BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-017904
Article Type:	Research
Date Submitted by the Author:	24-May-2017
Complete List of Authors:	Lee, Shing Fung; Department of Clinical Oncology Luque, Miguel-Angel; London School of Hygiene and Tropical Medicine, Epidemiology and Population Health
Primary Subject Heading:	Oncology
Secondary Subject Heading:	Patient-centred medicine, Haematology (incl blood transfusion)
Keywords:	neutrophils, monocytes, lymphocytes, Lymphoma < ONCOLOGY, survival, prognosis

Original Article

Title: Prognostic value of lymphocyte to monocyte ratio and neutrophil to lymphocyte ratio in follicular lymphoma: a retrospective cohort study

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Word count: abstract: 222; text: 2970; references: 48; tables: 3; figures: 2; supplementary tables: 2; supplementary figure: 1

ABSTRACT

Objectives Clinical course and prognosis of follicular lymphoma (FL) are diverse and associated with a patient's immune response. We investigated the lymphocyte to monocyte ratio (LMR) and neutrophil to lymphocyte ratio (NLR) as prognostic factors for patients with FL, including those receiving radiotherapy.

Design A retrospective cohort study.

Setting Regional cancer centre in Hong Kong.

Participants 88 patients with histologically proven FL diagnosed between 2000 and 2014.

Materials and methods The best LMR and NLR cut-off values were determined using receiver-operating characteristic curves. The extent to which progression-free survival (PFS) and overall survival differed by NLR and LMR cut-off values were assessed using Kaplan–Meier analysis and log-rank tests. A Cox proportional hazards model was fitted to adjust for confounders.

Results The best cut-off values for LMR and NLR were 3.43 and 2.78, respectively. The 5 year PFS was 73.6%. After multivariate adjustment, high LMR (>3.43) at diagnosis was associated with superior PFS, with a hazard ratio (HR) 0.31 (95% confidence interval [CI] 0.13, 0.72), whereas high NLR at relapse was associated with poorer post-progression survival (HR 1.26, 95% CI 1.06, 1.51).

Conclusions Baseline LMR and NLR at relapse were shown to be independent prognostic factors in FL. LMR and NLR are low cost and widely available biomarkers for clinicians who may use these in combination with FLIPI to better predict prognosis.

Keywords: neutrophils, monocytes, lymphocytes, lymphoma, survival, prognosis

ARTICLE SUMMARY

Strengths and limitations of this study

- We obtained strong evidence to support these prognostic factors to possess practical clinical utility and significance in follicular lymphoma.
- Our study included patients without exposure to rituximab so that our result is also applicable to regions where rituximab is less accessible.
- Sensitivity analysis was performed to determine the robustness of the main findings in different scenarios.
- Association between cell count ratios and systemic treatment choices, duration and number of cycles, and salvage treatment upon progression were not analysed because of limited sample size for subgroup analysis.

INTRODUCTION

Follicular lymphoma (FL) accounts for approximately 20% of all incident lymphoma cases, making it the most common indolent non-Hodgkin lymphoma (NHL). The clinical course and prognosis of FL are diverse.[1-6] Clinical and laboratory parameters assist in predicting prognosis, allow for tailoring appropriate therapies, and aid in selecting patients for appropriate clinical trials. The commonly used criteria include the groupe d'etude des lymphomes folliculaires criteria,[7] follicular lymphoma international prognostic index (FLIPI),[2] and FLIPI2.[8] FLIPI is a clinical prognostic score and classifies patients into risk categories: low, intermediate, and high risk. It does not include parameters associated with tumour microenvironment or host antitumour immune response.

About 20% of FL patients do not respond or experienced progression within two years of treatment, these early relapse represents a subgroup of patients who are at a substantially greater risk of death, and their median OS is only 5 years.[9] These higher risk FL may have a distinct biology, but it is not easily identified at diagnosis, even high-risk disease defined by the commonly employed FLIPI[2] could have prolonged survival with modern therapy. A biologic rationale to account for this heterogeneity in patient outcomes would provide insights that may influence disease monitoring, and treatment strategy.

Advances in gene expression profiling allow us to elucidate the role of stromal, non-malignant cells in the pathogenesis, and progression of lymphoma. Immune response-1 and immune response-2 are two types of immune responses.[10] Dave et al. discovered that most of the component genes in prognostically unfavourable immune response-2 signatures are expressed more highly in the nonmalignant component of tumours. Many genes in the signature of immune response-2 are highly expressed by peripheral blood monocytes. Furthermore, monocyte chemoattractant protein, a potent chemotactic factor for monocytes, and its receptor CC chemokine receptor 2 are shown to play roles in modulating

inflammatory responses, tumour proliferation, angiogenesis, and metastasis.[11, 12] Their levels of expression are correlated with prognosis in cancers. In addition, the myeloid-derived suppressor cells, a subpopulation of cells, are reported to have immune suppressive functions.[13-15] Increasing numbers of monocytes, macrophages, or their precursors have been detected in lymphomatous nodes.[13, 16] Recent studies have indicated that peripheral blood lymphocyte-to-monocyte ratio (LMR) at diagnosis can predict long-term outcome in patients with diffuse large B-cell lymphoma (DLBCL),[17] FL,[18, 19], and Hodgkin lymphoma (HL).[20-22] This evidence supports monocyte as an important component in tumour microenvironment.

On the other hand, absolute neutrophil count (ANC), a surrogate marker of inflammation produced by tumour,[23-26] is utilised in the form of peripheral blood neutrophil-to-lymphocyte ratio (NLR) at diagnosis to predict survival in DLBCL,[17, 27] and HL.[28] The rationale of utilising these cell count ratios is to consider the interaction among host immunity represented by lymphocytes, the inflammation produced by tumour, and the tumour microenvironment. However, studies on FL mainly focus on those who were treated with rituximab-containing chemotherapy, with little emphasis on those who had radiotherapy (RT) as a component of or as a primary treatment. Moreover, the prognostic role of NLR in FL in terms of survival outcomes has not been studied. Therefore, we aimed to investigate the extent to which NLR at diagnosis predict survival outcomes in patients with FL, including those who were treated with RT. We also evaluated whether NLR can be used in combination with FLIPI to improve prognosis prediction.

MATERIALS AND METHODS

Study design, setting, and participants

We performed a longitudinal study using retrospective information from electronic medical records of patients with incident FL treated in Tuen Mun Hospital, Hong Kong. All FL

incident cases from 2000 to 2014 were identified (n = 88). We restricted the analysis to patients with complete laboratory, pathology, and radiological data in the medical records regarding the variables in the analysis (supplementary figure S1). The sociodemographic information of the excluded patients was not different from that of the included patients in the final sample. Patients were followed up for a median of 5.88 (range 0.49–16.45) years. The peripheral blood count results were obtained from standard automated complete blood count machine. This study was approved by the Clinical and Research Ethics Committee of the Tuen Mun Hospital, Tuen Mun, Hong Kong (NTWC/CREC/16107). The research was conducted in accordance with the 1964 Declaration of Helsinki and its later amendments.

Data and variables

Overall survival (OS) and progression-free survival (PFS) were the main outcomes of the study. These outcomes were defined and measured as per criteria from the International Harmonization Project.[29] OS was defined as the time from diagnosis until death as a result of any cause or last follow-up visit. PFS was defined as the time from diagnosis until lymphoma progression (first date of documentation of a new lesion or enlargement of a previous lesion) or death as a result of any cause or last follow-up visit. For both OS and PFS, patients were censored at their last follow-up visit. Patients' demographics and disease factors were collected. FLIPI score was then calculated using those factors (nodal sites, age, serum lactate dehydrogenase [LDH], stage, and haemoglobin [Hb]) (see supplementary table S1).[2] Chemotherapy involved cyclophosphamide, vincristine, and prednisolone (CVP) or cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP) or CHOP-like regimens.

Statistical methods

We initially described the cohort of patients using means and standard deviations for continuous variables, and proportions and ranges (minimum, maximum) for categorical

variables. To evaluate LMR and NLR performance in predicting mortality, we fitted two logistic models with cancer death as outcome and LMR and NLR as continuous independent predictors. Bayesian and Akaike information criteria were used to determine the impact of any associated factors and to find out the best fitting model. Subsequently, we computed marginal probabilities for the outcome and derived the area under the curve (AUC), sensitivity, specificity, and the Youden's index (sensitivity + specificity −1). Respective LMR and NLR cut-off values were determined at a point with maximum Youden's index in receiver operating characteristic (ROC) curves.[30, 31] To evaluate the extent to which OS and PFS differ by LMR and NLR cut-off values, we used incidence rates, rate ratios, Kaplan–Meier analysis, and log-rank tests for statistical inference.[32, 33] We also used semi-parametric Cox proportional hazards models to evaluate OS and PFS for the computed LMR and NLR cut-off values adjusted for FLIPI, use of rituximab, and sex.[34, 35] Finally, we developed a sensitivity analysis to evaluate the robustness of our findings in multivariate analysis. The proportional hazard assumption for multivariate-adjusted Cox models was also assessed based on the analysis of the Schoenfeld residuals. We used Stata v.14.2 for statistical analysis (StataCorp, College Station, TX, USA).

RESULTS

Description of the cohort

The median age at diagnosis for the patients included in the study was 54 years (range 22–87). Among them, 18 deaths were encountered during the follow-up period. Thirteen patients died of lymphoma. Five deaths were non-lymphoma related; one patient developed prostate cancer, and died of pneumonia. Another three died of community-acquired pneumonia; one died of acute coronary syndrome and renal failure. The estimated 5-year PFS and OS were 73.6% and 85.6% respectively (figure 1). At diagnosis, 18.2%, 21.6%, and 60.2% were classified as being at low, intermediate, and high risk according to the FLIPI score. Table 1

shows descriptive summary statistics of patients included in the study.

Table 1 Descriptive summary statistics for the best cut-offs of LMR and NLR by patients' clinical characteristics, n = 88

Characteristics	All patients (n = 88)	LMR >3.43 (n = 49)	LMR ≤3.43 (n = 39)	NLR >2.78 (n = 44)	NLR ≤2.78 (n = 44)
Age, years					
Median (range)	54 (22–87)	53 (22–87)	54 (31–78)	55 (31–80)	53 (22–87)
>60, n (%)	29 (33.0)	16 (32.7)	13 (33.3)	17 (38.6)	12 (27.3)
≤60, n (%)	59 (67.0)	33 (67.3)	26 (66.7	27 (61.4)	32 (72.7)
Sex, n (%)					
Male	47 (53.4)	29 (59.2)	18 (46.2)	21 (47.7)	26 (59.1)
Female	41 (46.6)	20 (40.8)	21 (53.9)	23 (52.3)	18 (40.9)
FLIPI, n (%)					
Low risk (scores 0–1)	16 (18.2)	10 (20.4)	6 (15.4)	6 (13.6)	10 (22.7)
Intermediate risk (score 2)	19 (21.6)	13 (26.5)	6 (15.4)	8 (18.2)	11 (25.0)
High risk (scores 3–5)	53 (60.2)	26 (53.1)	27 (69.2)	30 (68.2)	23 (52.3)
ANC (10^9/L), median (range)	4.2 (1.9–10.7)	3.6 (1.9–7.9)	4.6 (2.1–10.7)	4.8 (2.5–10.7)	3.5 (1.9–7.9)
ALC (10^9/L), median (range)	1.6 (0.6–11.3)	1.9 (0.7–11.3)	1.1 (0.6–3.1)	1.1 (0.6–2.3)	2.0 (0.8–11.3)
AMC (10^9/L), median (range)	0.4 (0.1–1.2)	0.4 (0.1–0.9)	0.5 (0.2–1.2)	0.4 (0.1–1.2)	0.4 (0.2–1.2)
NLR, median (range)	2.76 (0.59–9.91)	2.15 (0.59–8.50)	3.83 (1.81–9.91)	4.04 (2.78–9.91)	1.90 (0.59–2.74)
LMR, median (range)	3.80 (0.55–22.60)	5.00 (3.00–22.60)	2.33 (0.55–3.43)	2.90 (0.55–8.00)	4.46 (1.29–22.60)
LDH >220 IU/L, n (%)	70 (80.5)	38 (79.2)	32 (82.1)	36 (81.8)	34 (79.1)
Stage, n (%)					
I/II	24 (27.3)	15 (30.6)	9 (23.1)	11 (25.0)	13 (29.5)
III/IV	64 (72.7)	34 (69.4)	30 (76.9)	33 (75.0)	31 (70.5)
Hb <12 g/dL, n (%)	23 (26.1)	14 (28.6)	9 (23.1)	10 (22.7)	13 (29.6)
Number of nodal sites >4, n (%)	50 (56.8)	23 (46.9)	27 (69.2)	28 (63.6)	22 (50.0)
Use of rituximab, n (%)	38 (43.2)	20 (40.8)	18 (46.2)	22 (50)	16 (36.4)
Treatment, n (%)					
Chemotherapy plus RT	14 (15.9)	10 (11.4)	4 (4.5)	6 (6.8)	8 (9.1)

Chemotherapy alone	54 (61.4)	25 (28.4)	29 (33.0)	30 (34.1)	24 (27.3)
RT alone	14 (15.9)	9 (10.2)	5 (5.7)	6 (6.8)	8 (9.1)

Only 18 patients had RT as definitive treatment for limited stages I and II. Involved-field irradiation, in which the RT fields were limited to the involved nodal region, was mostly given with two parallel opposed fields; the median radiation dose was 40 Gy (range 30–54 Gy). 10 other patients with stage III or IV received RT in their disease course, as part of palliation or as consolidation to sites with inadequate response to systemic treatment. High-grade transformation occurred in 6 out of 27 relapsed patients. Peripheral blood counts were available at the time of their relapse.

Progression-free survival

The predicted values from logistic regression models for LMR and NLR were used to create ROC curves and derive the area under the curve (AUC). The AUC of LMR and NLR were 0.84 (95% confidence interval [CI] 0.75, 0.93) and 0.84 (95% CI 0.75, 0.92), respectively, and they did not differ in predictive performance for PFS (test equality of ROC areas, p-value 0.838). An LMR cut-off value of 3.43 (positive predictive value 40.5% and negative predictive value 97.8%; sensitivity 94.4% and specificity 64.3%) and NLR cut-off value of 2.78 (positive predictive value 40.0% and negative predictive value 95.8%; sensitivity 88.9% and specificity 65.7%) showed the greatest Youden's index, corresponding to maximum joint sensitivity and specificity on the ROC curve (supplementary table S2).

NLR and LMR mortality predictive performance

The median NLR and LMR at diagnosis were 2.77 (range 0.59–9.91) and 3.80 (range 0.55–22.60), respectively. The median NLR and LMR at relapse were 2.67 (range 0.95–17.25) and 3.33 (range 0.48–8.5), respectively.

In univariate analysis presented in table 2, NLR at relapse was associated with post-progression survival as a continuous variable (hazard ratio [HR] 1.26, 95% CI 1.06, 1.51).

High LMR (>3.43) had a superior PFS with a rate ratio (RR) of 0.38 (95% CI 0.18, 0.82). Patients with high FLIPI score had 2.5 times greater risk of death or relapse than patients with a lower score (RR: 2.52, 95% CI 1.10, 5.75). However, patients treated with rituximab had 72% lower risk of death or relapse (RR: 0.28 95% CI 0.10, 0.81). We found evidence of a linear trend of PFS associated with the calendar period in analysis (p-value of trend = 0.003). Compared with the period 2000-2005 those patients diagnosed during 2010-2014 had 90% lower risk of death or relapse (RR: 0.10 95% CI 0.01, 0.75). Furthermore, there was no evidence of differences in PFS by sex (male vs female, RR: 1.09, 95% CI 0.51–2.32). LMR at relapse showed weak evidence of association with post-progression survival (HR 1.06, 95% CI 0.77, 1.45) (table 2).

Table 2 Rate ratios of PFS events for different factors, n = 88

		Cases/1000 person years	Rate (per 1000 person years)	Rate ratio	95% CI	p-value
LMR	>3.43	11	35.96	0.38	0.18–0.82	0.010
	≤3.43	16	96.40	1		
NLR	>2.78	13	59.72	1.10	0.52–2.34	0.806
	≤2.78	14	54.33	1		
FLIPI	High risk	19	82.30	2.52	1.10–5.75	0.023
	Low/ intermediate risk	8	32.72	1		
Sex	Male	15	58.96	1.09	0.51–2.32	0.831
	Female	12	54.29	1		
Rituximab	Yes	4	22.02	0.28	0.10–0.81	0.012
	No	23	78.31	1		
Year of diagnosis	2010–2014	1	8.71	0.10	0.01–0.75	0.006
	2006–2010	5	43.05	0.50	0.19–1.33	0.157
	2000–2005	21	85.91	1		

In multivariate analysis, patients with high LMR (>3.43) at diagnosis had better PFS, with an adjusted HR of 0.31 (95% CI 0.13, 0.72) (figure 2). However, NLR cut-off levels did not show strong evidence of association with PFS (adjusted HR: 1.10, 95% CI 0.50, 2.38) (table 3).

Table 3 Multivariate analyses for PFS of LMR and NLR at diagnosis, n = 88

	Adjusted HR*	95% CI	p-value
LMR: >3.43 vs. ≤3.43 (reference)	0.31	0.13–0.72	0.006
FLIPI: high risk vs. low/ intermediate risk (reference)	2.15	0.91–5.07	0.079
Sex: male vs. female (reference)	1.51	0.67–3.36	0.318
Rituximab: yes vs. no. (reference)	0.16	0.05–0.49	0.001
	Adjusted HR*	95% CI	p-value
NLR: >2.78 vs. ≤2.78 (reference)	1.10	0.50–2.38	0.817
FLIPI: high risk vs. low/ intermediate risk (reference)	2.64	1.13–6.14	0.024
Sex: male vs. female (reference)	1.14	0.53–2.45	0.745
Rituximab: yes vs. no. (reference)	0.20	0.07–0.58	0.003

*Adjusted for all other covariates in the table

Sensitivity analysis

Sensitivity analysis showed that LMR was consistently associated with PFS under different model specifications and multivariate adjustments. In multivariate analyses, the assumption of proportional hazard was met. However, the strength of the evidence for differences in OS by LMR and NLR levels was weak.

PFS was different across the levels of FLIPI categories and cut-off values of LMR (3.43). However, the strength of the evidence was low for the interaction between the FLIPI score and LMR (Interaction test p-value 0.050). PFS tends to increase with patients having LMR above cut-off and low FLIPI score (data not shown).

DISCUSSION

To our knowledge, this study is the first to report the clinical and prognostic implications of pre-treatment NLR in patients with FL. Our findings demonstrated notable differences in clinical behaviour and outcome between the low and high LMR groups at diagnosis and NLR groups at the time of relapse. Previous studies reported that NLR is a predictor of mortality in

several cancer types, including gastric,[36, 37] and colorectal cancer. [38] One possible underlying mechanism is inflammatory reaction, which has been reported to be involved in tumour growth, invasion, metastasis, and resistance to treatment.[23-26]

The factors included in FLIPI[2] are primarily related to tumour burden (stage, serum LDH, and number of nodal site involvement) and patient characteristics (age and haemoglobin). Cell count ratio at diagnosis is a simple tool that assesses the host's immune homoeostasis, inflammatory state,[23, 24] and the tumour microenvironment.[14, 15] We obtained strong evidence to support these prognostic factors to possess practical clinical utility and significance. A recent study[39] surveying on groups of haematologists and oncologists in the United States and emerging markets, including Brazil, Mexico, Russia, and Turkey, reported that across all markets, less than 50% of physicians considered rituximab easy to access from a cost perspective. Our study contained patients treated with and without rituximab and showed that the cell ratio is independently prognostic in FL, our result is also useful for area where rituximab is less accessible. Besides, inferring from the above survey, the cost is a major concern for lots of health systems around the globe, cell count ratio, compared with FLIPI, is a simpler and cheaper alternative. In the present study, LMR played a significant role in predicting the PFS and NLR in post-progression survival; however, the strength of the evidence for OS was weak. This weak evidence may be attributed to inadequate sample size and few patients in the study died, and interaction with other parameters or unknown confounding. Moreover, the availability of salvage treatments upon progression makes OS difference difficult to demonstrate.

Cell count or its ratio at diagnosis may be used to decide which among the treatment strategies to use, including watchful waiting, RT, or systemic treatment. Previous studies showed that lymphocytes have an important role in mediating the antitumour effect of rituximab.[40-42] For those with low LMR, the disease may progress earlier, and closer

follow-up may be indicated. We separated patients into FLIPI low/intermediate and high risk and then incorporated biological factor (LMR) into known clinical prognostic factor (FLIPI) for FL. Based on the findings, future study can be conducted to compare the utility of cell count ratios with other established prognostic factors in an independent validation cohort and to explore the therapeutic strategies based on cell count ratio (i.e., observation alone versus early initiation of treatment), ideally in a prospective manner. Most studies are subject to a certain degree of misclassification related to measurement error.[43] However, Our study population followed standardised investigation procedures; any misclassification is likely to be non-differential.

The incidence and spectrum of NHL differ between the Chinese and Western populations, and risk of FL is rarer in the former.[44-46] Our sample size is comparable to other retrospective FL studies in Asia, ranging between 40 to 50 patients.[44, 47] Both genetic and environmental factors play a part in governing the overall incidence, as shown by migration studies.[45] We analysed a group of patients with definitive RT as treatment, with a sample size of 18 only. The results did not reach statistical significance; a bigger cohort or even a dedicated prospective study would be interesting. In our data, the complete blood count did not differentiate the subtypes of B and T lymphocytes and monocytes. Therefore, information regarding patient outcomes with a combination of different subtypes of immune cells was not explored in this study. Furthermore, in our study the distribution of blood cells may be different when leukocytosis or leukopenia is present. Moreover, evidence of correlation of age and circulating white blood cell counts has been reported, and a decrease in total lymphocyte counts is observed in the elderly when compared to younger adults.[48] Also, the treatments may have interaction with other factors, such as age and performance status. We did not analyse the association between cell count ratios and systemic treatment choices, duration and number of cycles, and salvage treatment upon progression because of the limited

sample size for subgroup analysis. Given the unavailability of beta-2 microglobulin in most patients, we did not analyse FLIPI2.

One merit of our study is the performance of sensitivity analysis to determine the robustness of the main findings in different scenarios. Sensitivity analysis was not available in numerous studies assessing relationship between cell count ratio and survival. Furthermore, we explored the effect of calendar year of diagnosis to account for potential improvement in life expectancy over study period due to change in environment or technological advancement in general medical care. Also, we accounted for the impact of the inclusion of rituximab as therapeutic option in early 2005 which is strongly correlated with an improved OS and PFS.

The external validity of this study is limited to a single institution. Thus, further evidence for validation of our results and multi-institutional studies with larger sample size are warranted. However, the strength of the evidence of our findings is still important given the clinical relevance of LMR and NLR capability to predict prognosis.

CONCLUSION

In this study, we demonstrated that LMR and NLR may provide independent and additional prognostic information for risk classification when used along with FLIPI in FL. These can be determined using widely available complete blood count test, which can be used as non-invasive and cost-effective choice to complement prognosis information for FL. Future prospective studies are necessary to validate the results of our study and evaluate the exact clinical significance.

ACKNOWLEDGMENTS

None to declare.

COMPLETING INTERESTS

None to declare.

FUNDING

None to declare.

DATA SHARING STATEMENT

Raw data can be obtained by contacting the authors at the corresponding address.

AUTHORSHIP STATEMENT

SFL developed the concept and design of the study. SFL analysed the data with MALF guidance. SFL wrote the manuscript. Both authors interpreted the data, drafted and revised the manuscript critically, and approved the final version of the manuscript. SFL is the guarantor of the paper.

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2016.

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Figure legend:

Figure 1 Kaplan–Meier curves. Kaplan-Meier estimate for (A) progression-free survival, and (B) overall survival of the whole study cohort (n = 88)

Figure 2 Adjusted progression-free survival. Estimate of (A) high and low LMR at diagnosis and (B) high and low NLR at diagnosis (n = 88)

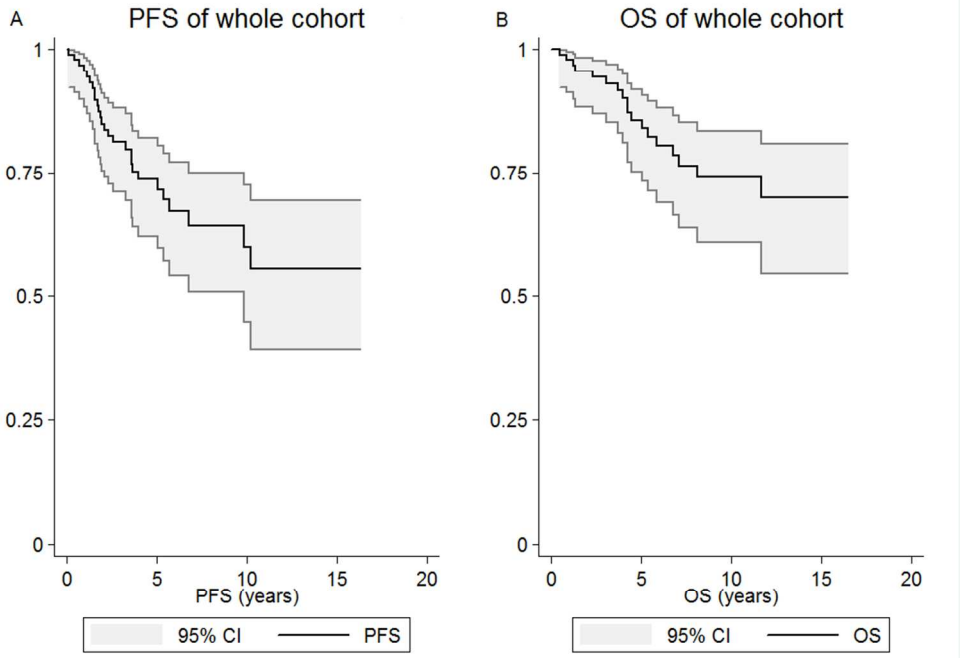

Figure 1 Kaplan–Meier curves. Kaplan-Meier estimate for (A) progression-free survival, and (B) overall survival of the whole study cohort (n = 88)

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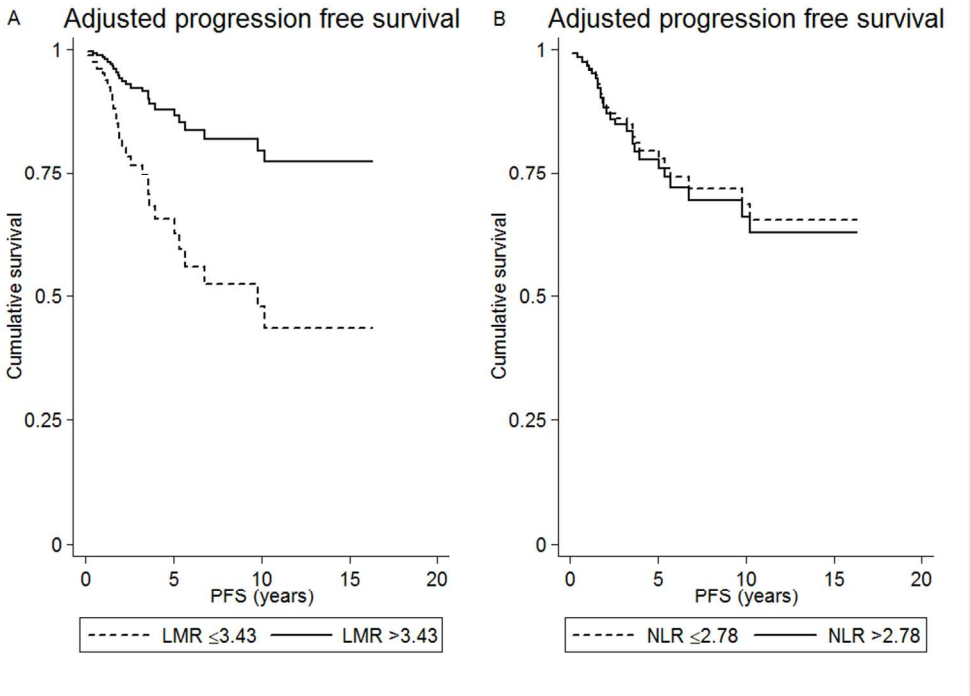


Figure 2 Adjusted progression-free survival. Estimate of (A) high and low LMR at diagnosis and (B) high and low NLR at diagnosis (n = 88)

364x265mm (300 x 300 DPI)

Supplementary table S1. FLIPI

Parameters	Definition of risk factors
Nodal sites	>4 lymph node regions
Age	>60 years
Serum marker	Elevated LDH
Stage	Advanced (stages III–IV according to Ann Arbor staging)
Hemoglobin	<12 g/dL

0–1 risk factors: low risk

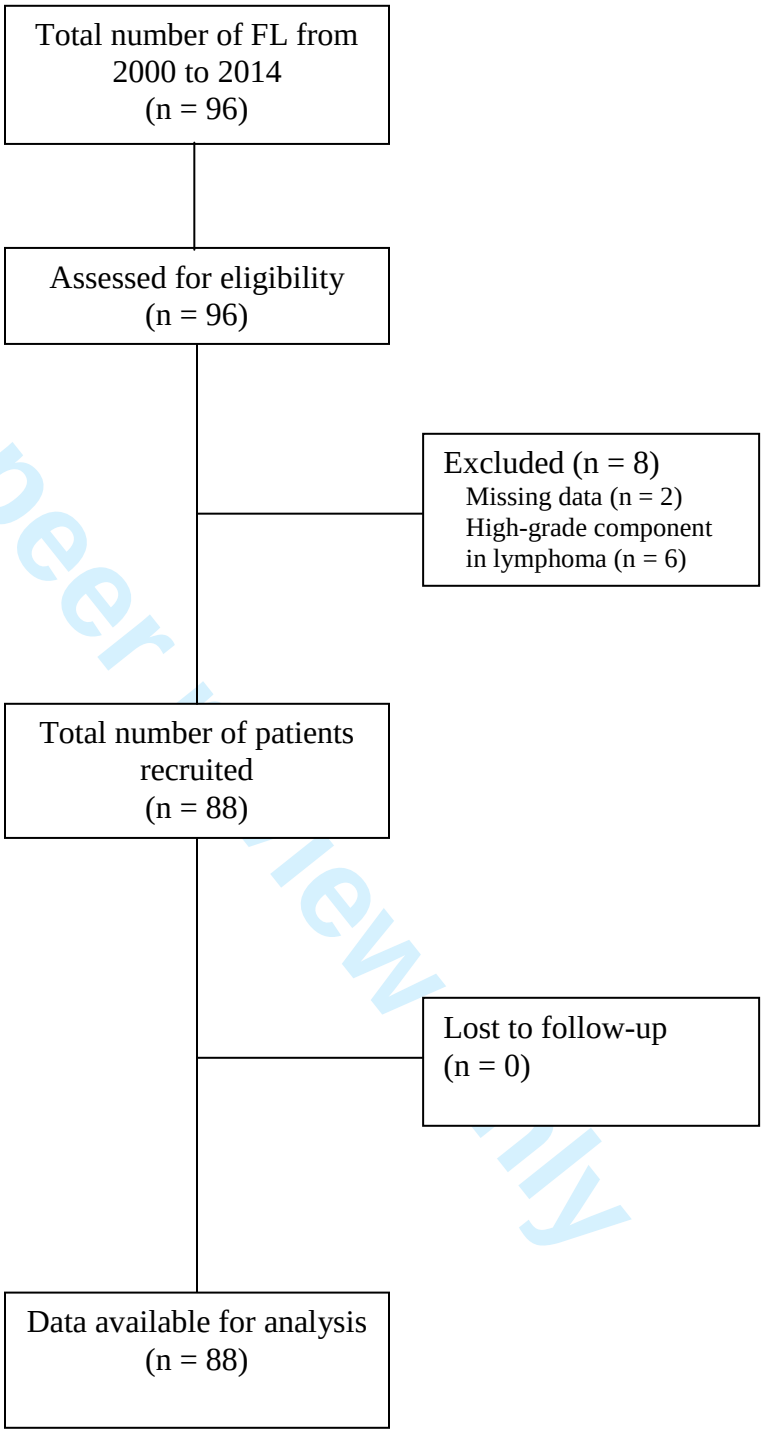
2 risk factors: intermediate risk

3–5 risk factors: high risk

Supplementary table S2. Sensitivity, specificity, positive predictive value, negative predictive value, likelihood ratio positive, likelihood ratio negative, and Youden's index for LMR and NLR

Cell count ratio cut-off values	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Likelihood ratio positive	Likelihood ratio negative	Youden's index
LMR: 3.43	94.4	64.3	40.5	97.8	2.6	0.1	0.60
NLR: 2.78	88.9	65.7	40.0	95.8	2.6	0.2	0.56

Supplementary figure S1 Patient recruitment and follow-up flow diagram



STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract **[Within the title page 1 and method section of abstract]**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **[See abstract page 1]**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported **[main manuscript page 1-2]**
Objectives	3	State specific objectives, including any prespecified hypotheses [**main manuscript page 2**]
Methods		
Study design	4	Present key elements of study design early in the paper **[page 2-3]**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection **[page 2-3]**
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up **[page 2-3]**
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable **[page 3]**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group **[page 3-4]**
Bias	9	Describe any efforts to address potential sources of bias **[page 4]**
Study size	10	Explain how the study size was arrived at **[page 2-3]**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why **[page 3-4]**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **[page 4]**
		(*b*) Describe any methods used to examine subgroups and interactions **[page 4]**
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses **[page 4]**
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed **[supplementary figure 1]**
		(b) Give reasons for non-participation at each stage **[supplementary figure 1]**
		(c) Consider use of a flow diagram **[supplementary figure 1]**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders **[Table 1 on page 5]**
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount) **[page 4]**
Outcome data	15*	Report numbers of outcome events or summary measures over time **[page 6-8]**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were

		adjusted for and why they were included **[page 6-7]**
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses **[page 8]**
Discussion		
Key results	18	Summarise key results with reference to study objectives page **[page8-9]**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias **[page 9-10]**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence **[page 11]**
Generalisability	21	Discuss the generalisability (external validity) of the study results **[page 11]**
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based **[Within Funding section]**

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

BMJ Open

Prognostic value of lymphocyte to monocyte ratio and neutrophil to lymphocyte ratio in follicular lymphoma: a retrospective cohort study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-017904.R1
Article Type:	Research
Date Submitted by the Author:	21-Aug-2017
Complete List of Authors:	Lee, Shing Fung; Department of Clinical Oncology Luque, Miguel-Angel; London School of Hygiene and Tropical Medicine, Epidemiology and Population Health
Primary Subject Heading:	Oncology
Secondary Subject Heading:	Patient-centred medicine, Haematology (incl blood transfusion)
Keywords:	neutrophils, monocytes, lymphocytes, Lymphoma < ONCOLOGY, survival, prognosis

Original Article

Title: Prognostic value of lymphocyte to monocyte ratio and neutrophil to lymphocyte ratio in follicular lymphoma: A retrospective cohort study

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Word count: abstract: 232; text: 3494 (including article summary, table and figure legends); references: 49; tables: 3; figures: 2; supplementary tables: 2; supplementary figure: 1

ABSTRACT

Objectives The clinical course and prognosis of follicular lymphoma (FL) are diverse and associated with the patient's immune response. We investigated the lymphocyte to monocyte ratio (LMR) and neutrophil to lymphocyte ratio (NLR) as prognostic factors in patients with FL, including those receiving radiotherapy.

Design A retrospective cohort study.

Setting Regional cancer centre in Hong Kong.

Participants 88 patients with histologically proven FL diagnosed between 2000 and 2014.

Materials and methods The best LMR and NLR cut-off values were determined using cross-validated areas under the receiver-operating characteristic curves. The extent to which progression-free survival (PFS) and overall survival differed by NLR and LMR cut-off values were assessed using Kaplan–Meier analysis and log-rank tests. A Cox proportional hazards model was fitted to adjust for confounders.

Results The best cut-off values for LMR and NLR were 3.20 and 2.18, respectively. The 5-year PFS was 73.6%. After multivariate adjustment, high LMR (>3.20) at diagnosis was associated with superior PFS, with a hazard ratio (HR) of 0.31 (95% confidence interval [CI] 0.13, 0.71), whereas high NLR at relapse was associated with poorer post-progression survival (HR 1.24, 95% CI 1.04, 1.49).

Conclusions Baseline LMR and NLR at relapse were shown to be independent prognostic factors in FL. LMR and NLR are cheap and widely available biomarkers that could be used in combination with the Follicular Lymphoma International Prognostic Index by clinicians to better predict prognosis.

Keywords: neutrophils, monocytes, lymphocytes, lymphoma, survival, prognosis

ARTICLE SUMMARY

Strengths and limitations of this study

- We obtained strong evidence in support of NLR and LMR as prognostic factors that possess practical clinical utility and significance in follicular lymphoma.
- Sensitivity analysis was performed to determine the robustness of the main findings in different scenarios.
- Association between cell count ratios and systemic treatment choices, duration and number of cycles, and salvage treatment upon progression were not analysed because of a limited sample size for subgroup analysis.

INTRODUCTION

Follicular lymphoma (FL) accounts for approximately 20% of all incident lymphoma cases, making it the most common indolent non-Hodgkin lymphoma (NHL). The clinical course and prognosis of FL are diverse.[1-6] Clinical and laboratory parameters assist in predicting prognosis, allow for tailoring appropriate therapies, and aid in selecting patients for appropriate clinical trials. The commonly used criteria include the Groupe d'Etude des Lymphomes Folliculaires criteria,[7] Follicular Lymphoma International Prognostic Index (FLIPI),[2] and FLIPI2.[8] FLIPI is a clinical prognostic score and classifies patients into risk categories: low, intermediate, and high risk. It does not include parameters associated with tumour microenvironment or host anti-tumour immune response.

About 20% of FL patients do not respond to or experience progression within 2 years of treatment; early relapse manifests in a subgroup of patients who are at a substantially greater risk of death, and their median OS is only 5 years.[9] These cases of high-risk FL may have a distinct biology, but it is not easily identified at diagnosis; even patients with high-risk disease defined by the commonly employed FLIPI [2] could have prolonged survival with modern therapy. A biologic rationale to account for this heterogeneity in patient outcomes would provide insights that may influence disease monitoring and treatment strategy.

Advances in gene expression profiling allow us to elucidate the role of stromal, non-malignant cells in the pathogenesis and progression of lymphoma. Immune response-1 and immune response-2 are two types of immune responses.[10] Dave et al. discovered that most of the component genes in prognostically unfavourable immune response-2 signatures are expressed more strongly in the non-malignant component of tumours.[10] Many genes in the immune response-2 signature are highly expressed by peripheral blood monocytes. Furthermore, monocyte chemoattractant protein, a potent chemotactic factor for monocytes, and its receptor CC chemokine receptor 2, are shown to play roles in modulating

inflammatory responses, tumour proliferation, angiogenesis, and metastasis.[11, 12] Their levels of expression are correlated with cancer prognosis. In addition, myeloid-derived suppressor cells are reported to have immune suppressive functions.[13-15] Increasing numbers of monocytes, macrophages, or their precursors have been detected in lymphomatous nodes.[13, 16] Recent studies have indicated that the peripheral blood lymphocyte-to-monocyte ratio (LMR) at diagnosis can predict long-term outcome in patients with diffuse large B-cell lymphoma,[17] FL,[18, 19], and Hodgkin lymphoma (HL).[20-22] This evidence indicates that monocytes are an important component of the tumour microenvironment.

On the other hand, absolute neutrophil count (ANC), a surrogate marker of inflammation produced by the tumour,[23-26] is utilised in the form of peripheral blood neutrophil-to-lymphocyte ratio (NLR) at diagnosis to predict survival in diffuse large B-cell lymphoma [17, 27] and HL.[28] The rationale behind utilising these cell count ratios is to consider the interaction among components of host immunity represented by lymphocytes, inflammation produced by the tumour, and the tumour microenvironment. However, studies on FL mainly focus on patients who were treated with rituximab-containing chemotherapy, with little emphasis on those who underwent radiotherapy (RT) as a component of or as a primary treatment. Moreover, the prognostic role of NLR in FL in terms of survival outcomes has not been studied. Therefore, we aimed to investigate the extent to which NLR at diagnosis predicts survival outcomes in patients with FL, including those who were treated with RT. We also evaluated whether NLR can be used in combination with FLIPI to improve prognosis prediction.

MATERIALS AND METHODS

Study design, setting, and participants

We performed a longitudinal study using retrospective information from electronic medical

records of patients with incident FL treated in Tuen Mun Hospital, Hong Kong. All FL incident cases from 2000 to 2014 were identified (n = 88). We restricted the analysis to patients with complete laboratory, pathology, and radiological data in the medical records (Supplementary Figure S1). The sociodemographic information of the excluded patients was not different from that of the included patients in the final sample. Patients were followed up for a median of 5.88 (range 0.49–16.45) years. The peripheral blood count results were obtained from a standard automated complete blood count machine. This study was approved by the Clinical and Research Ethics Committee of the Tuen Mun Hospital, Tuen Mun, Hong Kong (NTWC/CREC/16107). The research was conducted in accordance with the 1964 Declaration of Helsinki and its later amendments.

Data and variables

Overall survival (OS) and progression-free survival (PFS) were the main outcomes of the study. These outcomes were defined and measured as per criteria from the International Harmonization Project.[29] OS was defined as the time from diagnosis until death as a result of any cause or the last follow-up visit. PFS was defined as the time from diagnosis until lymphoma progression (first date of documentation of a new lesion or enlargement of a previous lesion) or death as a result of any cause or last follow-up visit. For both OS and PFS, patients were censored at their last follow-up visit. Patients' demographics and disease characteristics were collected. The FLIPI score was then calculated using those factors (nodal sites, age, serum lactate dehydrogenase, stage, and haemoglobin) (see Supplementary Table S1).[2] Chemotherapy involved cyclophosphamide, vincristine, and prednisolone or cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP) or CHOP-like regimens.

Statistical methods

We initially described the cohort of patients using ranges (minimum, maximum), means and

standard deviations for continuous variables, and proportions for categorical variables. To evaluate LMR and NLR performance in predicting mortality, we fitted two logistic models with cancer-related death as the outcome and LMR and NLR as continuous independent predictors. Data-adaptive methods based on Bayesian and Akaike information criteria were used to determine the impact of any associated factors and to identify the best fitting model. Subsequently, we computed marginal probabilities for the outcome based on two different weighted logistic models, with time and NLR as independent predictors for the first model and time and LMR for the second model. Weights were accounted for the inverse probability of censoring.[30] Then we derived cross-validated areas under the curve (AUC) [31]; afterward we chose the best cut-off values based on the cross-validated sensitivity, specificity, and the Youden's indices (sensitivity + specificity − 1). Respective LMR and NLR cut-off values were determined at a point with the maximum Youden's index on the receiver operating characteristic (ROC) curve.[32, 33] To evaluate the extent to which OS and PFS differ by LMR and NLR cut-off values, we used incidence, rate ratios, Kaplan–Meier analysis, and log-rank tests [34, 35] for statistical inference. We also used semi-parametric Cox proportional hazards models to evaluate OS and PFS for the computed LMR and NLR cut-off values adjusted for FLIPI, use of rituximab, and sex.[36, 37] Finally, we developed a sensitivity analysis to evaluate the robustness of our findings in the multivariate analysis including different model specifications to account for nonlinearities and the interaction between rituximab and LMR/NLR levels. The proportional hazard assumption for multivariate-adjusted Cox models was also assessed based on the analysis of the Schoenfeld residuals. We used Stata v.14.2 (StataCorp, College Station, TX, USA) for the statistical analysis.

RESULTS

Description of the cohort

The median age at diagnosis of the patients included in the study was 54 years (range 22–87 years). Among them, 18 died during the follow-up period. Thirteen patients died due to the lymphoma. Five deaths were non-lymphoma related: one patient developed prostate cancer and died of pneumonia, another three died of community-acquired pneumonia, and one died of acute coronary syndrome and renal failure. The estimated 5-year PFS and OS were 73.6% and 85.6% respectively (figure 1). At diagnosis, 18.2%, 21.6%, and 60.2% were classified as being at low, intermediate, and high risk according to the FLIPI score. Table 1 shows the descriptive summary statistics of patients included in the study.

Table 1 Descriptive summary statistics for the best cut-offs of LMR and NLR according to patient clinical characteristics, n = 88

Characteristics	All patients (n = 88)	LMR >3.20 (n = 49)	LMR ≤3.20 (n = 39)	NLR >2.18 (n = 57)	NLR ≤2.18 (n = 31)
Age, years					
Median (range)	54 (22–87)	53 (22–87)	54 (31–78)	55 (31–87)	52 (22–77)
>60, n (%)	29 (33.0)	16 (32.7)	13 (33.3)	24 (42.1)	5 (16.1)
≤60, n (%)	59 (67.0)	33 (67.4)	26 (66.7)	33 (57.9)	26 (83.9)
Sex, n (%)					
Male	47 (53.4)	29 (59.2)	18 (46.2)	27 (47.4)	20 (64.5)
Female	41 (46.6)	20 (40.8)	21 (53.9)	30 (52.6)	11 (35.5)
FLIPI, n (%)					
Low risk (scores 0–	16 (18.2)	9 (18.4)	7 (18.0)	6 (10.5)	10 (32.3)

1)					
Intermediate risk (score 2)	19 (21.6)	13 (26.5)	6 (15.4)	10 (17.5)	9 (29.0)
High risk (scores 3–5)	53 (60.2)	27 (55.1)	26 (66.7)	41 (71.9)	12 (38.7)
ANC (10^9/L), median (range)	4.2 (1.9–10.7)	3.7 (1.9–7.9)	4.6 (2.1–10.7)	4.6 (2.1–10.7)	3.5 (1.9–7.9)
ALC (10^9/L), median (range)	1.6 (0.6–11.3)	1.9 (0.7–11.3)	1.1 (0.6–3.1)	1.2 (0.6–3.1)	2.1 (1.4–11.3)
AMC (10^9/L), median (range)	0.4 (0.1–1.2)	0.4 (0.1–0.9)	0.5 (0.2–1.2)	0.4 (0.1–1.2)	0.4 (0.2–1.1)
NLR, median (range)	2.76 (0.59–9.91)	2.15 (0.59–8.50)	3.83 (1.81–9.91)	3.50 (2.20–9.91)	1.73 (0.59–2.18)
LMR, median (range)	3.80 (0.55–22.60)	5.00 (3.43–22.60)	2.33 (0.55–3.20)	3.00 (0.55–8.00)	5.33 (2.82–22.60)
LDH >220 IU/L, n (%)	70 (80.5)	39 (81.3)	31 (79.5)	47 (82.5)	23 (76.7)
Stage, n (%)					
I/II	24 (27.3)	14 (28.6)	10 (25.6)	11 (19.3)	13 (41.9)
III/IV	64 (72.7)	35 (71.4)	29 (74.4)	46 (80.7)	18 (58.1)

Hb <12 g/dL, n (%)	23 (26.1)	14 (28.6)	9 (23.1)	15 (26.3)	8 (25.8)
Number of nodal sites >4, n (%)	50 (56.8)	24 (49.0)	26 (66.7)	37 (64.9)	13 (41.9)
Use of rituximab, n (%)	38 (43.2)	19 (38.8)	19 (48.7)	27 (47.4)	11 (35.5)
Treatment, n (%)					
Chemotherapy plus RT	14 (15.9)	9 (10.2)	5 (5.7)	8 (9.1)	6 (6.8)
Chemotherapy alone	54 (61.4)	26 (29.5)	28 (31.8)	40 (45.5)	14 (15.9)
RT alone	14 (15.9)	9 (10.2)	5 (5.7)	6 (6.8)	8 (9.1)

Abbreviations: LMR, lymphocyte to monocyte ratio; NLR, neutrophil to lymphocyte ratio; FLIPI, Follicular Lymphoma International Prognostic Index; ANC, absolute neutrophil count; ALC, absolute lymphocyte count; AMC, absolute monocyte count; LDH, lactate dehydrogenase; RT, radiotherapy.

Only 18 patients underwent RT as a definitive treatment for limited stages I and II. Involved-field irradiation, in which the RT fields were limited to the involved nodal region, was mostly administered with two parallel opposed fields; the median radiation dose was 40 Gy (range 30–54 Gy). Ten other patients with stage III or IV disease received RT during their disease course, as part of palliation or as consolidation therapy to sites demonstrating an inadequate response to systemic treatment. High-grade transformation occurred in 6 out of 27 patients with relapse. Peripheral blood counts were available at the time of the relapse.

Progression-free survival

The AUCs of LMR and NLR were 0.90 (95% confidence interval [CI] 0.84, 0.97) and 0.87 (95% CI 0.77, 0.96), respectively, and they did not differ in terms of predictive performance for PFS (test equality of ROC areas, p-value 0.470). An LMR cut-off value of 3.20 (positive predictive value 22.4% and negative predictive value 82.1%; sensitivity 61.1% and specificity 45.7%) and NLR cut-off value of 2.18 (positive predictive value 21.1% and negative predictive value 80.6%; sensitivity 66.7% and specificity 35.7%) showed the greatest Youden's index, corresponding to maximum joint sensitivity and specificity on the ROC curve (Supplementary Table S2).

NLR and LMR mortality predictive performance

The median NLR and LMR at diagnosis were 2.77 (range 0.59–9.91) and 3.80 (range 0.55–22.60), respectively. The median NLR and LMR at relapse were 2.67 (range 0.95–17.25) and 3.33 (range 0.48–8.5), respectively.

NLR at relapse was associated with post-progression survival as a continuous variable (hazard ratio [HR] 1.24, 95% CI 1.04, 1.49). In the univariate analysis presented in Table 2, high LMR (>3.20) had a superior PFS with a rate ratio (RR) of 0.34 (95% CI 0.16, 0.74). Patients with a high FLIPI score had a 2.5 times greater risk of death or relapse than patients with a lower score (RR: 2.52, 95% CI 1.10, 5.75). However, patients treated with rituximab had a 72% lower risk of death or relapse (RR: 0.28 95% CI 0.10, 0.81). We found evidence of a linear association between PFS and the calendar period (p-value of trend = 0.003). Compared with the period 2000–2005, those patients diagnosed during 2010–2014 had a 90% lower risk of death or relapse (RR: 0.10 95% CI 0.01, 0.75). Furthermore, there was no evidence of differences in PFS by sex (male vs female, RR: 1.09, 95% CI 0.51–2.32). LMR at relapse showed a weak association with post-progression survival (HR 1.06, 95% CI 0.77, 1.45).

Table 2 Rate ratios of PFS events, n = 88

		Cases/1000 person years	Rate (per 1000 person years)	Rate ratio	95% CI	p-value
LMR	>3.20	11	34.66	0.34	0.16–0.74	0.004
	≤3.20	16	101.28	1		
NLR	>2.18	18	67.37	1.56	0.70–3.47	0.273
	≤2.18	9	43.23	1		
FLIPI	High risk	19	82.30	2.52	1.10–5.75	0.023
	Low/ intermediate risk	8	32.72	1		
Sex	Male	15	58.96	1.09	0.51–2.32	0.831
	Female	12	54.29	1		
Rituximab	Yes	4	22.02	0.28	0.10–0.81	0.012
	No	23	78.31	1		
Year of diagnosis	2010–2014	1	8.71	0.10	0.01–0.75	0.006
	2006–2010	5	43.05	0.50	0.19–1.33	0.157
	2000–2005	21	85.91	1		

In the multivariate analysis, patients with a high LMR (>3.20) at diagnosis had a longer PFS, with an adjusted HR of 0.31 (95% CI 0.13, 0.71) (Figure 2). However, NLR cut-off levels did not show strong evidence of an association with PFS (adjusted HR 1.33, 95% CI 0.57, 3.10) (Table 3).

Table 3 Multivariate analyses of PFS with LMR and NLR at diagnosis, n = 88

	Adjusted	95% CI	p-value

	HR*		
LMR: >3.20 vs. ≤3.20 (reference)	0.31	0.13–0.71	0.006
FLIPI: high risk vs. low/ intermediate risk (reference)	2.17	0.92–5.10	0.075
Sex: male vs. female (reference)	1.50	0.67–3.34	0.321
Rituximab use: yes vs. no. (reference)	0.16	0.05–0.48	0.001
	Adjusted HR*	95% CI	p-value
NLR: >2.18 vs. ≤2.18 (reference)	1.33	0.57–3.10	0.511
FLIPI: high risk vs. low/ intermediate risk (reference)	2.47	1.03–5.89	0.042
Sex: male vs. female (reference)	1.15	0.53–2.48	0.721
Rituximab use: yes vs. no. (reference)	0.19	0.07–0.57	0.003

*Adjusted for all other covariates in the table

Sensitivity analysis

Sensitivity analysis showed that LMR was consistently associated with PFS under different model specifications and multivariate adjustments. We evaluated the multivariate analyses, and found that the assumption for the proportional hazard was met. However, the strength of the evidence for differences in OS by LMR and NLR levels was weak.

PFS tended to increase with LMRs above the cut-off and low FLIPI scores (RR LMR >3.2 and low FLIPI 0.17, 95% CI 0.04, 0.70 vs. RR LMR>3.2 and high FLIPI 0.60, 95% CI 0.24, 1.50), but evidence of a statistical interaction was weak (interaction p-value 0.171). There was some evidence of a statistical interaction between LMR and rituximab (interaction p-value 0.024, HR of the interaction term 17.1, 95% CI 1.46, 199.36).

DISCUSSION

To our knowledge, this study is the first to report the clinical and prognostic implications of pre-treatment NLR in patients with FL. Our findings demonstrated notable differences in clinical behaviour and outcome between the low and high LMR groups at diagnosis and NLR groups at the time of relapse. Previous studies reported that NLR is a predictor of mortality in several cancer types, including gastric [38, 39] and colorectal cancer.[40] One possible underlying mechanism is the inflammatory reaction, which has been reported to be involved in tumour growth, invasion, metastasis, and resistance to treatment.[23-26]

The factors included in FLIPI [2] are primarily related to tumour burden (stage, serum lactate dehydrogenase, and number of nodal site involvement) and patient characteristics (age and haemoglobin). Cell count ratio at diagnosis is a simple tool that assesses the host's immune homoeostasis, inflammatory state,[23, 24] and the tumour microenvironment.[14, 15] We obtained strong evidence in support of these prognostic factors possessing practical clinical utility and significance. In the present study, LMR played a significant role in predicting the PFS and NLR in post-progression survival; however, the strength of the evidence for OS was weak. This weak evidence may be attributed to the inadequate sample size and the few deaths observed, along with the interaction with other parameters or unknown confounding. Moreover, the availability of salvage treatments upon progression makes the difference in OS difficult to demonstrate.

Cell count or its ratio at diagnosis may be used to decide which treatment strategy is most appropriate, including watchful waiting, RT, or systemic treatment. Previous studies showed that lymphocytes have an important role in mediating the antitumor effect of rituximab.[41-43] For those with low LMR, the disease may progress earlier, and closer follow-up may be indicated. We separated patients into FLIPI-based low/intermediate and high-risk groups and then incorporated a biological factor (LMR) into a known clinical prognostic factor (FLIPI). Based on the findings of our study, future studies should aim to understand the utility of cell

count ratios with other established prognostic factors in an independent validation cohort and to explore the therapeutic strategies based on cell count ratio (i.e., observation alone versus early initiation of treatment), ideally in a prospective manner.

Most studies are subject to a certain degree of misclassification related to measurement error.[44] However, our study population followed standardised investigation procedures; any misclassification is likely to be non-differential. The incidence and spectrum of NHL cases differ between the Chinese and Western populations, and the risk of FL is lower in the former group.[45-47] However, our sample size is comparable to those in other retrospective FL studies in Asia, ranging between 40 and 50 patients.[45, 48] Both genetic and environmental factors play a part in governing the overall incidence, as shown by migration studies.[46] Our lymphoma treatment regimens were not completely uniform in this analysis and involved a modest sample size, which may have introduced selection bias. We analysed a group of patients receiving definitive RT as treatment, with a sample size of only 18. The results did not reach statistical significance; a bigger cohort or even a dedicated prospective study would be interesting. In our data, the complete blood count did not differentiate the subtypes of B and T lymphocytes and monocytes. Therefore, information regarding patient outcomes with a combination of different subtypes of immune cells was not explored in this study. Furthermore, in our study, the distribution of blood cells may be different when leucocytosis or leukopenia is present. Moreover, evidence of a correlation between age and circulating white blood cell counts has been reported, and a decrease in total lymphocyte counts is observed more frequently in the elderly than in younger adults.[49] Also, the treatments may interact with other factors, such as age and performance status. There appears to be an interaction between rituximab and LMR. However, the 95% CI of the interaction term was wide; this reflected the small numbers and data sparsity for secondary analysis, and therefore, no conclusive evidence can be extrapolated. We did not analyse the association

between cell count ratios and systemic treatment choices, duration and number of cycles, and salvage treatment upon progression because of the limited sample size for the subgroup analysis. Given the unavailability of beta-2 microglobulin in most patients, we did not analyse FLIPI2.

One merit of our study is the performance of a sensitivity analysis to determine the robustness of the main findings in different scenarios. A sensitivity analysis was not conducted in numerous studies assessing the relationship between cell count ratio and survival. Furthermore, we explored the effect of calendar year of diagnosis to account for potential improvement in life expectancy over the study period due to changes in the environment or technological advancement in general medical care. Also, we accounted for the impact of the inclusion of rituximab as a therapeutic option in early 2005, which is strongly correlated with an improved OS and PFS.

The external validity of this study is limited to a single institution. Thus, further evidence for validation of our results and multi-institutional studies with larger sample sizes are warranted. However, the strength of the evidence of our findings is still important given the clinical relevance of LMR and NLR capability to predict prognosis.

CONCLUSION

In this study, we demonstrated that LMR and NLR may provide independent and additional prognostic information for risk classification when used along with FLIPI in FL. These can be determined using widely available complete blood count tests, which can be used as non-invasive and cost-effective alternatives to complement prognosis data for FL. Future prospective studies are necessary to validate the results of our study and evaluate the exact clinical significance.

ACKNOWLEDGMENTS

None to declare.

COMPLETING INTERESTS

None to declare.

FUNDING

None to declare.

DATA SHARING STATEMENT

Raw data can be obtained by contacting the authors at the corresponding address.

AUTHORSHIP STATEMENT

SFL developed the concept and design of the study. SFL analysed the data with MALF guidance. SFL wrote the manuscript. Both authors interpreted the data, drafted and revised the manuscript critically, and approved the final version of the manuscript. SFL is the guarantor of the paper.

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Figure legends:

Figure 1 Kaplan–Meier curves. Kaplan-Meier estimate for (A) progression-free survival and (B) overall survival of the whole study cohort (n = 88)

Figure 2 Adjusted progression-free survival. Estimate of (A) high and low LMR at diagnosis and (B) high and low NLR at diagnosis (n = 88)

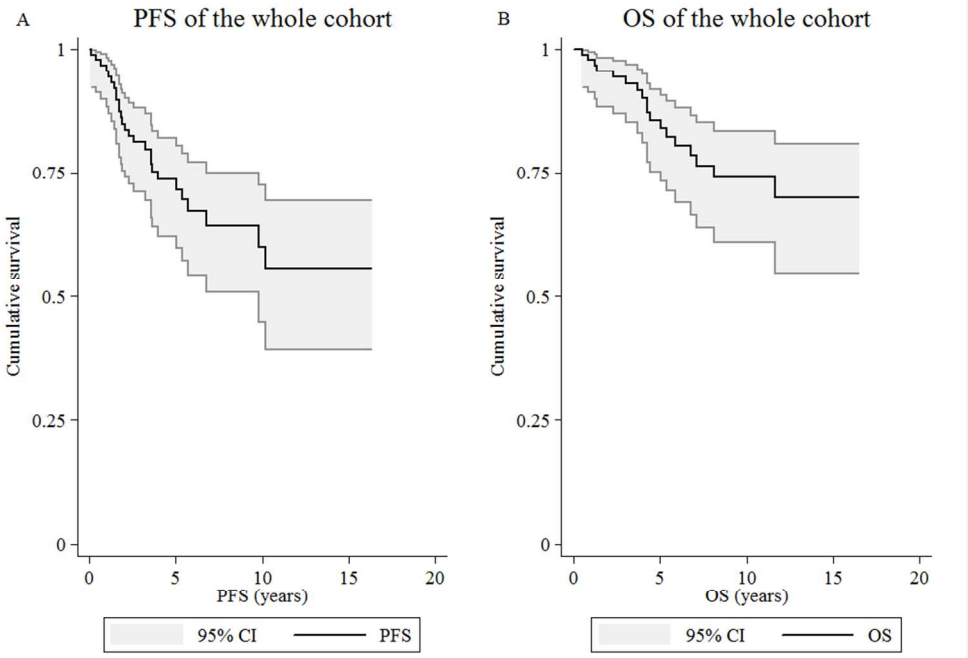

Figure 1 Kaplan–Meier curves. Kaplan-Meier estimate for (A) progression-free survival and (B) overall survival of the whole study cohort (n = 88)

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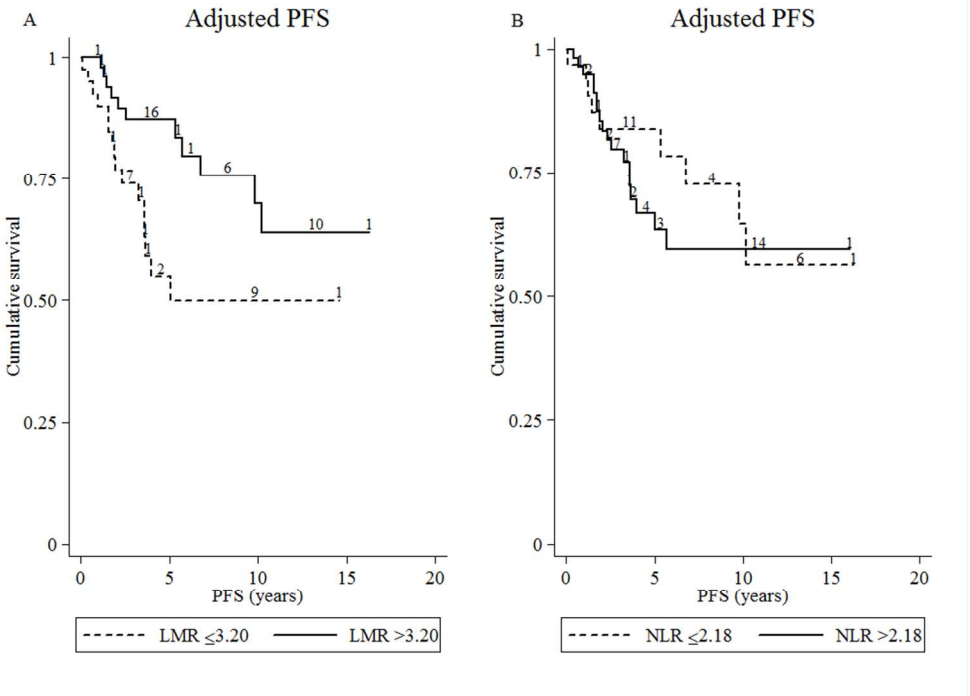


Figure 2 Adjusted progression-free survival. Estimate of (A) high and low LMR at diagnosis and (B) high and low NLR at diagnosis (n = 88)

364x265mm (300 x 300 DPI)

Supplementary Table S1. Follicular Lymphoma International Prognostic Index

Parameters	Definition of risk factors
Nodal sites	>4 lymph node regions
Age	>60 years
Serum marker	Elevated LDH
Stage	Advanced (stages III–IV according to Ann Arbor staging)
Haemoglobin	<12 g/dL

0–1 risk factors: low risk

2 risk factors: intermediate risk

3–5 risk factors: high risk

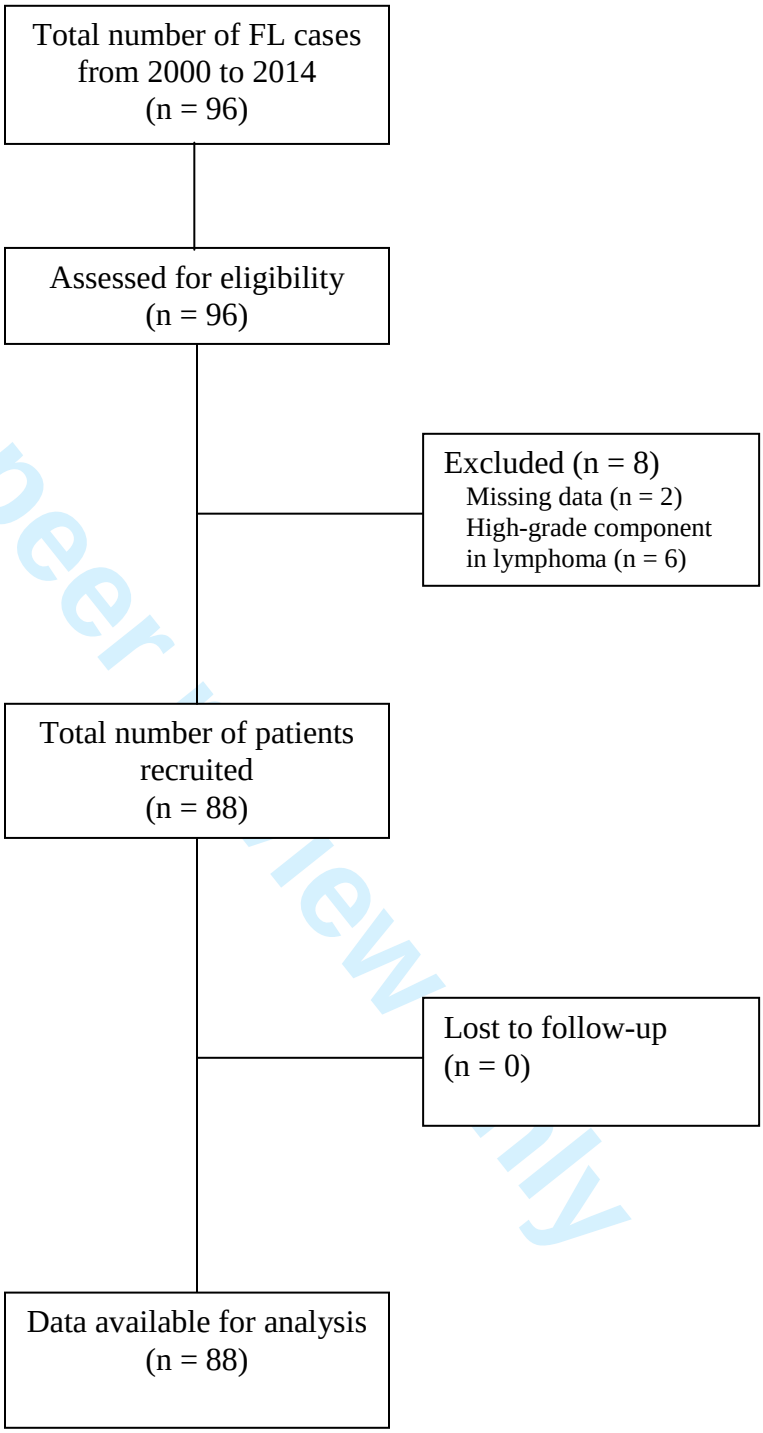
Abbreviations: LDH, lactate dehydrogenase

Supplementary Table S2. Sensitivity, specificity, positive predictive value, negative predictive value, likelihood ratio positive, likelihood ratio negative, and Youden's index for LMR and NLR

Cell count ratio cut-off values	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Likelihood ratio positive	Likelihood ratio negative	Youden's index
LMR: 3.20	61.1%	45.7%	22.4%	82.1%	1.1	0.9	0.60
NLR: 2.18	66.7%	35.7%	21.1%	80.6%	1.0	0.9	0.56

Abbreviations: LMR, lymphocyte to monocyte ratio; NLR, neutrophil to lymphocyte ratio

Supplementary Figure S1. Patient recruitment and follow-up flow diagram



STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract **[Within the title page 1 and method section of abstract]**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **[See abstract page 1]**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported **[main manuscript page 1-2]**
Objectives	3	State specific objectives, including any prespecified hypotheses [**main manuscript page 2**]
Methods		
Study design	4	Present key elements of study design early in the paper **[page 2-3]**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection **[page 2-3]**
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up **[page 2-3]**
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable **[page 3]**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group **[page 3-4]**
Bias	9	Describe any efforts to address potential sources of bias **[page 4]**
Study size	10	Explain how the study size was arrived at **[page 2-3]**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why **[page 3-4]**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **[page 4]**
		(*b*) Describe any methods used to examine subgroups and interactions **[page 4]**
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses **[page 4]**
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed **[supplementary figure 1]**
		(b) Give reasons for non-participation at each stage **[supplementary figure 1]**
		(c) Consider use of a flow diagram **[supplementary figure 1]**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders **[Table 1 on page 5]**
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount) **[page 4]**
Outcome data	15*	Report numbers of outcome events or summary measures over time **[page 6-8]**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were

		adjusted for and why they were included **[page 6-7]**
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses **[page 8]**
Discussion		
Key results	18	Summarise key results with reference to study objectives page **[page8-9]**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias **[page 9-10]**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence **[page 11]**
Generalisability	21	Discuss the generalisability (external validity) of the study results **[page 11]**
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based **[Within Funding section]**

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

BMJ Open

Prognostic value of lymphocyte to monocyte ratio and neutrophil to lymphocyte ratio in follicular lymphoma: a retrospective cohort study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-017904.R2
Article Type:	Research
Date Submitted by the Author:	21-Sep-2017
Complete List of Authors:	Lee, Shing Fung; Department of Clinical Oncology Luque, Miguel-Angel; London School of Hygiene and Tropical Medicine, Epidemiology and Population Health
Primary Subject Heading:	Oncology
Secondary Subject Heading:	Patient-centred medicine, Haematology (incl blood transfusion)
Keywords:	neutrophils, monocytes, lymphocytes, Lymphoma < ONCOLOGY, survival, prognosis

Original Article

Title: Prognostic value of lymphocyte to monocyte ratio and neutrophil to lymphocyte ratio in follicular lymphoma: A retrospective cohort study

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Word count: abstract: 232; text: 3494 (including article summary, table and figure legends); references: 49; tables: 3; figures: 2; supplementary tables: 2; supplementary figure: 1

ABSTRACT

Objectives The clinical course and prognosis of follicular lymphoma (FL) are diverse and associated with the patient's immune response. We investigated the lymphocyte to monocyte ratio (LMR) and neutrophil to lymphocyte ratio (NLR) as prognostic factors in patients with FL, including those receiving radiotherapy.

Design A retrospective cohort study.

Setting Regional cancer centre in Hong Kong.

Participants 88 patients with histologically proven FL diagnosed between 2000 and 2014.

Materials and methods The best LMR and NLR cut-off values were determined using cross-validated areas under the receiver-operating characteristic curves. The extent to which progression-free survival (PFS) and overall survival differed by NLR and LMR cut-off values were assessed using Kaplan–Meier analysis and log-rank tests. A Cox proportional hazards model was fitted to adjust for confounders.

Results The best cut-off values for LMR and NLR were 3.20 and 2.18, respectively. The 5-year PFS was 73.6%. After multivariate adjustment, high LMR (>3.20) at diagnosis was associated with superior PFS, with a hazard ratio (HR) of 0.31 (95% confidence interval [CI] 0.13, 0.71), whereas high NLR at relapse was associated with poorer post-progression survival (HR 1.24, 95% CI 1.04, 1.49).

Conclusions Baseline LMR and NLR at relapse were shown to be independent prognostic factors in FL. LMR and NLR are cheap and widely available biomarkers that could be used in combination with the Follicular Lymphoma International Prognostic Index by clinicians to better predict prognosis.

Keywords: neutrophils, monocytes, lymphocytes, lymphoma, survival, prognosis

ARTICLE SUMMARY

Strengths and limitations of this study

- We obtained strong evidence in support of NLR and LMR as prognostic factors that possess practical clinical utility and significance in follicular lymphoma.
- Sensitivity analysis was performed to determine the robustness of the main findings in different scenarios.
- Association between cell count ratios and systemic treatment choices, duration and number of cycles, and salvage treatment upon progression were not analysed because of a limited sample size for subgroup analysis.

INTRODUCTION

Follicular lymphoma (FL) accounts for approximately 20% of all incident lymphoma cases, making it the most common indolent non-Hodgkin lymphoma (NHL). The clinical course and prognosis of FL are diverse.[1-6] Clinical and laboratory parameters assist in predicting prognosis, allow for tailoring appropriate therapies, and aid in selecting patients for appropriate clinical trials. The commonly used criteria include the Groupe d'Etude des Lymphomes Folliculaires criteria,[7] Follicular Lymphoma International Prognostic Index (FLIPI),[2] and FLIPI2.[8] FLIPI is a clinical prognostic score and classifies patients into risk categories: low, intermediate, and high risk. It does not include parameters associated with tumour microenvironment or host anti-tumour immune response.

About 20% of FL patients do not respond to or experience progression within 2 years of treatment; early relapse manifests in a subgroup of patients who are at a substantially greater risk of death, and their median OS is only 5 years.[9] These cases of high-risk FL may have a distinct biology, but it is not easily identified at diagnosis; even patients with high-risk disease defined by the commonly employed FLIPI [2] could have prolonged survival with modern therapy. A biologic rationale to account for this heterogeneity in patient outcomes would provide insights that may influence disease monitoring and treatment strategy.

Advances in gene expression profiling allow us to elucidate the role of stromal, non-malignant cells in the pathogenesis and progression of lymphoma. Immune response-1 and immune response-2 are two types of immune responses.[10] Dave et al. discovered that most of the component genes in prognostically unfavourable immune response-2 signatures are expressed more strongly in the non-malignant component of tumours.[10] Many genes in the immune response-2 signature are highly expressed by peripheral blood monocytes. Furthermore, monocyte chemoattractant protein, a potent chemotactic factor for monocytes, and its receptor CC chemokine receptor 2, are shown to play roles in modulating

inflammatory responses, tumour proliferation, angiogenesis, and metastasis.[11, 12] Their levels of expression are correlated with cancer prognosis. In addition, myeloid-derived suppressor cells are reported to have immune suppressive functions.[13-15] Increasing numbers of monocytes, macrophages, or their precursors have been detected in lymphomatous nodes.[13, 16] Recent studies have indicated that the peripheral blood lymphocyte-to-monocyte ratio (LMR) at diagnosis can predict long-term outcome in patients with diffuse large B-cell lymphoma,[17] FL,[18, 19], and Hodgkin lymphoma (HL).[20-22] This evidence indicates that monocytes are an important component of the tumour microenvironment.

On the other hand, absolute neutrophil count (ANC), a surrogate marker of inflammation produced by the tumour,[23-26] is utilised in the form of peripheral blood neutrophil-to-lymphocyte ratio (NLR) at diagnosis to predict survival in diffuse large B-cell lymphoma [17, 27] and HL.[28] The rationale behind utilising these cell count ratios is to consider the interaction among components of host immunity represented by lymphocytes, inflammation produced by the tumour, and the tumour microenvironment. However, studies on FL mainly focus on patients who were treated with rituximab-containing chemotherapy, with little emphasis on those who underwent radiotherapy (RT) as a component of or as a primary treatment. Moreover, the prognostic role of NLR in FL in terms of survival outcomes has not been studied. Therefore, we aimed to investigate the extent to which NLR at diagnosis predicts survival outcomes in patients with FL, including those who were treated with RT. We also evaluated whether NLR can be used in combination with FLIPI to improve prognosis prediction.

MATERIALS AND METHODS

Study design, setting, and participants

We performed a longitudinal study using retrospective information from electronic medical

records of patients with incident FL treated in Tuen Mun Hospital, Hong Kong. All FL incident cases from 2000 to 2014 were identified (n = 88). We restricted the analysis to patients with complete laboratory, pathology, and radiological data in the medical records (Supplementary Figure S1). The sociodemographic information of the excluded patients was not different from that of the included patients in the final sample. Patients were followed up for a median of 5.88 (range 0.49–16.45) years. The peripheral blood count results were obtained from a standard automated complete blood count machine. This study was approved by the Clinical and Research Ethics Committee of the Tuen Mun Hospital, Tuen Mun, Hong Kong (NTWC/CREC/16107). The research was conducted in accordance with the 1964 Declaration of Helsinki and its later amendments.

Data and variables

Overall survival (OS) and progression-free survival (PFS) were the main outcomes of the study. These outcomes were defined and measured as per criteria from the International Harmonization Project.[29] OS was defined as the time from diagnosis until death as a result of any cause or the last follow-up visit for censored patients. PFS was defined as the time from diagnosis until lymphoma progression (first date of documentation of a new lesion or enlargement of a previous lesion) or death as a result of any cause or last follow-up visit for censored patients. Post-progression survival (PPS) was defined as the time from progression or relapse to the date of death as a result of any cause or last follow-up visit for censored patients. For the survival endpoints, patients were censored at their last follow-up visit. Patients' demographics and disease characteristics were collected. The FLIPI score was then calculated using those factors (nodal sites, age, serum lactate dehydrogenase, stage, and haemoglobin) (see Supplementary Table S1).[2] Chemotherapy involved cyclophosphamide, vincristine, and prednisolone or cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP) or CHOP-like regimens.

Statistical methods

We initially described the cohort of patients using ranges (minimum, maximum), means and standard deviations for continuous variables, and proportions for categorical variables. To evaluate LMR and NLR performance in predicting mortality, we fitted two logistic models with cancer-related death as the outcome and LMR and NLR as continuous independent predictors. Data-adaptive methods based on Bayesian and Akaike information criteria were used to determine the impact of any associated factors and to identify the best fitting model. Subsequently, we computed marginal probabilities for the outcome based on two different weighted logistic models, with time and NLR as independent predictors for the first model and time and LMR for the second model. Weights were accounted for the inverse probability of censoring.[30] Then we derived cross-validated areas under the curve (AUC) [31]; afterward we chose the best cut-off values based on the cross-validated sensitivity, specificity, and the Youden's indices (sensitivity + specificity − 1). Respective LMR and NLR cut-off values were determined at a point with the maximum Youden's index on the receiver operating characteristic (ROC) curve.[32, 33] To evaluate the extent to which OS and PFS differ by LMR and NLR cut-off values, we used incidence, rate ratios, Kaplan–Meier analysis, and log-rank tests [34, 35] for statistical inference. We also used semi-parametric Cox proportional hazards models to evaluate OS and PFS for the computed LMR and NLR cut-off values adjusted for FLIPI, use of rituximab, and sex.[36, 37] Finally, we developed a sensitivity analysis to evaluate the robustness of our findings in the multivariate analysis including different model specifications to account for nonlinearities and the interaction between rituximab and LMR/NLR levels. The proportional hazard assumption for multivariate-adjusted Cox models was also assessed based on the analysis of the Schoenfeld residuals. We used Stata v.14.2 (StataCorp, College Station, TX, USA) for the statistical analysis.

RESULTS

Description of the cohort

The median age at diagnosis of the patients included in the study was 54 years (range 22–87 years). Among them, 18 died during the follow-up period. Thirteen patients died due to the lymphoma. Five deaths were non-lymphoma related: one patient developed prostate cancer and died of pneumonia, another three died of community-acquired pneumonia, and one died of acute coronary syndrome and renal failure. The estimated 5-year PFS and OS were 73.6% and 85.6% respectively (figure 1). At diagnosis, 18.2%, 21.6%, and 60.2% were classified as being at low, intermediate, and high risk according to the FLIPI score. Table 1 shows the descriptive summary statistics of patients included in the study.

Table 1 Descriptive summary statistics for the best cut-offs of LMR and NLR according to patient clinical characteristics, n = 88

Characteristics	All patients (n = 88)	LMR >3.20 (n = 49)	LMR ≤3.20 (n = 39)	NLR >2.18 (n = 57)	NLR ≤2.18 (n = 31)
Age, years					
Median (range)	54 (22–87)	53 (22–87)	54 (31–78)	55 (31–87)	52 (22–77)
>60, n (%)	29 (33.0)	16 (32.7)	13 (33.3)	24 (42.1)	5 (16.1)
≤60, n (%)	59 (67.0)	33 (67.4)	26 (66.7)	33 (57.9)	26 (83.9)
Sex, n (%)					
Male	47 (53.4)	29 (59.2)	18 (46.2)	27 (47.4)	20 (64.5)
Female	41 (46.6)	20 (40.8)	21 (53.9)	30 (52.6)	11 (35.5)

FLIPI, n (%)					
Low risk (scores 0–1)	16 (18.2)	9 (18.4)	7 (18.0)	6 (10.5)	10 (32.3)
Intermediate risk (score 2)	19 (21.6)	13 (26.5)	6 (15.4)	10 (17.5)	9 (29.0)
High risk (scores 3–5)	53 (60.2)	27 (55.1)	26 (66.7)	41 (71.9)	12 (38.7)
ANC (10^9/L), median (range)	4.2 (1.9–10.7)	3.7 (1.9–7.9)	4.6 (2.1–10.7)	4.6 (2.1–10.7)	3.5 (1.9–7.9)
ALC (10^9/L), median (range)	1.6 (0.6–11.3)	1.9 (0.7–11.3)	1.1 (0.6–3.1)	1.2 (0.6–3.1)	2.1 (1.4–11.3)
AMC (10^9/L), median (range)	0.4 (0.1–1.2)	0.4 (0.1–0.9)	0.5 (0.2–1.2)	0.4 (0.1–1.2)	0.4 (0.2–1.1)
NLR, median (range)	2.76 (0.59–9.91)	2.15 (0.59–8.50)	3.83 (1.81–9.91)	3.50 (2.20–9.91)	1.73 (0.59–2.18)
LMR, median (range)	3.80 (0.55–22.60)	5.00 (3.43–22.60)	2.33 (0.55–3.20)	3.00 (0.55–8.00)	5.33 (2.82–22.60)
LDH >220 IU/L, n (%)	70 (80.5)	39 (81.3)	31 (79.5)	47 (82.5)	23 (76.7)
Stage, n (%)					

I/II	24 (27.3)	14 (28.6)	10 (25.6)	11 (19.3)	13 (41.9)
III/IV	64 (72.7)	35 (71.4)	29 (74.4)	46 (80.7)	18 (58.1)
Hb <12 g/dL, n (%)	23 (26.1)	14 (28.6)	9 (23.1)	15 (26.3)	8 (25.8)
Number of nodal sites >4, n (%)	50 (56.8)	24 (49.0)	26 (66.7)	37 (64.9)	13 (41.9)
Use of rituximab, n (%)	38 (43.2)	19 (38.8)	19 (48.7)	27 (47.4)	11 (35.5)
Treatment, n (%)					
Chemotherapy plus RT	14 (15.9)	9 (10.2)	5 (5.7)	8 (9.1)	6 (6.8)
Chemotherapy alone	54 (61.4)	26 (29.5)	28 (31.8)	40 (45.5)	14 (15.9)
RT alone	14 (15.9)	9 (10.2)	5 (5.7)	6 (6.8)	8 (9.1)

Abbreviations: LMR, lymphocyte to monocyte ratio; NLR, neutrophil to lymphocyte ratio; FLIPI, Follicular Lymphoma International Prognostic Index; ANC, absolute neutrophil count; ALC, absolute lymphocyte count; AMC, absolute monocyte count; LDH, lactate dehydrogenase; RT, radiotherapy.

Only 18 patients underwent RT as a definitive treatment for limited stages I and II. Involved-field irradiation, in which the RT fields were limited to the involved nodal region, was mostly administered with two parallel opposed fields; the median radiation dose was 40 Gy (range 30–54 Gy). Ten other patients with stage III or IV disease received RT during their disease course, as part of palliation or as consolidation therapy to sites demonstrating an inadequate

response to systemic treatment. High-grade transformation occurred in 6 out of 27 patients with relapse. Peripheral blood counts were available at the time of the relapse.

Progression-free survival

The AUCs of LMR and NLR were 0.90 (95% confidence interval [CI] 0.84, 0.97) and 0.87 (95% CI 0.77, 0.96), respectively, and they did not differ in terms of predictive performance for PFS (test equality of ROC areas, p-value 0.470). An LMR cut-off value of 3.20 (positive predictive value 22.4% and negative predictive value 82.1%; sensitivity 61.1% and specificity 45.7%) and NLR cut-off value of 2.18 (positive predictive value 21.1% and negative predictive value 80.6%; sensitivity 66.7% and specificity 35.7%) showed the greatest Youden's index, corresponding to maximum joint sensitivity and specificity on the ROC curve (Supplementary Table S2).

NLR and LMR mortality predictive performance

The median NLR and LMR at diagnosis were 2.77 (range 0.59–9.91) and 3.80 (range 0.55–22.60), respectively. The median NLR and LMR at relapse were 2.67 (range 0.95–17.25) and 3.33 (range 0.48–8.5), respectively.

NLR at relapse was associated with PPS as a continuous variable (hazard ratio [HR] 1.24, 95% CI 1.04, 1.49). In the univariate analysis presented in Table 2, high LMR (>3.20) had a superior PFS with a HR of 0.34 (95% CI 0.16, 0.74). Patients with a high FLIPI score had a 2.5 times greater risk of death or relapse than patients with a lower score (HR: 2.52, 95% CI 1.10, 5.75). However, patients treated with rituximab had a 72% lower risk of death or relapse (HR: 0.28 95% CI 0.10, 0.81). We found evidence of a linear association between PFS and the calendar period (p-value of trend = 0.003). Compared with the period 2000–2005, those patients diagnosed during 2010–2014 had a 90% lower risk of death or relapse (HR: 0.10 95% CI 0.01, 0.75). Furthermore, there was no evidence of differences in PFS by sex (male vs female, HR: 1.09, 95% CI 0.51–2.32). LMR at relapse showed a weak

association with PPS (HR 1.06, 95% CI 0.77, 1.45).

Table 2 Hazard Ratios of PFS events, n = 88

		Cases/1000 person years	Rate (per 1000 person years)	Hazard Ratio	95% CI	p-value
LMR	>3.20	11	34.66	0.34	0.16–0.74	0.004
	≤3.20	16	101.28	1		
NLR	>2.18	18	67.37	1.56	0.70–3.47	0.273
	≤2.18	9	43.23	1		
FLIPI	High risk	19	82.30	2.52	1.10–5.75	0.023
	Low/ intermediate risk	8	32.72	1		
Sex	Male	15	58.96	1.09	0.51–2.32	0.831
	Female	12	54.29	1		
Rituximab	Yes	4	22.02	0.28	0.10–0.81	0.012
	No	23	78.31	1		
Year of diagnosis	2010–2014	1	8.71	0.10	0.01–0.75	0.006
	2006–2010	5	43.05	0.50	0.19–1.33	0.157
	2000–2005	21	85.91	1		

In the multivariate analysis, patients with a high LMR (>3.20) at diagnosis had a longer PFS, with an adjusted HR of 0.31 (95% CI 0.13, 0.71) (Figure 2). However, NLR cut-off levels did not show strong evidence of an association with PFS (adjusted HR 1.33, 95% CI 0.57, 3.10)

(Table 3).

Table 3 Multivariate analyses of PFS with LMR and NLR at diagnosis, n = 88

	Adjusted HR*	95% CI	p-value
LMR: >3.20 vs. ≤3.20 (reference)	0.31	0.13–0.71	0.006
FLIPI: high risk vs. low/ intermediate risk (reference)	2.17	0.92–5.10	0.075
Sex: male vs. female (reference)	1.50	0.67–3.34	0.321
Rituximab use: yes vs. no. (reference)	0.16	0.05–0.48	0.001
	Adjusted HR*	95% CI	p-value
NLR: >2.18 vs. ≤2.18 (reference)	1.33	0.57–3.10	0.511
FLIPI: high risk vs. low/ intermediate risk (reference)	2.47	1.03–5.89	0.042
Sex: male vs. female (reference)	1.15	0.53–2.48	0.721
Rituximab use: yes vs. no. (reference)	0.19	0.07–0.57	0.003

*Adjusted for all other covariates in the table

Sensitivity analysis

Sensitivity analysis showed that LMR was consistently associated with PFS under different model specifications and multivariate adjustments. We evaluated the multivariate analyses, and found that the assumption for the proportional hazard was met. However, the strength of the evidence for differences in OS by LMR and NLR levels was weak.

PFS tended to increase with LMRs above the cut-off and low FLIPI scores (HR LMR >3.2 and low FLIPI 0.17, 95% CI 0.04, 0.70 vs. HR LMR>3.2 and high FLIPI 0.60, 95% CI 0.24, 1.50), but evidence of a statistical interaction was weak (interaction p-value 0.171). There was some evidence of a statistical interaction between LMR and rituximab (interaction p-

value 0.024, HR of the interaction term 17.1, 95% CI 1.46, 199.36).

DISCUSSION

To our knowledge, this study is the first to report the clinical and prognostic implications of pre-treatment NLR in patients with FL. Our findings demonstrated notable differences in clinical behaviour and outcome between the low and high LMR groups at diagnosis and NLR groups at the time of relapse. Previous studies reported that NLR is a predictor of mortality in several cancer types, including gastric [38, 39] and colorectal cancer.[40] One possible underlying mechanism is the inflammatory reaction, which has been reported to be involved in tumour growth, invasion, metastasis, and resistance to treatment.[23-26]

The factors included in FLIPI [2] are primarily related to tumour burden (stage, serum lactate dehydrogenase, and number of nodal site involvement) and patient characteristics (age and haemoglobin). Cell count ratio at diagnosis is a simple tool that assesses the host's immune homoeostasis, inflammatory state,[23, 24] and the tumour microenvironment.[14, 15] We obtained strong evidence in support of these prognostic factors possessing practical clinical utility and significance. In the present study, LMR played a significant role in predicting the PFS and NLR in PPS; however, the strength of the evidence for OS was weak. This weak evidence may be attributed to the inadequate sample size and the few deaths observed, along with the interaction with other parameters or unknown confounding. Moreover, the availability of salvage treatments upon progression makes the difference in OS difficult to demonstrate.

Cell count or its ratio at diagnosis may be used to decide which treatment strategy is most appropriate, including watchful waiting, RT, or systemic treatment. Previous studies showed that lymphocytes have an important role in mediating the antitumor effect of rituximab.[41-43] For those with low LMR, the disease may progress earlier, and closer follow-up may be

indicated. We separated patients into FLIPI-based low/intermediate and high-risk groups and then incorporated a biological factor (LMR) into a known clinical prognostic factor (FLIPI). Based on the findings of our study, future studies should aim to understand the utility of cell count ratios with other established prognostic factors in an independent validation cohort and to explore the therapeutic strategies based on cell count ratio (i.e., observation alone versus early initiation of treatment), ideally in a prospective manner.

Most studies are subject to a certain degree of misclassification related to measurement error.[44] However, our study population followed standardised investigation procedures; any misclassification is likely to be non-differential. The incidence and spectrum of NHL cases differ between the Chinese and Western populations, and the risk of FL is lower in the former group.[45-47] However, our sample size is comparable to those in other retrospective FL studies in Asia, ranging between 40 and 50 patients.[45, 48] Both genetic and environmental factors play a part in governing the overall incidence, as shown by migration studies.[46] Our lymphoma treatment regimens were not completely uniform in this analysis and involved a modest sample size, which may have introduced selection bias. We analysed a group of patients receiving definitive RT as treatment, with a sample size of only 18. The results did not reach statistical significance; a bigger cohort or even a dedicated prospective study would be interesting. In our data, the complete blood count did not differentiate the subtypes of B and T lymphocytes and monocytes. Therefore, information regarding patient outcomes with a combination of different subtypes of immune cells was not explored in this study. Furthermore, in our study, the distribution of blood cells may be different when leucocytosis or leukopenia is present. Moreover, evidence of a correlation between age and circulating white blood cell counts has been reported, and a decrease in total lymphocyte counts is observed more frequently in the elderly than in younger adults.[49] Also, the treatments may interact with other factors, such as age and performance status. There appears

to be an interaction between rituximab and LMR. However, the 95% CI of the interaction term was wide; this reflected the small numbers and data sparsity for secondary analysis, and therefore, no conclusive evidence can be extrapolated. We did not analyse the association between cell count ratios and systemic treatment choices, duration and number of cycles, and salvage treatment upon progression because of the limited sample size for the subgroup analysis. Given the unavailability of beta-2 microglobulin in most patients, we did not analyse FLIPI2.

One merit of our study is the performance of a sensitivity analysis to determine the robustness of the main findings in different scenarios. A sensitivity analysis was not conducted in numerous studies assessing the relationship between cell count ratio and survival. Furthermore, we explored the effect of calendar year of diagnosis to account for potential improvement in life expectancy over the study period due to changes in the environment or technological advancement in general medical care. Also, we accounted for the impact of the inclusion of rituximab as a therapeutic option in early 2005, which is strongly correlated with an improved OS and PFS.

The external validity of this study is limited to a single institution. Thus, further evidence for validation of our results and multi-institutional studies with larger sample sizes are warranted. However, the strength of the evidence of our findings is still important given the clinical relevance of LMR and NLR capability to predict prognosis.

CONCLUSION

In this study, we demonstrated that LMR and NLR may provide independent and additional prognostic information for risk classification when used along with FLIPI in FL. These can be determined using widely available complete blood count tests, which can be used as non-invasive and cost-effective alternatives to complement prognosis data for FL. Future prospective studies are necessary to validate the results of our study and evaluate the exact

clinical significance.

ACKNOWLEDGMENTS

None to declare.

COMPLETING INTERESTS

None to declare.

FUNDING

None to declare.

DATA SHARING STATEMENT

Raw data can be obtained by contacting the authors at the corresponding address.

AUTHORSHIP STATEMENT

SFL developed the concept and design of the study. SFL analysed the data with MALF guidance. SFL wrote the manuscript. Both authors interpreted the data, drafted and revised the manuscript critically, and approved the final version of the manuscript. SFL is the guarantor of the paper.

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Figure legends:

Figure 1 Kaplan–Meier curves. Kaplan-Meier estimate for (A) progression-free survival and (B) overall survival of the whole study cohort (n = 88)

Figure 2 Adjusted progression-free survival. Estimate of (A) high and low LMR at diagnosis and (B) high and low NLR at diagnosis (n = 88)

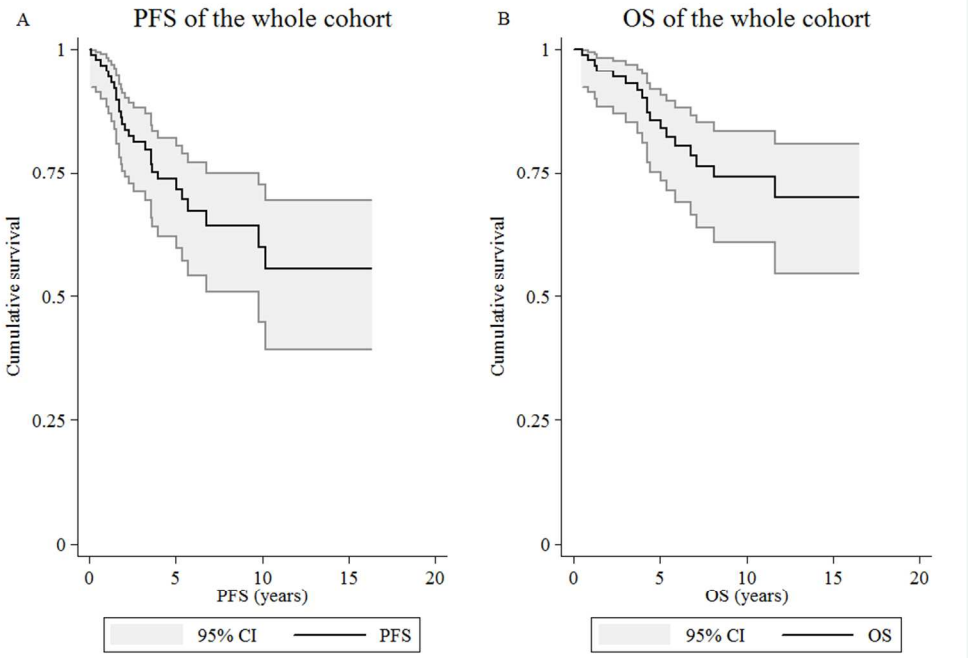

Figure 1 Kaplan–Meier curves. Kaplan-Meier estimate for (A) progression-free survival and (B) overall survival of the whole study cohort (n = 88)

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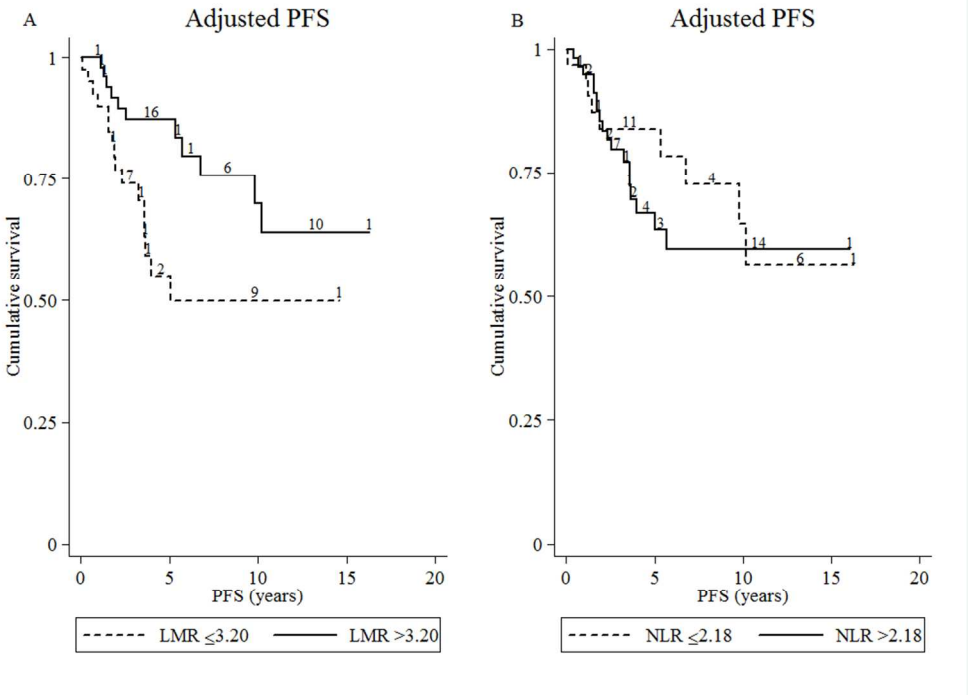

Figure 2 Adjusted progression-free survival. Estimate of (A) high and low LMR at diagnosis and (B) high and low NLR at diagnosis (n = 88)

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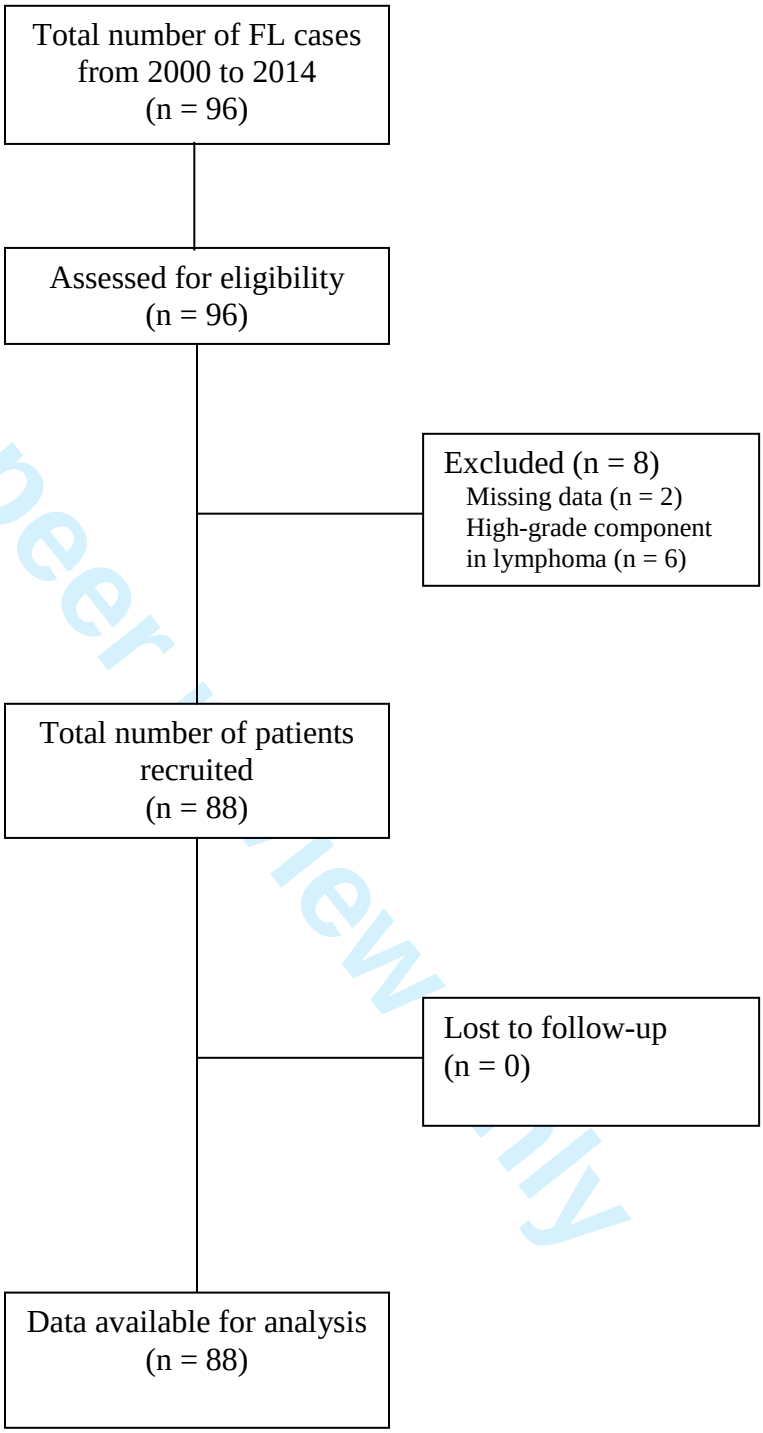
Supplementary Figure S1. Patient recruitment and follow-up flow diagram



Supplementary Table S1. Follicular Lymphoma International Prognostic Index

Parameters	Definition of risk factors
Nodal sites	>4 lymph node regions
Age	>60 years
Serum marker	Elevated LDH
Stage	Advanced (stages III–IV according to Ann Arbor staging)
Haemoglobin	<12 g/dL

0–1 risk factors: low risk

2 risk factors: intermediate risk

3–5 risk factors: high risk

Abbreviations: LDH, lactate dehydrogenase

Supplementary Table S2. Sensitivity, specificity, positive predictive value, negative predictive value, likelihood ratio positive, likelihood ratio negative, and Youden's index for LMR and NLR

Cell count ratio cut-off values	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Likelihood ratio positive	Likelihood ratio negative	Youden's index
LMR: 3.20	61.1%	45.7%	22.4%	82.1%	1.1	0.9	0.60
NLR: 2.18	66.7%	35.7%	21.1%	80.6%	1.0	0.9	0.56

Abbreviations: LMR, lymphocyte to monocyte ratio; NLR, neutrophil to lymphocyte ratio

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract **[Within the title page 1 and method section of abstract]**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **[See abstract page 1]**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported **[main manuscript page 1-2]**
Objectives	3	State specific objectives, including any prespecified hypotheses [**main manuscript page 2**]
Methods		
Study design	4	Present key elements of study design early in the paper **[page 2-3]**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection **[page 2-3]**
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up **[page 2-3]**
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable **[page 3]**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group **[page 3-4]**
Bias	9	Describe any efforts to address potential sources of bias **[page 4]**
Study size	10	Explain how the study size was arrived at **[page 2-3]**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why **[page 3-4]**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **[page 4]**
		(*b*) Describe any methods used to examine subgroups and interactions **[page 4]**
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses **[page 4]**
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed **[supplementary figure 1]**
		(b) Give reasons for non-participation at each stage **[supplementary figure 1]**
		(c) Consider use of a flow diagram **[supplementary figure 1]**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders **[Table 1 on page 5]**
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount) **[page 4]**
Outcome data	15*	Report numbers of outcome events or summary measures over time **[page 6-8]**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were

		adjusted for and why they were included **[page 6-7]**
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses **[page 8]**
Discussion		
Key results	18	Summarise key results with reference to study objectives page **[page8-9]**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias **[page 9-10]**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence **[page 11]**
Generalisability	21	Discuss the generalisability (external validity) of the study results **[page 11]**
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based **[Within Funding section]**

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.