and recruited to the tumor site by soluble tumor-derived chemotactic factors. The ALC/AMC-DX as a surrogate marker of host immunity (that is, ALC) and tumor microenvironment (that is, AMC) has been reported as a prognostic biomarker of clinical outcomes in cHL. However, the ALC/AMC-DX is only obtained at diagnosis and it does not provide a sequential assessment of the host/tumor interaction during treatment. Therefore, the ALC/AMC ratio was analyzed during treatment to assess implications on prognosis and survival. This study demonstrated that patients maintaining a high ALC/AMC ratio during treatment experienced better clinical outcomes compared with those who did not. These observations suggest that the surrogate markers of the interaction between host immunity and tumor microenvironment not only at diagnosis but also during treatment directly impact survival in CHL using a dynamic real-time biomarker in the ALC/AMC ratio.

To minimize the inherent biases due to the nature of retrospective studies, the following steps were taken. With regards to selection bias, we only included patients with CHL and excluded any patient with NLPHL, as NLPHL is considered to be a different disease entity. Only patients treated with ABVD chemotherapy with or without subsequent radiation therapy were included as this chemotherapy regimen is currently considered the standard of care in North America. Therefore, we excluded any patient treated up-front with palliative care or radiation therapy alone, as chemotherapy with or without radiation is considered also the current standard of care in CHL. With regard to confounding factors, our study included currently used clinical prognostic factors such as the IPS and int-PET-scan. In the multivariate analysis, the ALC/AMC ratio during treatment cycles remained an independent predictor for survival when compared with these clinical prognostic factors.

The strength of the study is the follow-up of a well-defined group of patients with CHL with a median follow-up of 3.7 years for the entire cohort of patients and 4.6 years for living patients. Second, the ALC/AMC ratio combines the clinical surrogate biomarkers for the inflammatory, pathological biomarkers—tumor-infiltrating lymphocytes and tumor-associated macrophages—which directly affect the biology of CHL. In addition, an inverse correlation has also been reported between the ALC/AMC ratio and tumor-associated macrophages in CHL, suggesting an association between the host biological response in the macroenvironment (peripheral blood) and microenvironment (tumor bed). Third, the ALC/AMC ratio is a simple, easily determined clinical biomarker that can be used to assess the clinical outcomes in CHL patient at any time during the course of treatment.

In conclusion, the ALC/AMC ratio recovery during treatment cycles in CHL is prognostic biomarker for clinical outcomes and provides a platform to develop therapeutic intervention to manipulate the ALC/AMC ratio during the treatment to improve clinical outcomes in CHL. Further studies are warranted to confirm our findings.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Porrata LF, Ristow K, Colgan JP, Habermann TM, Witzig TE, Inwards DJ et al. Peripheral blood lymphocyte/monocyte ratio at diagnosis and survival in classical Hodgkin’s lymphoma. Hematologica 2012; 97: 262–269.
2. Koh YW, Kang HJ, Park C, Yoon DH, Kim S, Suh C et al. The ratio of the absolute lymphocyte count to the absolute monocyte count is associated with prognosis in Hodgkin’s lymphoma: correlation with tumor-associated macrophages. Oncologist 2012; 17: 871–880.
3. Cox DR, Habermann TM, Payne BA, Klee GC, Pierre RV. Evaluation of the Coulter counter model S-Plus IV. Am J Clin Pathol 1985; 84: 297–306.
4. Hasenclever D, Diehl VA. A prognostic score for advanced Hodgkin disease. N Engl J Med 1998; 339: 1506–1514.
5. Cheson BD, Pfistner B, Juweid ME, Gascoyne RD, Specht L, Horning SJ et al. Revised response criteria for malignant lymphoma. J Clin Oncol 2007; 25: 579–586.
6. Kaplan E, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958; 53: 457–481.
7. Cox DR. Regression models and life-tables. J R Stat Soc Ser B 1972; 34: 187–202.
8. Sun D, Elson P, Liedtke M, Medeiros BC, Earl M, Alizadeh A et al. Absolute lymphocyte count at day 28 independently predicts event-free and overall survival in adults with newly diagnosed acute lymphoblastic leukemia. Am J Hematol 2012; 87: 957–960.
9 Behl D, Porrata LF, Markovic SN, Letendre L, Pruthi PK, Hook CC et al. Absolute lymphocyte count recovery after induction chemotherapy predicts superior survival in acute myelogenous leukemia. *Leukemia* 2006; 20: 29–34.

10 Chae YS, Shin H, Sohn SK, Lee SJ, Moon JH, Kang BW et al. Absolute lymphocyte count at day +21 predicts survival in patients with early-stage diffuse large B-cell lymphoma treated with rituximab, cyclophosphamide, Adriamycin, vincristine and prednisone. *Leuk Lymph* 2012; 53: 1757–1763.

11 Porrata LF, Ristow K, Habermann TM, Ozsan N, Dogan A, Macon W et al. Absolute monocyte/lymphocyte count prognostic score is independent of immunohistochemically determined cell of origin in predicting survival in diffuse large B-cell lymphoma. *Leuk Lymph* 2012; 53: 2159–2165.

12 Steidl C, Lee T, Shah SP, Farinha P, Han G, Nayar T et al. Tumor-associated macrophages and survival in classical Hodgkin’s lymphoma. *N Engl J Med* 2010; 362: 875–885.

13 Ribatti D, Nico B, Crivellato E, Vacca A. Macrophages and tumor angiogenesis. *Leukemia* 2007; 21: 2085–2089.

14 Green CE, Liu T, Montel V, Hsiao G, Lester RD, Subramaniam S et al. Chemoattractant signaling between tumor cells and macrophages regulates cancer cell migration, metastasis, and neovascularization. *PLoS ONE* 2009; 4: e6713.

15 Roca H, Varsos ZS, Sud S, Craig MJ, Ying C, Piena KJ. CCL2 and interleukin 6 promote survival of human CD11 b+ peripheral blood mononuclear cells and induce M2-type macrophages polarization. *J Biol Chem* 2009; 284: 34342–34354.

16 Dirkx AE, Oude Egbrink MG, Wagstaff J, Griffioen AW. Monocyte/macrophages infiltration in tumors modulators of angiogenesis. *J Leukocyte Bio* 2006; 80: 1183–1196.

This work is licensed under a Creative Commons Attribution 3.0 Unported License. To view a copy of this license, visit http://creativecommons.org/licenses/by/3.0/

Supplementary Information accompanies this paper on Blood Cancer Journal website (http://www.nature.com/bcj)