



Prognostic meaning of neutrophil to lymphocyte ratio (NLR) and lymphocyte to monocyte ration (LMR) in newly diagnosed Hodgkin lymphoma patients treated upfront with a PET-2 based strategy

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Abstract

Recent reports identify NLR (the ratio between absolute neutrophils counts, ANC, and absolute lymphocyte count, ALC), as predictor of progression-free survival (PFS) and overall survival (OS) in cancer patients. We retrospectively tested NLR and LMR (the ratio between absolute lymphocyte and monocyte counts) in newly diagnosed Hodgkin lymphoma (HL) patients treated upfront with a PET-2 risk-adapted strategy. NLR and LMR were calculated using records obtained from the complete blood count (CBC) from 180 newly diagnosed HL patients. PFS was evaluated accordingly to Kaplan-Meier method. Higher NLR was associated to advanced stage, increased absolute counts of neutrophils and reduced count of lymphocytes, and markers of systemic inflammation. After a median follow-up of 68 months, PFS at 60 months was 86.6% versus 70.1%, respectively, in patients with $NLR \geq 6$ or $NLR < 6$. Predictors of PFS at 60 months were PET-2 scan ($p < 0.0001$), $NLR \geq 6.0$ ($p = 0.02$), $LMR < 2$ ($p = 0.048$), and ANC ($p = 0.0059$) in univariate analysis, but only PET-2 was an independent predictor of PFS in multivariate analysis. Advanced-stage patients ($N = 119$) were treated according to a PET-2 risk-adapted protocol, with an early switch to BEACOPP regimen in case of PET-2 positivity. Despite this strategy, patients with positive PET-2 still had an inferior outcome, with PFS at 60 months of 84.7% versus 40.1% (negative and positive PET-2 patients, respectively, $p < 0.0001$). Independent predictors of PFS by multivariate analysis were PET-2 status and to a lesser extend NLR in advanced stage, while LMR maintained its significance in early stage. By focusing on PET-2 negative patients, we found that patients with $NLR \geq 6.0$ or $LMR < 2$ had an inferior outcome compared to patients with both ratios above the cutoff (78.7 versus 91.9 months, $p = 0.01$). We confirm NLR as predictor of PFS in HL patients independently from stage at diagnosis. Integration of PET-2 scan, NLR and LMR can result in a meaningful prognostic system that needs to be further validated in prospective series including patients treated upfront with PET-2 adapted-risk therapy.

Keywords PET-2 · Hodgkin's disease · NLR · LMR

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Introduction

Clinical outcome in Hodgkin lymphoma (HL) is partly due to the loss of critical mediators of immune surveillance, due to expansion of dysfunctional and immune-suppressive myeloid progenitors [1–3]. Despite the high rate of deep and durable responses, relapses after first-line therapy can occur in 20–30% of patients with advanced-stage HL [4].

Unfortunately, current prognostic models have not been shown to be very accurate in early detection of patients at high risk of shortened survival. A major prognostic system used in advanced HL is the International Prognostic System (IPS), based on the retrospective international series of more than

5000 patients treated with ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) or ABVD-like regimens. However, IPS has scarce clinical utility because only 19% of patients with scores 4 and 5 had a probability of 7-year-progression-free survival (PFS) < 50% [5, 6].

Positive ^{18}F -fluoro-2-deoxy-glucose (FDG) uptake in positron emission tomography (PET) scan performed early after first two cycles of chemotherapy (PET-2) [7–10] is the most reliable tool to predict outcome [11] and to base a risk-adapted strategy [11–13]. PET-2 is considered a surrogate test of tumor chemosensitivity and when positive indicates the persistence of the reactive microenvironment with high glycolytic activity [11, 14, 15].

Indeed, PET-2 information is available only during treatment and biological mechanisms leading to chemoresistance may be activated as early as after two cycles of treatment; thus, a warning to address patients to treatment switch to a more aggressive regimen in an escalating, risk-adapted strategy could be helpful in clinical management.

Starting from the clinical observation that the amount of tumor-associated macrophages (TAM) in the diagnostic biopsy [16] is predictive of outcome mirroring the complex network of cells and cytokines in HL microenvironment, our group previously showed the clinical impact of myeloid-derived suppressor cells and their key enzyme arginase-1 at diagnosis [1, 2].

Since MDSC count and Arg-1 represent the effect of the myeloid axis on the immune cells, we hypothesized that the ratio between absolute neutrophils (ANC) and lymphocyte count (ALC), known as NLR, could mirror the subtle relationship between the myeloid microenvironment and the lymphoid system. In this perspective, we asked if NLR could be integrated with available information at diagnosis to identify early patients with high risk of poor outcome.

Indeed, ALC as a surrogate of host immunity impairment is a prognostic factor for advanced-stage HL, being part of the international prognostic factor (IPS) [6]. In particular, T cell function is impaired in HL, with a depressed T cell-mediated response, mainly due to a defective T cell receptor CD3 ζ chain expression, accumulation of Th2 cells, and immunosuppressive regulatory T cells (T-reg) [3].

The increased amount of granulocytes is common in HL, as a consequence of inflammation status, associated to a negative prognosis [2, 17] despite that it is not included in the International Prognostic Index (IPS) [6]. Our previous work showed that granulocytes in HL are dysfunctional, since they exhibit a reduced phagocytic activity despite presence of activation signaling as shown by high levels of CD11b on the surface and they are able to reduce CD3 ζ expression and other activation markers on the cell surface of T lymphocytes [2]. In addition, we have demonstrated that neutrophils correlate with myeloid-derived suppressor cells (MDSC) and indeed they may represent their mature counterpart [1, 2, 18]. Therefore,

the NLR ratio may be representative of both the number of immunosuppressive mediators and the ability of the host to mount an immune response against the tumor.

NLR has been reported as an independent prognostic marker in solid and hematological cancers [19], including myeloma [20] and lymphoma [21, 22].

Another easily assessable variable is the LMR (lymphocytes to monocytes ratio) that could be a projection in peripheral blood of both tumor-infiltrating lymphocytes and TAM. LMR has been identified as an independent prognostic factor for survival in patients with classical HL [19, 23]. However, the impact of both NLR and LMR on the outcome of patients treated upfront with PET-2 risk-adapted strategy and its role in PET-2 era has never been investigated in HL.

Methods

Study design

In this retrospective study, we reviewed 190 files of patients with HL newly diagnosed at our institution between January 2006 and June 2012. Ten patients were excluded because history of HIV or immune-suppression therapy. Thirty healthy subjects were included in the study to evaluate normal range of NLR and LMR.

In all patients, CBC and routine biochemical examinations were taken before start treatment. White blood cell count and types (neutrophil, lymphocyte, eosinophil, and monocyte) were determined by electrical impedance method in automatic blood counter device (Beckman Coulter LH 750).

The study was approved by the local institutional review board. All participants gave a written informed consent in accord to the Declaration of Helsinki.

Basic characteristics, treatment, and clinical outcome are summarized respectively in Tables 1 and 2.

For patients with advanced disease (Ann-Arbor stage \geq IIB), treatment consisted of six cycles according to ABVD regimen (doxorubicin, bleomycin, vinblastine, and dacarbazine) [24, 25], followed by involved field radiotherapy if clinically indicated. PET-2 positive patients were shifted to BEACOPP regimen (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone) for eight cycles and, in case of persistent disease, a bone marrow autologous transplant was performed [12]. Early-stage patients received two or four or six courses of ABVD chemotherapy plus involved field radiotherapy as clinically indicated.

Both baseline and PET-2 were performed using standard technique and a semi-quantitative Deauville score was attributed to each image [26, 27]. A minimally positive scan was defined as any scan with any residual FDG uptake outside the

Table 1 Characteristics at baseline of 180 newly diagnosed HL patients

		NLR < 6	NLR ≥ 6	<i>p</i> value	LMR ≤ 2	LMR > 2	<i>p</i> value
N	180 (100%)	105	75	–	82	98	–
Median age (range)	31.7 (14.8–76.9)	32.5 (14.8–76.9)	28.6 (16.2–70.7)	0.41	31.8 (16.2–70.7)	31.3 (14.8–76.9)	0.96
Sex (M/F)	90/90	55/50	35/40	0.96	41/41	49/49	0.99
Bulky disease (yes/no)	54/130	26/79	28/47	0.09	31/51	23/75	0.12
B-symptoms (yes/no)	106/74	56/49	50/25	0.09	58/24	48/50	0.09
Lymphocyte count/10 ³ μL median (range)	1.4 (0.2–3.9)	1.9 (0.2–3.9)	1.1 (0.2–3.2)	< 0.0001	1.1 (0.2–3.4)	1.8 (0.2–3.9)	< 0.0001
Neutrophil count/10 ³ μL median (range)	7.7 (0.8–21.8)	6.19 (0.8–15.8)	9.3 (3.2–21.8)	< 0.0001	8.6 (1.6–21.8)	6.9 (0.8–19.3)	< 0.0001
Monocyte count/10 ³ μL median (range)	0.6 (0.2–1.9)	0.6 (0.2–1.9)	0.7 (0.2–1.8)	0.45	0.8 (0.2–1.9)	0.6 (0.2–1.6)	< 0.0001
LMR median (range)	2.1 (0.2–8.4)	2.7 (0.4–7.0)	1.5 (0.2–4.3)	< 0.0001	1.5 (0.2–3.3)	3.1 (0.9–7.0)	< 0.0001
NLR median (range)	5.0 (0.6–16.2)	3.8 (0.60–6.5)	8.2 (3.9–16.2)	< 0.0001	7.5 (2.0–16.2)	3.9 (0.6–9.4)	< 0.0001
Histotype							
Nodular sclerosis (%)	130 (72)	72	58	0.96	60	70	0.96
Mixed cellularity (%)	28 (16)	18	10	0.95	15	13	0.95
Lymphocyte-rich (%)	15 (8)	8	7	0.93	5	10	0.83
Lymphocyte depleted (%)	7 (4)	7	0	0.91	2	5	0.90
Stage							
Early (I–IIA)	61 (34)	42	19	0.05	20	41	0.02
Advanced (≥ IIB)	119 (66)	63	56		62	57	
IPS (for advanced stage only)							
0–1 (%)	35 (29)	19	16	0.08	6	29	< 0.0001
2–7 (%)	84 (71)	44	40		57	27	
LDH median (U/L, range)	319 (125–914)	297 (148–914)	328 (125–791)	0.63	318 (125–791)	300 (125–914)	0.78
ESR median (mm/h, range)	47 (2–135)	35 (2–119)	55 (6–135)	0.004	65 (6–135)	55 (6–125)	0.32
Beta-2 microglobulin median (ng/mL)	2.0 (0.3–6.3)	2.2 (0.3–5.9)	2.3 (0.8–6.3)	0.76	2.3 (0.80–6.34)	2.2 (0.3–6.3)	0.22
Ferritin median (ng/L, range)	190 (7–4982)	198 (11–1530)	182 (7–4982)	0.45	192 (7–4582)	190 (7–4982)	0.75
C-RP median (mg/L, range)	8.0 (0.1–8.9)	7.9 (0.2–8.5)	8.6 (0.1–8.9)	0.68	8.3 (0.1–8.9)	8.0 (0.1–8.7)	0.36
Fibrinogen median (mg/dL, range)	553 (145–1271)	503 (156–900)	595 (145–1271)	0.17	575 (145–1180)	550 (145–1271)	0.09

Abbreviations: NLR, neutrophil to lymphocyte ratio; LMR, lymphocyte to monocyte ratio; LDH, lactate dehydrogenase; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein

Differences in continuous parameters were evaluated using Wilcoxon signed rank test. Significant values are in bold.

physiological areas of the tracer concentration (mediastinal blood pool and liver) [28].

Statistical analysis

Qualitative results were summarized in counts and percentages. Descriptive statistics were generated for analysis of results and *p* value under 0.05 was considered significant.

The correlation of ANC, ALC, and NLR with various parameters was assessed with Pearson's chi-square test (or Fisher's exact test) for categorical parameters and with Mann-Whitney or Wilcoxon signed rank test for continuous parameters.

PFS was calculated from the time of inclusion until the date of progression, relapse, death, or the date the patient

was last known to be in remission; positivity of PET-2 was not considered as an event. PFS was analyzed by Kaplan-Meier test. Standard errors were calculated by the method of Greenwood; the 95% confidence intervals are computed as 1.96 times the standard error in each direction. The Cox proportional hazards model was used to evaluate NLR or LMR at diagnosis as a prognostic marker for PFS, as well as to assess and adjust with other known prognostic factors.

All calculations were performed using Graph Pad Prism version 6.00 for Windows, Graph Pad Software, San Diego, CA, USA. A stepwise multivariate regression analysis of survival was performed using MedCalc Statistical Software version 13.0.6 (MedCalc Software bvba, Ostend, Belgium; <http://www.medcalc.org>; 2014).

Table 2 First-line treatment and clinical outcome in 180 newly diagnosed HL patients

	<i>N</i> = 180 (100%)
Early-stage patients	
ABVD 2 cycles + i.f. RT (%)	9 (5)
ABVD 4 cycles + i.f. RT (%)	20 (11)
ABVD 6 cycles + i.f. RT (%)	28 (16)
ABVD 6 cycles (%)	4 (2)
Advanced-stage patients	
ABVD 6 cycles (%) + i.f. RT (%)	24 (22)
ABVD 6 cycles (%)	59 (33)
ABVD 2 cycles + 8 BEACOPP ± i.f. RT (%)	16 (9)
Continuous complete remission (cCR, %)	136 (76)
Treatment failure (%)	44 (24)

Results

NLR and LMR are increased in HL

In this series, the median age was 31.7 years old (range 14.8–76.9), and half patients were males (Table 1). The median ANC, ALC, and NLR at diagnosis were $7.74 \times 10^3/\mu\text{L}$, $1.47 \times 10^3/\mu\text{L}$, and 5.00, respectively.

Median NLR was significantly higher in patients with HL than controls (5.0, range 0.6–16.2 versus 1.6, range 0.2–3.1, respectively, $p < 0.001$). Higher NLR was associated to advanced stage ($p = 0.05$), increased ANC ($p < 0.0001$) and reduced ALC ($p < 0.0001$), increased marker of systemic inflammation ESR ($p = 0.004$), but not C-reactive protein or ferritin.

Median LMR was significantly lower in patients with HL than controls (2.1, range 0.2–8.4 versus 3.1, range 0.6–4.0, respectively, $p < 0.0001$), significantly associated to lower ALC, increased ANC, AMC, and NLR ($p < 0.0001$), advanced stage ($p = 0.002$), and high IPS ($p < 0.0001$) as shown in Table 1.

NLR and LMR are associated to PET-2 positivity and clinical outcome

After two ABVD cycles, out of 180 patients included in the study, 25 (14%) had a positive PET-2 (10 with score 4 and 15 with score 5) and 155 (86%) a negative PET-2 (Fig. 1). PET-2 positive patients had a higher median NLR (7.4 versus 4.8, $p = 0.0015$, Fig. 2a), higher in patients with DS5 than DS4 or DS3 ($p = 0.01$, Fig. 2b), and lower median LMR (1.7 versus 2.3, $p = 0.0027$, Fig. 2c), lower in patients with DS5 than DS4 or DS3 ($p = 0.001$, Fig. 2d).

Advanced-stage PET-2 positive patients (21/119) were allocated to the escalated BEACOPP program, refused, or not completed in five cases, while 98 PET-2 negative patients

continued with four additional ABVD, followed by consolidation radiotherapy on initial bulky nodal site of disease in 24 cases (Table 2). One early death for infection occurred among PET-2 advanced-stage negative patients. A third PET evaluation was performed at the end of planned treatment (PET-3) and a treatment failure within 2 years was registered in 9/97 PET-2 negative as compared to 14/21 PET-2 positive (Fig. 1).

In early-stage patients, therapy was not modified according to the PET-2 results. Based on disease extension, ABVD cycles were followed by involved field 30-Gy radiotherapy (ifRT); except in four cases in which for medical conditions and patient's will, treatment was extended to six cycles ABVD, two of them without success and later treated with salvage regimen IGEV and autologous stem cell transplantation (Table 2).

After a median follow-up of 68 months (range 5.4–84.4 months), 136 patients (75.5%) were in continued complete remission (cCR), 23 patients (13%) progressed during therapy or immediately after (during the first 6 months), 20 relapsed (11%) within 2 years from end treatment, and one died for infection before completing the planned cycles (Table 2). Achieving and maintaining cCR were associated to lower median NLR (4.8 versus 7.7, $p < 0.0001$) and higher LMR (2.4 versus 1.3, $p = 0.002$) than treatment failure (Fig. 2e–f).

To define a cutoff point, the choice of $\text{NLR} \geq 6$ was based on two strategies: (i) a value higher than mean plus two standard deviations in healthy subjects and (ii) the greatest differential to segregate cohorts, based on the X^2 value ($X^2 = 96.6$, p value < 0.0001) analyzed at different cutoff points between the 25 and 75% quartiles from the log-rank test. In addition, $\text{NLR} \geq 6$ and $\text{LMR} < 2$ have been previously identified in large series previously published [19, 21, 23, 29, 30].

Receiver-operator characteristic curves (ROCs) were generated and area under curves (AUCs) were calculated to assess the utility of NLR and LMR to predict treatment failure at diagnosis. The AUC for NLR was 0.71 (95% CI = 0.61–0.82, $p = 0.0001$); using $\text{NLR} \geq 6$ as cutoff, treatment failure was identified with sensitivity of 66.4% (95% CI = 58.1–74.0) and specificity of 67.6 (95% CI = 49.4–82.6). The AUC for LMR was 0.71 (95% CI = 0.61–0.82, $p = 0.0001$); $\text{LMR} < 2$ could identify treatment failure with sensitivity of 73.5% (95% CI = 55.6–87.1) and specificity of 62.3 (95% CI = 53.9–70.2).

NLR and LMR can predict clinical outcome in HL patients

In the whole cohort, 5-year PFS was 79.8%, lower in patients carrying $\text{NLR} \geq 6$ at diagnosis compared to $\text{NLR} < 6$ (86.6% versus 70.1%, $p = 0.002$, Fig. 3a). Similarly, 5-year PFS was lower in patients carrying $\text{LMR} < 2$ at diagnosis compared to $\text{LMR} \geq 2$ (70.2% versus 87.7%, $p = 0.004$, Fig. 3b). Predictors

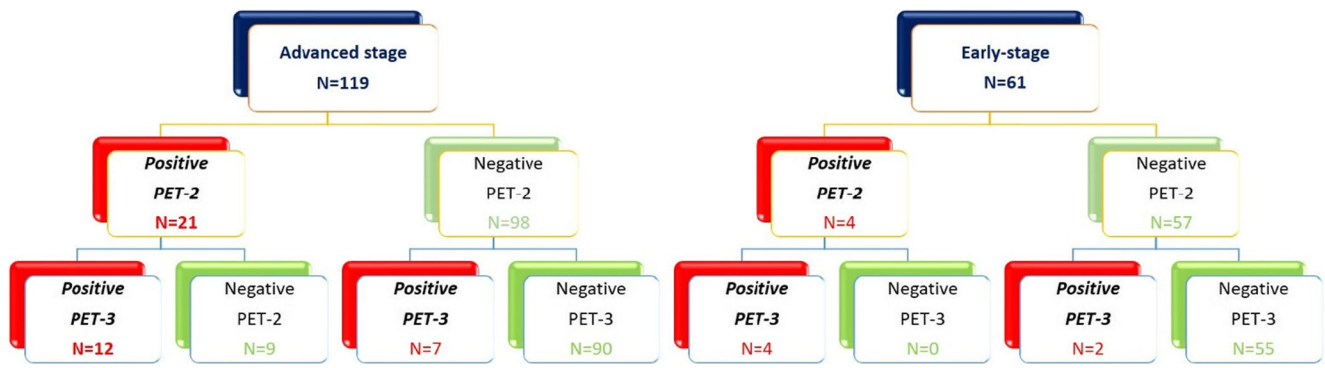
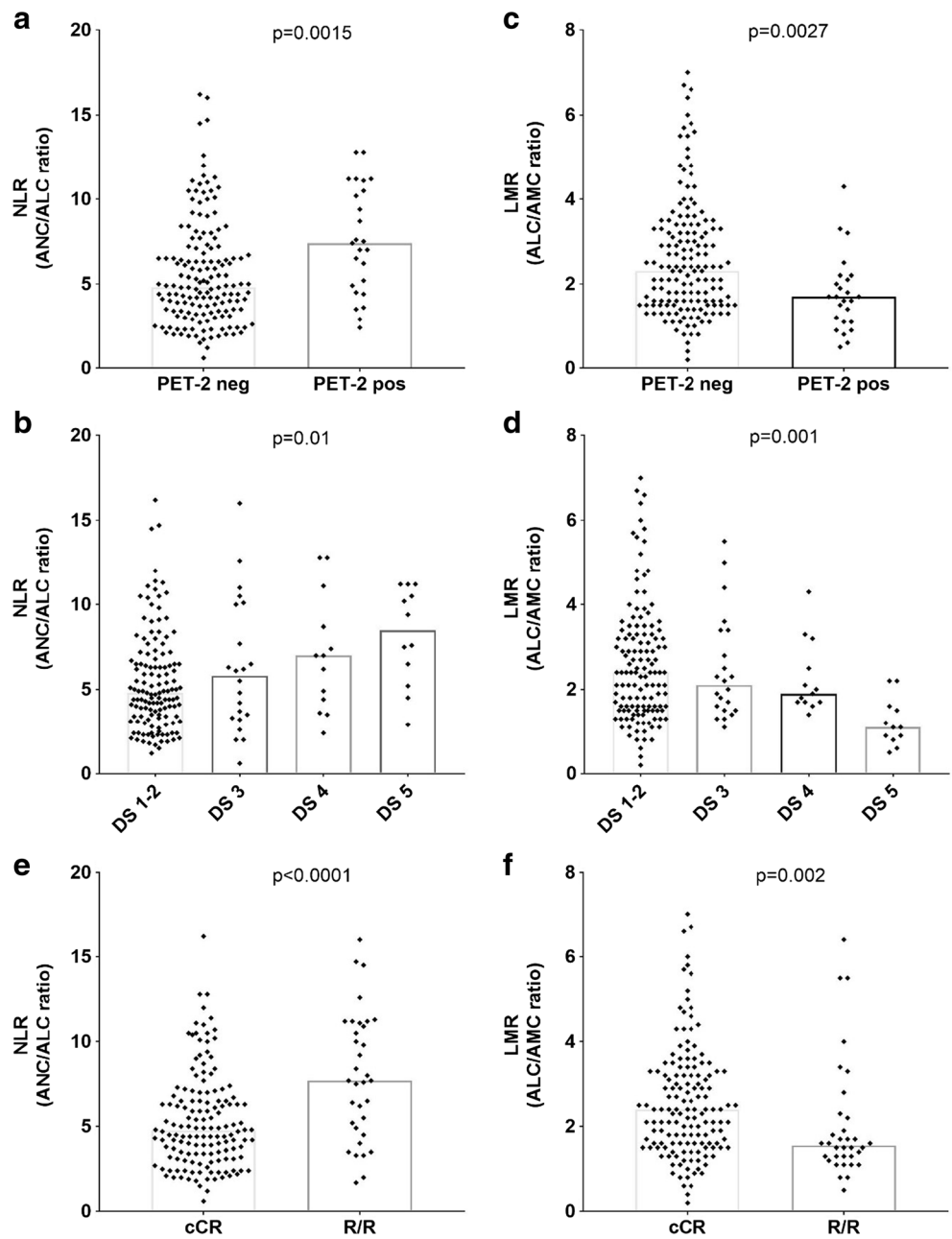


Fig. 1 Allocation of patients based on PET-2 and PET-3 scan

Fig. 2 Positive PET-2 is associated to increased NLR and reduced LMR at diagnosis. Pre-treatment median NLR was increased in patients with positive PET-2 (a), higher in patients with Deauville score (DS) 5 (b)—carrying new lesion, than DS4 (residual activity). Similarly, pre-treatment median LMR was reduced in patients with positive PET-2 (c), lower in DS5 than in DS4 patients (d)



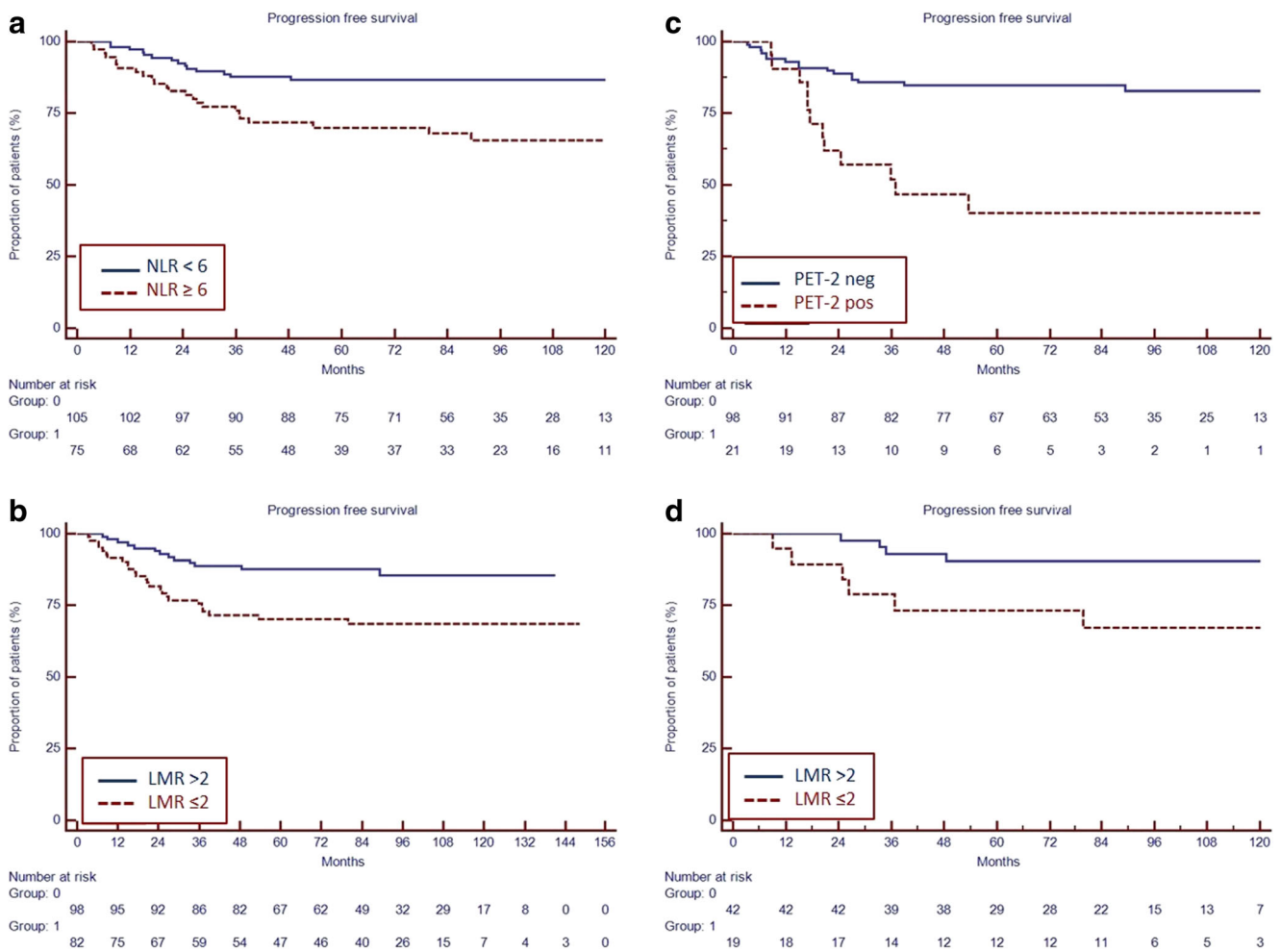


Fig. 3 PFS in newly diagnosed HL patients based on pre-treatment NLR, LMR, stage, and PET scan after two cycles of chemotherapy. Progression-free survival in 180 newly diagnosed HL patients based on NLR ≥ 6 (a) and LMR < 2 (b). In 119 advanced-stage patients treated

with a PET-2 risk-adapted strategy PET-2 status maintained its prognostic role (c). In 61 early-stage patients, LMR < 2 at diagnosis was associated to inferior outcome (d)

of 5-year PFS were PET-2 scan ($p < 0.0001$), NLR ≥ 6.0 ($p = 0.002$), and LMR < 2 ($p = 0.004$) in univariable analysis, but only PET-2 was maintained in multivariate analysis (Table 3).

Advanced-stage patients with positive PET-2 had an inferior outcome, with 5-year PFS of 84.7% versus 40.1%, respectively, in negative and positive PET-2 patients, $p < 0.0001$, Fig. 3c). Thus, in advanced-stage patients, ALC ($p = 0.017$), PET-2 ($p < 0.0001$), and NLR ≥ 6 ($p = 0.013$) were predictors of 5-year PFS in univariate analysis, but only PET-2 and barely NLR were maintained in multivariate analysis (Table 3). In early-stage patients, only LMR < 2 could predict 5-year PFS in both univariate analysis (73.3 versus 90.4%, $p = 0.03$) and multivariate analysis ($p = 0.02$, Table 3, Fig. 3d).

Finally, since the heterogeneity of treatment due to a PET-2 risk-adapted strategy, we analyzed separately NLR ≥ 6 and LMR < 2 to predict clinical outcome in PET-2 negative patients ($N = 155$, $N = 98$ advanced stage and $N = 57$ early stage). We found that carrying NLR ≥ 6 or LMR < 2 at diagnosis was associated to inferior outcome, with 5-year PFS of

78.7 versus 91.9% in NLR < 6 and LMR ≥ 2 (HR 3.1, 95% CI 1.4–6.8, $p = 0.01$ Fig. 4).

Discussion

A high NLR has been reported to have a negative prognostic impact in several solid and liquid tumors [20, 30–34]. A recent report indicates that a NLR ≥ 6 or LMR < 2 could be considered robust and negative prognostic factor for both PFS and OS also in HL patients [19, 30]. However, in both series, a formal comparison with long-term outcome in patients treated upfront with a PET-2 risk-adapted strategy is missed.

Thus, we retrospectively evaluated the prognostic significance of NLR in newly diagnosed patients treated upfront with a PET-2 risk-adapted strategy and our findings confirmed the importance of NLR in predicting PFS, in both early- and advanced-stage patients. Since higher NLR was associated to reduced LMR and some reports in pre-PET-2 era suggested a

Table 3 Progression-free survival in HL patients based on NLR and LMR

	Clinical variable	N	5-Year PFS	Univariable analysis			Multivariable analysis ^a		
				HR	95% CI	p value	HR	95% CI	p value
Whole cohort	ANC								
	ANC < 10,000	134	82.6	1.8	0.9 to 3.8	0.07	1.4	0.7 to 2.8	0.37
	ANC ≥ 10,000	46	71.6						
	ALC								
	ALC < 600	12	62.5	0.4	0.1 to 2.4	0.18	1.6	0.5 to 5.5	0.46
	ALC ≥ 600	168	81.3						
	Hb								
	Hb < 12	61	78.1	0.9	0.5 to 1.8	0.91	0.8	0.4 to 1.2	0.39
	Hb ≥ 12	119	81.2						
	NLR								
	NLR < 6	105	86.6	2.7	1.4 to 5.1	0.0022	1.6	0.7 to 3.7	0.25
	NLR ≥ 6	75	70.1						
	LMR								
	LMR ≤ 2	82	70.2	2.6	1.4 to 4.9	0.004	1.6	0.7 to 3.7	0.21
LMR > 2	98	87.7							
PET status									
Negative	155	85.1	4.2	1.5 to 11.5	< 0.0001	3.4	1.7 to 6.7	0.0006	
Positive	25	45.7							
Advanced-stage cohort	ANC								
	ANC < 10,000	80	79.8	1.6	0.7 to 3.6	0.19	n.a.	n.a.	n.a.
	ANC ≥ 10,000	39	71.6						
	ALC								
	ALC < 600	5	40.6	0.25	0.1 to 2.3	0.017	n.a.	n.a.	n.a.
	ALC ≥ 600	114	78.7						
	Hb								
	Hb < 12	51	77.4	1.5	0.6 to 3.4	0.37	1.1	0.9 to 1.4	0.31
	Hb ≥ 12	53	82.3						
	NLR								
	NLR < 6	63	85.7	2.6	1.2 to 5.6	0.013	2.2	0.1 to 5.1	0.06
	NLR ≥ 6	56	66.7						
	LMR								
	LMR ≤ 2	56	69.3	2.1	0.9 to 4.3	0.07	n.a.	n.a.	n.a.
LMR > 2	63	85.7							
PET status									
Negative	98	84.7	4.3	1.5 to 12.4	< 0.0001	3.8	1.7 to 8.3	0.0008	
Positive	21	40.1							
Early-stage cohort	ANC								
	ANC < 10,000	54	86.9	2.1	0.3 to 15.0	0.35	2.9	0.6 to 15.1	0.2
	ANC ≥ 10,000	7	71.4						
	ALC								
	ALC < 600	3	84.3	n.a.	n.a.	0.44	n.a.	n.a.	n.a.
	ALC ≥ 600	58	100						
	Hb								
	Hb < 12	21	81	0.7	0.2 to 2.7	0.59	0.5	0.1 to 2.3	0.41
	Hb ≥ 12	40	87.4						
	NLR								
	NLR < 6	42	87.9	2.3	0.6 to 8.9	0.17	n.a.	n.a.	n.a.
	NLR ≥ 6	19	78.9						
	LMR								
	LMR ≤ 2	42	90.4	3.8	0.9 to 15.0	0.03	6.5	1.4 to 31.1	0.02
LMR > 2	19	73.3							

Table 3 (continued)

Clinical variable	N	5-Year PFS	Univariable analysis			Multivariable analysis ^a		
			HR	95% CI	<i>p</i> value	HR	95% CI	<i>p</i> value
PET status								
Negative	57	83.3	1.9	0.1 to 28.9	0.53	4.8	0.5 to 45.7	0.18
Positive	4	75.1						

Abbreviations: 95% CI, 95% confidence interval; PFS, progression-free survival; HR, hazard ratio; NLR, neutrophil/lymphocyte ratio. Significant *p* values are in bold

^a Backward elimination was used to isolate predictors in the multivariable model

prognostic role for LMR [23], we tested the prognostic meaning of LMR that resulted to be a significant variable in predicting PFS in univariate but not in multivariate analysis, confirming our previous observations in a similar setting [8].

In our series, advanced-stage patients with PET-2 positive scan received a more aggressive regimen, switching from ABVD to BEACOPP. Recently, three phase 3 clinical trials, the U.K. RATHL [35], the American S081626 trial [36], and Italian GITIL/FIL HD0607 (Gallamini, manuscript in press) demonstrated the clinical benefit of this escalating PET-adapted strategy.

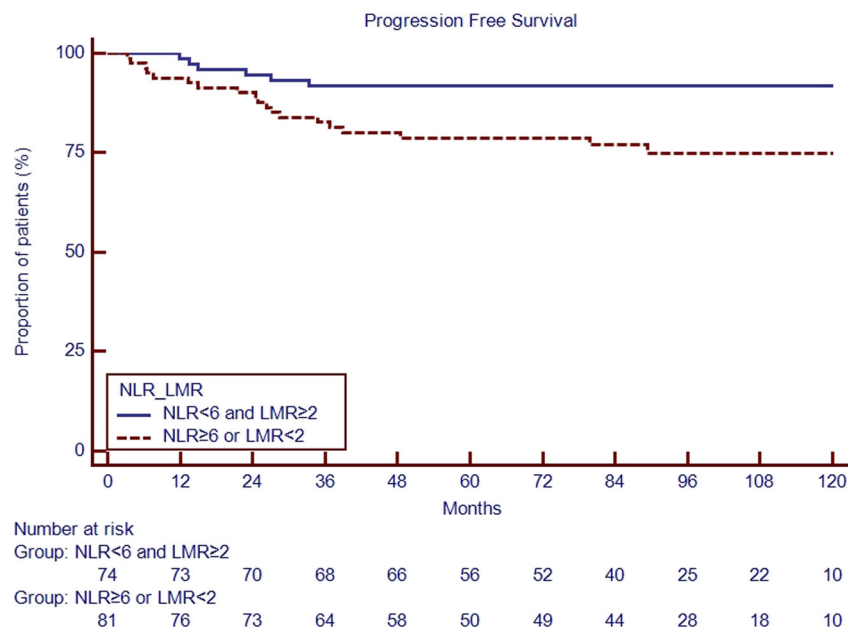
While a PET-2-adapted strategy can improve outcome in PET-2 positive patients, a backbone of around 10% of PET-2 negative patients is still at risk of relapse. For these patients, a bio-PET has been proposed, to integrate information about immune system and cancer features [18]. NLR, more than LMR, could reflect the immunosuppressive capacity of both mature and immature myeloid cells that are elevated in the peripheral blood, secrete arginase, and are positively associated to TAM [1, 2]. Several groups identified LMR as

independent prognostic factor [19, 23], but we and others have found its role in the cohort of early-stage patients only [37].

In HL microenvironment, the immune-suppressive component has prognostic meaning also when evaluated in the lymph-node biopsies, integrating the prognostic importance of PET-2 scan [18]. Especially in advanced-stage patients, the current IPS has low sensitivity and specificity, taking in account clinical parameters like hemoglobin and sex that have lost their importance in the last years thanks to supportive care improvement [38, 39]. Interestingly, in our series, the IPS was unable to provide any clear distinction in the outcomes for PFS. In contrast, the addition of NLR to PET-2 scan was the only independent variable able to identify patients with poor prognosis, giving NLR a clear and cheap means of prognostication at diagnosis.

Our study has several important limitations. First, this study is retrospective and, therefore, subject to inherent limitations and biases, including change of therapy based on PET-2 status. Thus, validation of the utility of NLR and LMR in an independent multicenter cohort of PET-2 negative patients treated with the same scheme is needed.

Fig. 4 PFS in 155 newly diagnosed HL patients with negative PET-2 based on NLR/LMR status



In conclusion, pre-treatment NLR is significantly associated with increased risk of treatment failure in advanced stage HL, while LMR in early-stage patients, even if in case of negative PET-2.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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