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Original Article

Diagnostic Usefulness of Neutrophil / Lymphocyte Count Ratio in Gram Negative Bacterial Infection

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ABSTRACT

Neutrophil to Lymphocyte Count ratio (NLCR) is used as a marker of subclinical inflammation. It is calculated by dividing the number of neutrophils by the number of lymphocytes counts which are measured in peripheral blood sample. The initial stages of severe infection may be characterised by increased neutrophils and decreased lymphocyte counts in the peripheral blood sample. Hence during gram negative bacterial infection, NLCR may be useful to find out the degree of Infection and the type of bacteria involved. There are no separate measurements required and the results available for CBC may be used to calculate NLCR. The objective of this study is to quantify the prognostic value of peripheral blood NLCR on clinical outcome in various types of bacterial infections in comparison with age and sex matched controls.

Keywords: NLCR, CBC, ESR, PLCR, Critical Ratio

INTRODUCTION

Viral or bacterial upper respiratory infections are the most common cause of Acute Exacerbation of Chronic Obstructive Pulmonary Disease (AECOPD). Based on available data, no reliable parameter has been presented to distinguish between bacterial and nonbacterial exacerbations. Procalcitonin (PCT) was found to be better than C-Reactive Protein (CRP), however NLCR is very useful in predicting a bacterial infection in hospitalized patients with AECOPD. PCT is not very reliable in predicting bacterial infection in AECOPD patients due to its sensitivity and specificity of less than 80 % and a low Area Under Curve Value (AUC) [1].

Absolute lymphocytopenia has been reported as a predictor of bacteremia in medical emergencies. Likewise, NLCR has been shown to be a simple promising method to evaluate systemic inflammation in critically ill patients. Significant differences between patients with positive and negative blood cultures were detected with respect to the CRP level, lymphocyte count and NLCR but not with White Blood Cell (WBC) and neutrophil counts alone. Sensitivity, specificity, positive and negative predictive values were highest for the NLCR. The area under the receiver operating characteristic curve was highest for the lymphocyte count. In an emergency care setting, both lymphocytopenia and NLCR are better predictors of bacteremia than routine parameters like CRP, WBC and neutrophil counts. Attention to these markers is easy to integrate in daily practice and without extra costs [2].

The NLCR can be used as a biomarker of disease severity even in *Escherichia coli* infections. The biomarker reacts rapidly, is cheap and needs no extra sampling. The higher the value, the higher the probability for severe sepsis. A high value can even precede the development of severe sepsis or septic shock. However, a low value never excludes neither bacteremia nor severe sepsis. The method cannot be used in patients with disturbances in neutrophil or lymphocyte levels due to other causes than sepsis [3]. Serum NLCR levels were significantly lower in patients with pulmonary tuberculosis (TB) than in patients with bacterial

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Community Acquired Pneumonia (CAP). NLCR value <7 is an optimal cut-off value to discriminate patients with pulmonary TB from patients with bacterial CAP (sensitivity 91.1%, specificity 81.9%, positive predictive value 85.7%, negative predictive value 88.5%). The NLCR value at the initial diagnostic stage in patients with pulmonary TB is a useful laboratory marker to discriminate patients [4]. The mean eosinophil count in survivors showed a tendency to increase rapidly and to achieve normal values between the second and third day. In these patients, the NLCR was <7 between the second and third day. Both sustained eosinopenia and persistence of an NLCR >7 were independent markers of mortality in patients with bacteremia [5].

NLCR < 2 along with a decrease in WBC count can be used as a screening tool in patients presenting with influenza like symptoms, while awaiting throat swab culture reports for confirmation [6]. NLCR has been identified as a predictor of bacteremia in medical emergencies in patients with CAP and on admission and NLCR at the emergency department predicts severity and outcome of CAP with a higher prognostic accuracy as compared with traditional infection markers [7].

Malaria is a major mosquito-borne public health problem in Thailand with varied haematological consequences. The following parameters were significantly lower in malaria-infected patients; red blood cells (RBCs), haemoglobin (Hb), platelets (PLT), WBC, neutrophil, monocyte, lymphocyte and eosinophil counts, while mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), NLCR and monocyte-lymphocyte count ratio (MLCR) were higher in comparison to non-malaria infected patients. Patients with platelet counts < 150,000/uL were 31.8 times (odds ratio) more likely to have a malaria infection. Thrombocytopenia was present in 84.9% of malaria-infected patients and was independent of age, gender and nationality. Patients infected with malaria exhibited important changes in most of haematological parameters with low platelet, WBCs, and lymphocyte counts being the most important predictors of malaria infection. When used in combination with other clinical and microscopy methods, these parameters could improve malaria diagnosis and treatment [8].

In a study, NLCR was evaluated in 45 patients with bacterial infections: 24 patients with viral infections and 18 healthy adults. The medians of NLCR were 11.73 in bacterial infections, 2.86 in viral infections and 1.86 in controls suggesting a diagnostic potential for NLCR in bacterial infections. The NLCR cut-off value of 6.2 exhibited a sensitivity value of 0.91 and a specificity value of 0.96 for bacterial infection [9].

Serum PCT levels alone or in combination with WBC/PLT measurements seem to provide a satisfactory early diagnostic biomarker in decompensated liver cirrhotic ascites patients (DCPs) with infections, especially for patients with spontaneous bacterial peritonitis (SBP) [10]. The NLCR is an independent prognostic marker for time to recurrence (TTR) and overall survival (OS) in a large cohort of patients with soft-tissue sarcoma (STS). The potential confounding factors mentioned above should be considered in further prospective studies investigating inflammatory biomarkers in various diseases to elucidate their true prognostic impact [11]. The most appropriate cut-off value of NLCR to distinguish TB from sarcoidosis was determined as 2.55. For this cut-off value of NLCR there was 79% sensitivity, 69% specificity, 73% positive predictive value (PPV), 75% negative predictive value (NPV), and AUC was 0.788. For differentiation of sarcoidosis from TB, accuracy of the NLCR test according to this cut-off value was found as 76%.NLCR as a little known marker in respiratory medicine was found to be supportive in differentiation of TB and sarcoidosis suggesting more studies on this issue [12].

NLCR was found to be a useful prognostic biomarker in hepatocellular carcinoma (HCC) patients. The prognostic value of NLCR ≥2.7 is superior to that of End-Stage Liver Disease (ESLD) stage or Child–Pugh class, and correlates with that of Barcelona-Clinic Liver Cancer (BCLC) and Tumor, Node, Metastasis (TNM) staging score [13]. Calprotectin was related to neutrophil granulocyte count and NLCR in patients with moderate to very severe Chronic Obstructive Pulmonary Disease (COPD) in stable phase and not in treatment with systemic glucocorticoids. Lymphopenia, higher plasma calprotectin and higher NLCR were independent predictors of increased all-cause mortality in this group. All these suggests that treatment with systemic glucocorticoids has a significant impact on the ability of inflammatory biomarkers to predict all-cause mortality [14]. NLCR values were significantly associated with brucellosis and this situation can help clinicians during diagnosis of brucellosis [15].

Platelet to lymphocyte count ratio (PLCR) is a prognostic factor for various tumors, but the current opinion on the prognostic value of PLCR in liver transplantation (LT) for HCC is still controversial. Pre-transplant PLCR for predicting

post-LT HCC recurrence and further evaluate the correlation of PLCR with tumor-related characteristics. Pre-transplant PLCR ≥125 was associated with advanced tumor stage and aggressive tumor behavior, and it can be used as a prognostic factor for post-transplant HCC recurrence [16].

The individual maximum bearable bacterial concentration depended on neutrophil concentration, phagocytic activity, and patient barrier integrity; thus, the resulting maximal bearable bacterial concentration may vary by orders of magnitude between patients. Understanding the interplay between neutrophils and bacteria may enhance the development of new therapeutic approaches to bacterial infections [17].

MATERIAL AND METHODS

60 patients in the age group of 10-81 years consisting of 38 males and 22 females who were found to be positive for blood culture and were investigated for other routine haematological parameters were enrolled for this study. Laboratory results for 60 patients who attended routine master health check-up and who were not infected with any microorgansims and who were investigated for the analytes undertaken in this study were used as control groups. Routine haematological parameters were processed by Flowcytometry using Siemens Advia 2120i fully automatic analyser.

Inclusion Criteria: Patients who are positive for (*Klebsiella, Pseudomonas, Enterobacter, Escherichia Coli*) blood culture were included as study group.

Exclusion Criteria: Patients who are not positive for (*Klebsiella, Pseudomonas, Enterobacter, Escherichia Coli*) blood culture were excluded from this study.

Statistical Analysis: Critical ratios (CR) [18] were calculated using sample mean and Standard Deviation (SD) in comparison with population mean.

RESULTS

The statistical results obtained (Mean, SD, CR & P) for all bacterial infected patients as well as individual bacterial infected patients in comparison with controls are presented in a series of Tables from I to V. Each Table gives the probability for a series of analytes compared between patients and controls. From Table I, it is clear that except WBC & Platelet, all other analytes counts including the principal parameter NLCR shows very good probability of <0.001 indicating that individual parameters picked up in WBC counts are valuable to predict all gram negative bacterial infections. In Table II and III it is clear that NLCR, ESR and Platelets shows good probability when E.Coli infected patients are compared with controls and it is interesting to observe that only NLCR and WBC gives very good correlation in the case of *Enterobacter* infection confirming the usefulness of both NLCR and WBC as valuable analytes.

Tables IV and V presents data for both Klebeisella and pseudomonas infections, all three analytes NLCR, WBC and ESR are equally found to be useful to predict the infection by the above two bacterial organisms.

DISCUSSIONS

Many studies carried out in the past about the usefulness of NLCR has shown results for a particular type of gram negative bacterial infection, but studies involving the predictive parameter in all 4 major types of gram negative bacteria are lacking. Further, individual fractional counts in WBC were not totally used but only isolated analyte were compared. Previous studies have shown the clinical usefulness of both NLCR and WBC as useful analytes for bacteria and our study findings agrees with it [2]. Our study has established that NLCR, is the sole parameter which was found to be very useful in all generalised gram negative bacteremia positive patients as well as in all four major gram negative bacterial infections. Although WBC shows good correlations between patients and controls in most of the gram negative bacterias except *E.Coli*, it should not be used alone for predicting the degree of infection but should be done in conjunction with NLCR parameter. ESR was found to be a predictor in both *Klebeisella* and *Pseudomonas* but other studies did not predict this usefulness. However, we strongly recommend the use of NLCR for infections involving all four types of gram negative bacterias. Some studies have predicted PLCR as a useful prognostic marker for various tumour, but its useful in gram negative bacterial infections is lacking [17]. The individual concentrations of neutrophil has been recommended by some authors, but we could not find its usefulness as a single parameter in our study (18). Many previous studies have proved that NLCR is a very useful tool to predict infections, but only in some isolated cases [13, 14,16], but our study has strongly proved its usefulness in all four major gram negative bacteremia.

CONCLUSION

Our study has proved that NLCR as a diagnostic tool for predicting the degree of infections by the four major classes of gram negative bacteria. This study has also brought out the salient features about the usefulness of other inflammatory markers and some other individual cell counts in various disease conditions. PLCR is now emerging as a marker to identify cancer patients and along with NLCR, PLCR may be explored to identify different type of cancers in humans. The outcome of this study has clearly established beyond doubt the usefulness of NLCR in all four major classes of gram negative bacterial infections. The content of this paper will be very useful to research scholars to extend this type of study to all possible infections associated with humans and to establish a set of laboratory tests as a standard protocol to be investigated in any gram negative bacterial infection.

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Table 1: Statistical Parameters (Mean, SD, CR & P)-- All patients Vs Controls (n=60)

S.No	Analyte	All Bacteriai infected Patients		Normal Patients Mean	CR	P
		Mean	SD			
1	NLCR	10.7	6.3	2.27	10.4	<0.001
2	WBC	31.2	140	8.08	1.30	0.10
3	ESR	61	41	8.36	10.0	<0.001
4	Neutrophil	83	6.7	60.8	25.8	<0.001
5	Lymphocyte	10.93	5.37	29.25	26.86	<0.001
6	Monocyte	4.71	2.04	5.92	14.8	<0.001
7	Eosinophil	2.2	1.4	3.15	6.166	<0.001
8	Platelet	258	170	252.5	0.252	0.10

Table II: Statistical Parameters (Mean, SD, CR & P)
***E.Coli* Bacteria Vs Controls (n=23)**

S.No	Analyte	*E.Coli* Bacteria infected Patients		Normal Patients Mean	CR	P
		Mean	SD			
1	NLCR	10.26	6.1	2.184	6.354	<0.001
2	WBC	12.15	4.5	7.884	0.4550	0.10
3	ESR	66.9	41.1	6.636	7.038	<0.001
4	PLT	203.5	89.53	269.3	3.528	<0.001

Table III Statistical Parameters (Mean, SD, CR & P)
***Enterobacter* Vs Controls (n=7)**

S.No	Analyte	*Enterobacter* infected Patients		Normal Patients Mean	CR	P
		Mean	SD			
1	NLC	11.8	9.583	1.834	2.76	<0.001
2	WBC	17.47	9.148	7.201	2.97	<0.001
3	ESR	26	37.65	8	1.267	0.10
4	PLT	211	132.8	284.1	1.459	0.10

Table IV Statistical Parameters (Mean, SD, CR & P)
***Klebeisella* Vs Controls (n=7)**

S.No	Analyte	Klebeisella infected Patients		Normal Patients (Mean)	CR	P
		Mean	SD			
1	NLCR	10.04	5.972	2.236	5.841	<0.001
2	WBC	66.82	243	8.022	1.0816	<0.001
3	ESR	55.45	37.45	6.7	5.819	<0.001
4	PLT	289	232.2	257.8	0.60	0.10

Table V Statistical Parameters (Mean, SD, CR & P)
***Pseudomonas* Vs Controls (n=20)**

S.No	Analyte	*Pseudomonas* infected Patients		Normal Patients Mean	CR	P
		Mean	SD			
1	NLCR	7.386	4.197	1.789	4.0	<0.001
2	WBC	13.01	4.592	7.87	3.357	<0.001
3	ESR	89.9	24.1	8	10.2375	<0.001
4	PLT	375	155	278.1	1.8753	0.05

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