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Could neutrophil to lymphocyte ratio be a prognostic predictor of relapse in patients with extra-pulmonary tuberculosis?

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ABSTRACT

Background: We aimed to evaluate whether neutrophil to lymphocyte ratio (NLR) was associated with relapse occurrence during the follow-up of extra-pulmonary tuberculosis (EPTB).

Material and Methods: We retrospectively collected data from 408 EPTB patients hospitalized between 1996 and 2015. We evaluated the performance of NLR in predicting relapse and to stratify patients into high and low level groups. Kaplan-Meier method was used to generate regression-free survival (RFS) curves, which were compared by Logrank test according to NLR categories.

Results: Fourteen cases developed a relapse (3.4%). The median value of NLR was significantly higher in patients with relapse (3.8±1.4 vs 2.7±1.4; p=0.008). Receiving operating characteristics (ROC) curves analysis showed that NLR had an Area under the ROC (AUROC) of 0.73 in predicting relapse (p=0.003). At a cut-off of 3, NLR had a sensitivity of 64% and a specificity of 70% to predict relapse. Survival curves showed that patients with a high NLR (≥3) had shorter RFS compared with those with a low NLR (<3) (24.7 vs 44.2 months; p=0.04). High NLR was significantly associated with relapse both in univariate (HR=3.7; p=0.041) and multivariate Cox regression analysis (HR=5.24; p=0.027).

Conclusion: NLR may have a prognostic value in the follow up of patients with EPTB.

Keywords: Extra-Pulmonary Tuberculosis, Neutrophil to Lymphocytes Ratio, Relapse, Prognosis

INTRODUCTION

Tuberculosis (TB) continues to be a major health problem worldwide. It has been declared as a worldwide health emergency since the last two decades (1). According to annual surveys conducted by the World Health Organization (WHO), 10.4 million cases of TB and 1.7 million deaths occurred in 2016 (2). Although the conventional focus of TB programs has been on pulmonary TB due to its transmissibility, there is a renewed interest in extra-pulmonary forms of tuberculosis since its relative frequency increases progressively. Extra-pulmonary tuberculosis (EPTB) is a multisystem disease with myriad presentations and manifestations that can affect any organ or tissue. Some EPTB sites, such as neuromeningeal, pericardial, and hematopoietic TB, can have several serious outcomes and may require specific care.

Despite the availability of effective treatment regimens, EPTB is a common cause of morbidity and mortality, particularly tuberculous meningitis (3). Consequently, EPTB prognosis factors influencing survival must be sufficiently understood. The peripheral blood neutrophil to lymphocyte ratio (NLR), representing a combination of circulating neutrophil and lymphocyte counts, has been reported as a representative index of systemic inflammatory response that correlated with the prognosis of many acute or chronic diseases (4). NLR has been proposed as an independent predictor of poor survival in several diseases such as tumors (5,6) and cardiovascular diseases (7). To our knowledge, this is the first study dealing with the relationship between NLR levels and clinical prognosis of EPTB, mainly in case of relapse.

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In this perspective, we aimed to identify clinical and biological factors correlated with relapse in patients with EPTB and to evaluate whether NLR at admission was associated with a relapse-free survival (RFS) during their follow-up.

METHODS

Study Design

We performed an observational study using retrospective information from medical records of patients with EPTB who were hospitalized in a department of infectious diseases from January 1996 to December 2016. A follow-up had been performed during the study period in order to identify relapse cases in patients with EPTB.

Data Collection and Case Definitions

We recorded patients' demographic data, clinical features and laboratory parameters at the time of the diagnosis. These data included age, gender, risk factors, co-morbidities and clinical signs. Main laboratory findings were leukocyte counts, blood biochemistry, blood cultures and peripheral samples from other sites such as cerebrospinal and pleural liquid. NLR was calculated as follow: NLR= Neutrophil count (/mm^3)/Lymphocyte count (/mm^3). The peripheral blood count was obtained automatically from ethylene-diamine tetra-acetic acid (EDTA) tubes.

The inclusion criteria were patients of any age with EPTB. Diagnosis was confirmed through histological or microbiological findings having shown a positive culture or molecular biology result in the infection site sample. Otherwise, the diagnosis was based on strong clinical, radiological criteria compatible with TB with clinical improvement after specific treatment. All included patients received anti-tuberculosis drugs according to the WHO treatment guidelines [8]. During the follow-up, EPTB relapse was defined as a patient in whom the initial onset has been treated but the existing *Mycobacterium* reactivates into a second onset of TB after achieving treatment. All EPTB cases were adherent to anti-tuberculous drugs and followed correctly the treatment protocol.

A RFS was defined as the occurrence of relapse from the date of initial confirmed diagnosis of EPTB.

Statistical Analysis

Statistical analysis was performed using SPSS.20. The results of quantitative variables were presented as mean ± standard deviation (SD) or median and interquartile range (IQR), those of qualitative variables as percentages. When the variables distribution was normal, we used the *t*-test to compare 2 means. For categorical variables, we used the Chi-square test in independent samples. When the variables were not normally distributed, we used non-parametric tests such as Wilcoxon, Mann-Whitney and Kruskall-Wallis tests when appropriate. Receiving operating curve (ROC) analysis was performed to determine the best cut-off of NLR as the value with the highest sensitivity and specificity in order to evaluate its performance in identifying a relapse. Then, we derived cross-validated areas under the curve (AUROC) to measure best predictive accuracies. Afterwards, EPTB patients were stratified into high and low-level groups according to the NLR cut-off value. Kaplan-Meier method was used to calculate the cumulative relapse rate during the follow-up period and to generate RFS curves. We performed log-rank tests for statistical inference to delineate the extent to which RFS curves differ by NLR cut-off values and to compare the survival rates according to NLR categories. A Cox proportional hazard regression analysis was used to determine independent prognostic factors of EPTB patients by adjusting on the confounding variables. In the remaining cases, patients were censored at their last follow-up visit. *P* values lower than 0.05 were considered statistically significant.

RESULTS

Baseline Characteristics of the Study Population
Clinical and Biological Features

During a period of 21 years, a total of 408 patients were included among which 256 cases (62.7%) were females. The median age was 38 years (IQR= [26-57.7 years]). Main co-morbidities were diabetes (5.1%) and a history of cancer (5.1%). There were 58 smokers (14.2%). Main symptoms were fever (64.4%) and asthenia (62%) (**Table 1**).

The median white blood cells (WBC) count was 6500/mm^3 (IQR= [4870-8100/mm^3]) and median value of NLR was 2.4 (IQR= [1.7-3.66]). Median values of C-reactive protein and sedimentation rate were respectively 23 mg/L (IQR= [6-158]) and 40 mm/hour (IQR= [16-70]) (**Table 1**).

Table 1: *Clinical and biological features of the study population*

Variables		
Total (N%)	408	100
Age (median, IQR) (years)	38	[26-57.7]
Men (N, %)	152	37.2
Co-morbidities (N,%)	53	13
Diabetes	21	5.1
History of cancer	21	5.1
Chronic renal failure	8	2
Immunosuppression	3	0.73
Smoking	43	10.5
Alcohol consumption	15	3.6
Clinical signs (N,%)		
Fever	263	64.4
Asthenia	253	62
Anorexia	208	50.9
Weight loss	156	38.2
Night sweats	127	31.1
Laboratory parameters (median; IQR)		
WBC count (E/mm^3)	6500	[4870-8100]
Neutrophil count (E/mm^3)	4230	[3250-5700]
Lymphocyte count (E/mm^3)	1300	[950-1700]
Natremia (mmol/L)	137	[136-140]
C-reactive protein (mg/L)	23	[6-158]
Sedimentation rate (mm/hour)	40	[16-70]

EPTB: extra-pulmonary tuberculosis; N: number; IQR: Interquartile range; WBC: White blood cells.

Table 2: *Diagnosis, therapeutic regimen and evolution in patients with extra-pulmonary tuberculosis*

Variables		
EPTB Diagnosis (N %)		
Histopathological findings	249	61
Clinical and radiological arguments	116	28.4
Microbiological diagnosis	43	10.5
Positive culture specimen	30	7.3
PCR-positive sample	13	3.2
EPTB sites (N, %)		
Lymph node	168	41.2
Abdominal	64	15.7
Neuro-meningeal	63	15.4
Bone and joint	63	15.4
Multifocal	60	14.7
Urogenital	51	12.5
Skin	16	3.9
Pleural	9	2.2
Other sites*	32	7.8
Treatment regimen		
Separate tablets (N, %)	259	63.5
Fixed drugs combination (N, %)	149	36.5
Treatment duration first episode (months) (median; IQR)	16	[10-18]
Evolution (N, %)		
Relapse	14	3.4
Death	3	0.7

*Other sites: mammary, ocular, pericardia, cavum, and hematopoietic. EPTB: Extra-pulmonary tuberculosis. N: number; IQR: Interquartile range.

Diagnosis and Treatment of Extra-pulmonary Tuberculosis Patients

The diagnosis was based on histopathological findings in 249 cases (61%). There were 30 positive culture specimens (7.3%) and 13 PCR-positive samples (3.2%) (**Table 2**). The main EPTB sites were lymph node in 168 cases (41.2%), followed by abdominal site in 64 cases (15.7%). An anti-tuberculous therapy was based on separate tablets in 259 cases (63.5%). The disease evolution was marked by the occurrence of relapse in 14 cases (3.4%) and death in 3 cases (0.7%) (**Table 2**).

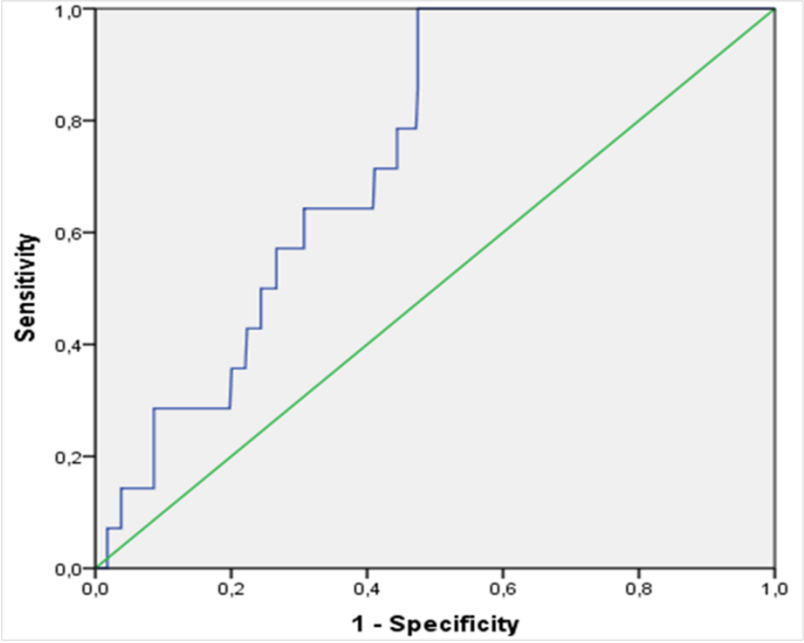

Figure 1: *Receiving operating curve of neutrophil to lymphocyte ratio in predicting clinical relapse in patients with extra-pulmonary tuberculosis*

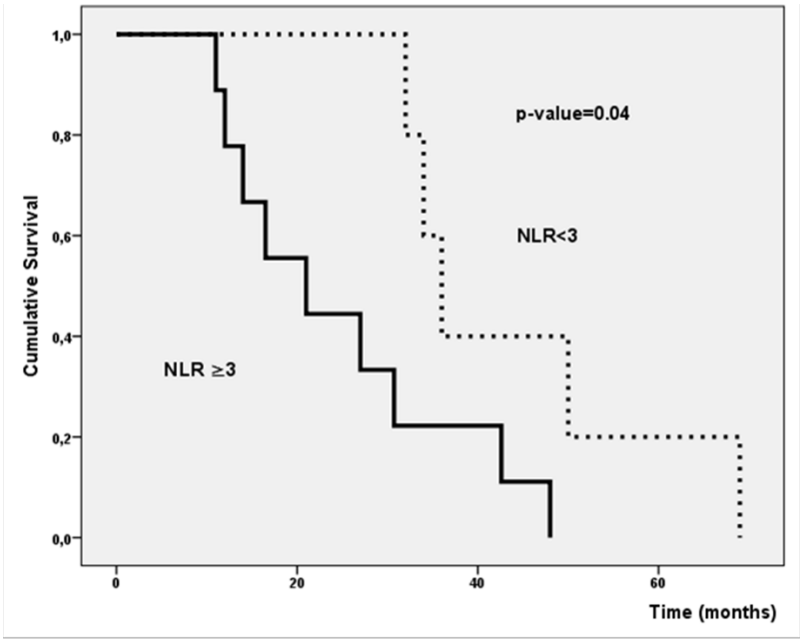

Figure 2: *Kaplan–Meier relapse-free survival curves stratified by neutrophil to lymphocyte ratio cut-off value (NLR=3) in patients with extra-pulmonary tuberculosis*

Performance of NLR in Predicting Clinical Relapse in Extra-pulmonary Tuberculosis

The median period between the first episode of EPTB and the occurrence of relapse was 11 months (IQR= [4-19.3 months]). The mean value of NLR was significantly higher in patients with relapse (3.8 ± 1.4 vs. 2.7 ± 1.4; p= 0.008). Roc curve analysis showed that NLR had an AUROC of 0.73 in predicting relapses (p=0.003). At a cut-off of 3, NLR had a sensitivity of 64% and a specificity of 70%. Of all EPTB patients, 144 cases had a high-level (≥3) of NLR (35.3%) (**Figure 1**).

Relapse-Free Survival (RFS)

Overall, the median RFS was 30.7 months (CI95% = [31.7-40.29 months]). When stratified by NLR cut-off value, survival curve analysis showed that patients with a high NLR (≥3) had shorter RFS (24.7 months (CI95% = [15.9-33.5] months) compared with those with low NLR (44.2 months (CI95% = [30.5-57.8] months) (Logrank test; p=0.04) (**Figure 2**).

Table 3: *Prognostic factors associated with relapse: Univariate and multivariate analysis for overall relapse free survival using the Cox proportional hazards model*

Covariates		Univariate analysis		Multivariate analysis	
		HR (CI95%)	p	Adjusted HR (CI95%)	p
Neuro-meningeal	Yes	4.9 [1.2-20]	0.028	8.8 [1.5-42]	0.014
	No	1	-	1	-
NLR groups	High (≥3)	3.7 [1.1-14]	0.041	5.24	0.027
	Low (<3)	1	-	1	-

NLR: Neutrophil to lymphocyte ratio; HR: Hazard ratio; CI: Confidence interval

Factors Associated with Relapse: Results of Univariate and Multivariate Cox Analysis

Univariate analysis showed that there was no significant difference in terms of age, gender, co-morbidities and clinical signs between patients with and without relapse during follow-up. Otherwise, neuro-meningeal site (HR=4.9; p=0.028) and a high level of NLR (≥3) (HR=3.7; p=0.041) were significantly associated with relapse.

In multivariate Cox regression analysis, NLR≥3 was found to be independently associated with relapse, after adjustment on neuro-meningeal site of EPTB (HR=5.24; p=0.027) (**Table 3**).

DISCUSSION

Tuberculosis is an infectious disease which progression and outcomes critically depend on host immune reactivity. Clinical post chemotherapy relapses remain a major problem in patients with EPTB especially in countries with high TB incidence (9). Effectiveness of tuberculosis control programs is assessed in part by relapse rates. Better understanding of the prognosis factors associated with relapse is an important issue to identify patients at high risk of relapse and promises to reduce the disease burden through risk factors interventions. Several prognosis factors of TB have been reported such as noncompliance to anti-tuberculosis therapy, smoking, longer duration of illness prior to treatment and low body weight (1).

Of the factors determined to be predictive of tuberculosis recurrence, HIV-infection and immune-suppression have been previously identified in countries of high and low TB incidence where most recurrences after successful TB treatment are due to endogenous reactivation (10,11). In fact, the physiological immune responses of circulating leukocytes to various stressful events are characterized by an increased neutrophil count and decreased lymphocyte count (12). An increase in total WBC and neutrophils is an inflammatory reaction, particularly when caused by a bacterial infection (5). Moreover, lymphocytopenia has been reported as a potential factor predictive of bacterial infection (13). Therefore, combining these two inflammatory biomarkers as the ratio of neutrophil and lymphocyte count serves as a representative index of systemic inflammation, and could be attractively interesting in evaluating the disease severity. In this context, a meta-analysis conducted by Yin Y and coll. reported that an elevated NLR predicted worse overall survival in lung cancer, with an adjusted HR of 1.2 in multivariate analysis (14). Similarly, in a narrative literature review, Faria SS and coll. reported that NLR had the potential to be a sensitive prognostic marker of breast cancer (15). Furthermore, several previous studies showed that NLR was significantly associated with higher mortality (16) and correlated with higher risk of exacerbation (17) in chronic obstructive pulmonary disease patients. On the other hand, NLR was identified as a potential marker representing the disease severity in bacterial infections and predicting unfavorable outcomes, such as urinary tract infection (18), chronic hepatitis B (19) and sepsis (20). Recently, useful biomarkers that reflect disease severity and respond to treatment are receiving increasing attention. In a previous study conducted in China, NLR ≥ 2.53 was proposed to be a prognosis factor predictive of pulmonary tuberculosis retreatment (21). In our study, we demonstrated that NLR ≥ 3 was an independent prognostic factor of EPTB relapse, with an adjusted HR of 5.24. These findings remained robust after adjusting for several potential covariates, such as neuro-meningeal site of TB infection. One of the most convincing explanations is based primarily on the physiological link between neutrophilia and lymphopenia with systemic inflammation and stress. In case of tuberculosis infection, the enhanced neutrophil response might promote *Mycobacterium tuberculosis* proliferation and transmission, and the suppression of lymphocytes might inhibit the immune response and delay therapy duration, which together lead to a higher NLR (21). Therefore, NLR can play a special role as a predictive marker of bacterial infection compared to neutrophilia or lymphocytopenia alone (12). Similarly, Yaranal PJ and coll. reported that hematological abnormalities are more commonly observed in cases of severe tuberculosis (22). Moreover, increased numbers of neutrophil implied that nidus of infection was not eradicated, which further induced depression of lymphocyte (5). Thus, the monitoring of systemic inflammatory biomarker blood levels,

notably neutrophil and lymphocyte count may potentially provide an additional level of risk stratification in patients with EPTB. Besides, NLR can be easily determined from a routine laboratory exam available in most institutions, since it was calculated from peripheral blood test results. Compared to other biomarkers such as CRP and procalcitonin, it is less expensive and an easier method in predicting post chemotherapy relapse. To the best of our knowledge, this is the first study to evaluate the role of NLR in predicting EPTB prognosis. Our results revealed that this ratio was found to be a sensitive (64%) and a specific (70%) marker in predicting relapse.

Our study had several limitations. First, we conducted a single-center observational study and thus, it may increase the likelihood of selection bias. Second, in this retrospective study, data were collected from medical records which make survival studies less accurate. Further prospective studies are still needed to better evaluate its prognosis relevance. Although our study included 408 patients who were treated over the study period, additional studies with larger sample sizes, particularly relapsing patients are needed to confirm our results.

In conclusion, our study revealed new insights into the prognosis value of NLR in predicting post chemotherapy relapse in EPTB patients. The survival analysis identified cut-off values of NLR that may help to predict patient relapse and to assist physicians in selecting the best therapy regimen for individual patients, in order to identify high-risk patients who require specific care.

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