Neutrophil–Lymphocyte Ratio as a Predictor of Disease Severity in Ulcerative Colitis

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Background: Blood neutrophil-to-lymphocyte (N/L) ratio is an indicator of the overall inflammatory status of the body, and an alteration in N/L ratio may be found in ulcerative colitis (UC) patients. The aims of this study were to investigate the utility of N/L ratio as a simple and readily available predictor for clinical disease activity in UC. *Methods:* Twenty-six patients and 28 healthy controls were enrolled in the study. The neutrophil and lymphocyte counts were recorded, and the N/L ratio was calculated from these parameters. The extent of disease classified according to the Montreal classification, clinical disease activity was evaluated using a modified Truelove–Witts severity index, and endo-scopic disease activities were classified according to Schroder et al. *Results:* The serum N/L ratios of active patients were significantly higher than those of inactive UC and controls ($P < 0.001$). The optimum N/L ratio cut-off point for active UC was 2.47. There was no significant difference between inflammation parameters, disease extension, and disease activity. *Conclusion:* Our results demonstrate that N/L ratio is higher in patients with active UC compared with controls and UC patients in remission and a cut-off value of 2.47 can be used to identify patients with active ulcerative colitis. J. Clin. Lab. Anal. 27:72–76, 2013. © 2012 Wiley Periodicals, Inc.

Key words: Neutrophil-to-lymphocyte ratio; ülcerative colitis; noninvasive monitoring

INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory disease causing continuous mucosal inflammation with an unknown precise etiology (1, 2). It is known as an incurable disease with patients usually requiring lifelong medication. Although the development of biologic agents has led to new therapeutic options in recent years, surgery continues to play an important role in the therapeutic alternative approach in severe UC (3). It is important to determine disease activity early as this will significantly reduce the surgery rate, and therefore reduce mortality in patients with serious UC (4). Noninvasive markers, such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), white blood cells (WBCs), fecal calprotectin, and polymorphonuclear neutrophil elastase are widely acknowledged as important, both for initial diagnosis and for precisely monitoring disease activity in UC (5,6). Nevertheless, an optimal test has not yet been developed. Therefore, the adjunctive use of additional serum markers may add a significant advantage for predicting disease severity and achieving diagnostic precision.

Blood neutrophil-to-lymphocyte (N/L) ratio is a simple marker of subclinical inflammation that can be easily obtained from the differential WBC count. The N/L ratio has been used to predict outcomes in patients with cancer and coronary artery disease (7, 8). We can obtain information about two different immune pathways from the N/L ratio. First of all about the neutrophils that are responsible for lasting inflammation and the second about the lymphocytes that demonstrate the regulatory pathway (9, 10).

Thus, the N/L ratio is an indicator of the overall inflammatory status of the body, and an alteration in N/L ratio may be found in UC patients with active disease. The aims of this study were to investigate the utility of N/L ratio as a simple and readily available predictor for clinical disease activity in UC and also to determine the association between N/L ratio with the disease widespread and endoscopic severity.

MATERIALS AND METHODS

Patients

Twenty-six patients with UC (18 male and 8 female) and 28 healthy controls (10 male and 18 female) attending the Gastroenterology Clinic of Erciyes University Hospital between March 2010 and September 2011 were enrolled in the study. The diagnosis of UC was based on standard clinical, radiological, endoscopic, and histological criteria. Patients' age, disease duration, drug intake, and other medical history were all noted for each subject. Complete blood count, ESR, and CRP were established for both patients and controls. The WBC, neutrophil, and lymphocyte counts were recorded, and the N/L ratios were calculated from these parameters. All of these parameters were determined again after disease remission was achieved in active UC patients. All UC patients underwent colonoscopy at study entry.

The endoscopic disease activities of UC patients were classified according to Schroder et al. (11). Based on initial colonoscopic examination, patients were divided into three subgroups with mild disease (erythema, decreased vascular pattern, mild friability), moderate disease (marked erythema, absent vascular pattern, friability, erosions), and severe disease (spontaneous bleeding, ulceration). Clinical disease activity was evaluated using a modified Truelove–Witts severity index (MTWSI) and details are shown in Table 1 (2, 12, 13). Clinical active disease was defined as an estimated MTWSI score of 4 or higher and patients with a lower score than 4 were considered in to be remission (inactive). The extent of the disease was

TABLE 1. Demographic Characteristics of Control Group and UC Patients

Variables	Control ($n = 28$)	UC ($n = 26$)	P
Sex (male/female)	10(35.7)/18(64.3)	18(69.2)/8(30.8)	0.028
Age (years)	44.50 ± 8.92	44.88 ± 13.23	0.900
Disease duration (years)	-	2.75(1.03–6.00)	-

Values are expressed as n (%), mean ± SD, or median (25th and 75th percentiles).

classified according to the Montreal classification (1). If limited to the rectum, it was defined as ulcerative proctitis; involvement up to the splenic flexure was defined as left sided colitis; and involvement extends proximal to the splenic flexure was defined as extensive UC.

Statistical Analyses

Shapiro–Wilk's and Levene's tests were used to test the normality and variance homogeneity of data. Independent-samples t-test, paired t-test, one-way analysis of variance parametric tests, and Mann–Whitney U, Wilcoxon-t, and Kruskal–Wallis H nonparametric tests were used for the comparison of continuous variables and chi-square analysis was used for categorical variables. Tukey and Tamhane's T2 tests were used for multiple comparisons. Values are expressed as frequencies and percentages, mean ± standard deviation or median and 25th–75th percentiles. Receiver operating characteristic (ROC) curves were constructed for the WBC, CRP, Sedimentation, and N/L variables, and the areas under the ROC curve values with 95% CIs were calculated and compared with each other. Optimal cut-off values were determined, sensitivity, specificity, positive predictive rate, negative predictive rate, and accuracy rate diagnosing measures were calculated with (95% CI) and Kappa tests were performed for each variable. Analysis was conducted using MedCalc (Version 9.2.0.1) and SPSS 15.0 (SPSS Inc., Chicago, IL) softwares by considering $P < 0.05$ as significantly different.
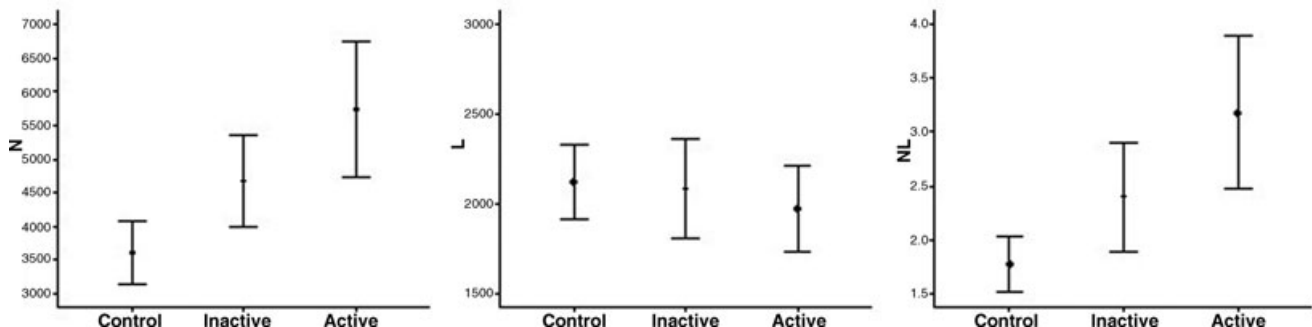

Fig. 1. Box-plots to display the variation of serum N, L, and N/L ratio values between control and UC patients.

TABLE 2. Comparison of Inflammation Markers Between Groups of Disease Activity

Variables	Control ($n = 28$)	Inactive UC ($n = 26$)	Active UC ($n = 26$)	P
WBC (/mm^3)	6,530.36 ± 1,461.91[a]	7,535.79 ± 1,672.55[a, b]	8,152.16 ± 3,016.71[b]	0.028
CRP (mg/l)	3.34 (3.34–3.34)[a]	3.08 (3.41–3.62)[a]	6.11 (3.41–21.93)[b]	<0.001
Sedimentation (mm/hr)	15.50 (10.25–20.75)[a]	17.00 (11.00–36.00)[a, b]	34.50 (14.75–57.00)[b]	0.004
N/L ratio	1.77 ± 0.68[a]	2.40 ± 1.05[a, b]	3.18 ± 1.76[b]	<0.001

Values are expressed as mean ± SD or median (25th and 75th percentiles). Different subscripts in a row indicate statistically significance difference.

RESULTS

Twenty-six patients with UC and 18 control subjects were enrolled in the present study. There were 8 females and 18 males in the UC group and 18 females and 10 males in the control group ($P = 0.028$). The median disease duration in UC patients was 2.75 years. The demographic characteristics of patients and control subjects are summarized in Table 1. There was no statistically significant difference between the ages of the study participants. The mean N/L ratios of the control, inactive, and active UC patients were $1.77 ± 0.68$, $2.40 ± 1.05$, and $3.18 ± 1.76$, respectively ($P < 0.001$). The serum N/L ratios of active patients were significantly higher than those of inactive UC and controls. While the serum neutrophil count increases in active patients unlike in inactive patients and controls, the serum lymphocyte count decreases, respectively (Fig. 1). Table 2 shows the serum N/L ratio and other laboratory values of the study participants. ROC curve analysis suggested that the optimum N/L ratio cut-off point for active UC was 2.47, with sensitivity, specificity, PPV, and NPV of 53.9% (33.4–73.4), 63.2% (38.4–83.7), 66.7% (43.0–85.4), and 50.0% (29.1–70.9), respectively (Fig. 2). The overall accuracy of the N/L ratio in determination of active UC was 57.8%. The same analyses for other inflammation markers are summarized in Table 3. There was no significant pair wise difference for AUC values ($P > 0.05$).

Ten patients were classified as having extensive disease and 16 patients as left sided according to the endoscopic examination on study entry. On the initial examination, only one patient was classed as having proctitis and this patient was included in the left-sided group to compare inflammation parameters between two groups in active
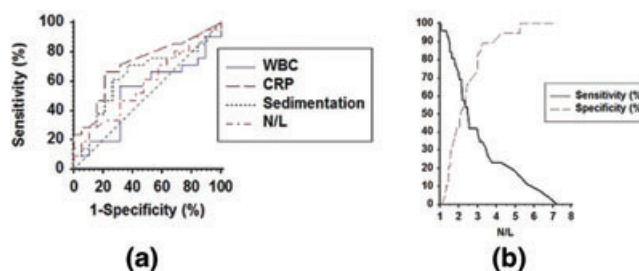

Fig. 2. (a) Comparison of ROC curves among WBC, CRP, sedimentation, and N/L ratio variables. AUC values were 0.52(0.36–0.68), 0.72(0.55–0.85), 0.64(0.48–0.79), 0.59(0.42–0.74), respectively, and there was not any significant pair wise difference for AUC values ($P > 0.05$). (b) Determination of the optimal cut-off value for N/L ratio.

disease. There was no significant difference between inflammation parameters and disease extension ($P > 0.05$; Table 4).

The endoscopic disease activity was mild in four, moderate in 17, and severe in 5 patients at the initial endoscopic examination. We compared the inflammation markers and disease activity in active disease and did not find any significant association ($P > 0.05$; Table 5).

DISCUSSION

The main finding of our study relates to the identification of the N/L ratio as a novel noninvasive marker of disease severity in patients with UC. Our results demonstrate that N/L ratio is higher in patients with active UC compared with controls and UC patients in remission and a cut-off value of 2.47 can be used to identify patients

TABLE 3. Diagnosing Measures and Kappa Test Results of WBC, CRP, Sedimentation, and N/L Variables for the Determined Cut-Off Values in the Detection of Ulcerative Collitis Activity

Variables and cut-off values	Diagnosing measures					Kappa test	
	SEN (95%CI)	SPE (95%CI)	PPR (95%CI)	NPR (95%CI)	AR (95%CI)	κ	P
WBC (>9,350/mm^3)	32.0 (15.0–53.5)	84.2 (60.4–96.6)	72.7 (39.0–94.0)	48.5 (30.8–66.5)	54.6 (38.9–69.6)	0.149	0.219
CRP (>3.62 mg/l)	66.7 (44.7–84.4)	73.7 (48.8–90.9)	76.2 (52.8–91.8)	63.6 (40.7–82.8)	69.8 (53.9–82.8)	0.397	0.009
Sedimentation (>25 mm/hr)	62.5 (40.6–81.2)	68.4 (43.5–87.4)	71.4 (47.8–88.7)	59.1 (36.4–79.3)	65.1 (49.1–79.0)	0.304	0.044
N/L (>2.47)	53.9 (33.4–73.4)	63.2 (38.4–83.7)	66.7 (43.0–85.4)	50.0 (29.1–70.9)	57.8 (42.2–72.3)	0.164	0.259

SEN, sensitivity; SPE, specifity; PPR, positive predictive rate; NPR, negative predictive rate; AR, accuracy rate.

TABLE 4. Comparison of Inflammation Markers and Disease Extension in Active Disease

Variables	Left side ($n = 16$)	Extensive ($n = 10$)	P
WBC (/mm^3)	$7,700.88 \pm 3,177.75$	$8,954.44 \pm 2,690.03$	0.329
CRP (mg/l)	5.30 (3.36–13.43)	8.62 (5.16–37.35)	0.192
Sedimentation (mm/hr)	26.00 (10.00–48.00)	46.00 (28.00–72.50)	0.108
N/L ratio	2.79 ± 1.57	3.81 ± 1.96	0.155

Values are expressed as mean $\pm$ SD or median (25th and 75th percentiles).

with active UC. High levels of N/L ratio in the sera of active UC patients, compared to inactive UC and controls, support the view that neutrophils may have a role in the inflammatory cascade of UC and disease pathophysiology.

Endoscopic examination, histologic findings, and radiological imaging modalities are commonly used to monitor intestinal inflammation. A great number of methods have also been assessed in different studies as potential noninvasive/invasive markers for UC diagnosis and for determining disease activity (14, 15). Although novel disease specific biomarkers have been identified, most of them are time consuming and expensive. Unfortunately the role of N/L ratio in UC has not been clearly elucidated until now.

Elevated levels of N/L ratio were also found to be associated with poor survival in patients after percutaneous coronary intervention and in those undergoing coronary artery bypass graft (7, 16). Many cancer survival studies have suggested that the N/L ratio is a significant predictor of overall and disease specific survival of patients (8,17,18). The N/L ratio is correlated with disease severity in patients with nonalcoholic fatty liver disease (19). It is superior to total WBC in predicting adverse outcomes of acute pancreatitis (20). N/L ratio could be an important measure of systemic inflammation as it is cost effective, readily available, and can be calculated easily. During the preparation of this article, a study was concluded that patients with active UC have elevated N/L ratios similar to our findings (21). Torun et al. found optimum cut-off value of 2.16 to indicate active disease with a higher sensitivity, specificity, positive predictive, and negative predictive value of 81.8%, 80.5%, 86.8%, and 73.8%, respectively (21). Moreover from Torun et al., in the present study, we evaluated the relation of N/L ratio with the disease extension and the endoscopic disease activity and did not find any significant association. Due to the lack of detailed knowledge, the current study was conducted to investigate the N/L ratio as a measure of systemic inflammation and its relationship with disease activity. Our study showed that N/L ratio is higher in patients with active UC compared with controls and UC patients in remission.

Physiological malfunction of the intestinal epithelium leads to the infiltration of bacteria and their components and the recruitment of neutrophils mediating inflammatory bowel disease (22,23). Neutrophil accumulation and abscess formation within intestinal crypts at the apical epithelial surface is a pathological feature of UC (22). In the present study increased N/L ratio in active UC patients confirms the key role of neutrophils in the inflammation process.

The study has some limitations including, (i) the cross-sectional design and the relatively small sample size, and (ii) our study was not designed to elucidate the mechanistic pathways that lead to higher N/L ratio in patients with UC.

In summary, our study demonstrates that in patients with UC, the N/L ratio is strongly associated with active disease. Unlike many other noninvasive markers of UC, the N/L ratio is inexpensive and readily available. Although the accuracy of the N/L ratio for detecting active UC is suboptimal, the ratio is an easily derived measure that might, in combination with other markers, assist in identifying patients at increased risk of active and severe disease. Future studies are needed to externally cross-validate our findings in a larger cohort of UC patients.

TABLE 5. Comparison of Inflammation Markers and Endoscopic Disease Activity in Active Disease

Variables	Mild ($n = 4$)	Moderate ($n = 17$)	Severe ($n = 5$)	P
WBC (/mm^3)	$8,592.50 \pm 3,008.75$	$8,137.75 \pm 3,438.03$	$7,846.00 \pm 1,782.32$	0.939
CRP (mg/l)	4.23 (3.16–6.81)	5.74 (3.41–29.80)	13.90 (7.98–46.50)	0.129
Sedimentation (mm/hr)	26.00 (10.00–40.00)	42.50 (12.50–64.50)	29.00 (23.50–65.50)	0.700
N/L ratio	2.40 ± 0.72	3.17 ± 1.63	3.85 ± 2.71	0.558

Values are expressed as mean $\pm$ SD or median (25th and 75th percentiles).

CONFLICT OF INTEREST

None.

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