Peripheral Blood Inflammatory Markers and Crohn's Disease: Lymphocytes Counts Could Predict Stricture Lesions

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Abstract

Background: Intestinal stricture is a complication of Crohn's disease (CD) due to fibrosis, but there are no biomarkers for predicting intestinal strictures before clinical obstruction. It is reported that several types of lymphocytes (LC) are involved in the pathogenesis of intestinal fibrosis. However, few studies have focused on the peripheral blood LC in patients with CD associated stricture.

Aim To analyze the relationships between peripheral blood inflammatory markers especially LC and CD to provide evidence for CD diagnosis and therapy.

Methods: A total of 158 CD patients who underwent single-balloon enteroscopy from January 2016 to June 2019 in Jinling Hospital were retrospectively enrolled. The Montreal classification, maintenance medicines, CD activity index (CDAI), simple endoscopic score for CD (SES-CD), full blood count and C-reactive protein (CRP) level were recorded. The relationships among peripheral blood inflammatory markers, disease activity and intestinal strictures were analyzed using SPSS 22.0.

Results: After excluding 8 patients treated with azathioprine, which severely affects blood counts, 150 patients were divided into two groups: a stricture group (n=82) and non-stricture group (n=68). LC and the proportion of lymphocytes (LC%) were significantly lower in the stricture group than in the non-stricture group, p was 0.000 and 0.018, respectively, and LC was an independent risk factor of stricture lesion. In the subgroup analysis, 30 strictures without obstruction were classified as mild strictures, and 52 cases of obstruction were in the severe stricture group. LC notably decreased following stricture aggravation, p=0.000. The area under the curve (AUC) of LC predicting strictures was 0.711 with sensitivity of 73.5% and a specificity of 63.4% (cutoff: 1.245).

Conclusion: LC gradually decreases as intestinal strictures aggravated and could be a new marker for predicting intestinal strictures in CD patients.

Background

Crohn´s disease (CD) is a type of chronic inflammatory bowel disease characterized by segmental and transmural involvement of any portion of the gastrointestinal tract [1]. The terminal ileum is most commonly involved in CD patients [2], and approximately one-third of CD patients develop intestinal strictures, which cause obstructive symptoms and require surgical resection [3]. Ileocolonoscopy and histology remain the "gold standard" for diagnosing CD,and these methods can investigate only the few centimeters distal to the ileum and can´t detect small bowel lesions. Balloon enteroscopy can provide direct visualization of the majority of the small bowel, enable biopsy histology, and is helpful for diagnosing and treating of CD [4].

The etiology and pathogenesis of CD are not yet entirely understood, and an aberrant immune response plays an important role in the pathogenesis. It was reported that lymphocytes (LC) play a key role in CD, such as in the recruitment of circulation to the inflamed intestine, mesenteric lymph nodes (MLNs) and

intestinal strictures [5–10]. However, few studies have focused on the change of LC in circulation. This study summarized patients with CD who underwent single-balloon enteroscopy to analyze the relationships between peripheral blood inflammatory markers, especially LC and disease activity as well as intestinal strictures respectively to provide evidence for CD diagnosis and therapy.

Methods

This was a retrospective study. We collected CD patients who underwent single-balloon enteroscopy between January 2016 and June 2019. Patients with CD diagnosed by standard clinical, radiological, endoscopic and histopathologic findings were included [11]. The exclusion criterion was a history of intestinal surgery. Full blood counts including white blood cells (WBC), neutrophils (NEUT), LC, proportion of neutrophils (NEUT%), proportion of lymphocytes (LC%) and C-reactive protein (CRP) were recorded. The clinical disease activity was graded according to Crohn's disease activity index (CDAI) score, and patients were divided into active group and the inactive group. Endoscopies were performed and assessed with Simple Endoscopic Score for CD (SES-CD). Patients were divided into stricture group and non-stricture group based on whether strictures were present. The phenotype of disease was defined according to the Montreal classification. The protocol for this research project has been approved by the Institutional Ethics Committee of Jinling Hospital.

Statistical analysis

Data were analyzed using SPSS 22.0. Continuous variables were expressed as the mean ± SD, and noncontinuous variables were expressed as the range, median, frequency and percentage. Data analyses between two groups were performed with unpaired, two-tailed Student's t tests or $\chi 2$ tests. One-way ANOVA was used to compare all groups. The association between two variables was assessed by the Spearman rank correlation coefficient (r). Significant differences indicated by $p < 0.05$. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the predictive value as defined by the area under the curve (AUC).

Results

A total of 158 CD patients who underwent single-balloon enteroscopy were enrolled, and the clinical characteristics are shown in Table 1. A total of 130 patients underwent enteroscopy performed with the anal approach, 18 patients underwent the oral approach, and 10 patients underwent both approaches. Enteroscopy the anal approach was performed from the upper ileocecal valve for approximately 5-280 cm in 140 cases, with an average of 133.7 cm. 28 cases of oral enteroscopy were performed approximately 120–350 cm beyond the ligament of Treitz, with an average of 225.71 cm. The SES-CD scores of 158 patients ranged from 1–28 with a median of 7.25 scores, and the CDAI scores was 40–418 with a median of 152.28 scores.

Table 1
Clinical characteristics of CD patients

	Total n = 158	Stricture group n = 87	Non-stricture group n = 71	p
Age (years)	34.47 ± 13.41	35.08 ± 11.91	33.72 ± 15.10	0.527
Male [n (%)]	120(75.95%)	67(77.01%)	53(74.65%)	0.852
First visit/Subsequent visit (n)	69/89	30/57	39/32	0.010
Course of disease (years)	3.26 ± 2.67	3.40 ± 2.91	3.00 ± 2.18	0.497
Age at diagnosis (years)	32.64 ± 13.57	32.86 ± 11.93	32.37 ± 15.43	0.820
Montreal Classification				
Age [n (%)]				
A1	13(8.23%)	4(4.60%)	9(12.68%)	0.066
A2	106(67.09%)	63(72.41%)	43(60.56%)	0.115
A3	39(24.68%)	20(22.99%)	19(26.76%)	0.584
Disease location [n (%)]				
L1	73(46.20%)	43(49.43%)	30(42.25%)	0.145
L3	76(48.10%)	39(44.83%)	37(52.11%)	0.362
L1 + L4	4(2.53%)	3(3.45%)	1(1.41%)	0.628
L3 + L4	5(3.16%)	2(2.30%)	3(4.23%)	0.493
Disease behavior [n (%)]				
B1	70(44.30%)		70(98.59%)	0.000
B2	84(53.16%)	84(96.55%)		0.000
B3	1(0.63%)		1(1.41%)	0.449
B2 + 3	3(1.90%)	3(3.45%)		0.253
+ P (perianal)	36 (22.78%)	18(20.69%)	18(25.35%)	0.487
Medication of subsequent visit patients [n (%)]				
no	16(17.98%)	10(17.54%)	6(18.75%)	0.887

	Total n = 158	Stricture group n = 87	Non-stricture group n = 71	p
Mesalazine	21(23.60%)	13(22.81%)	8(25%)	0.815
Mesalazine□EN	5(5.62%)	4(7.02%)	1(3.13%)	0.650
Azathioprim	2(2.25%)		2(6.25%)	0.127
Azathioprim□EN	6(6.74%)	5(8.78%)	1(3.13%)	0.413
Sulfasalazine	1(1.12%)		1(3.13%)	0.360
EN	34(38.20%)	23(40.35%)	11(34.38%)	0.578
Thunder god vine	1(1.12%)	1(1.75%)		0.451
Infliximab	3(3.37%)	1(1.75%)	2(6.25%)	0.293

After excluding 8 patients treated with azathioprine which severely affects blood counts, there were 40 patients with active disease (active group) and 110 patients in remission (inactive group) based on the CDAI scores. When comparing inflammatory markers in the two groups, we found that the WBC, NEUT, NEUT% and CRP were significantly higher in the active group than in the inactive group, p values were 0.009, 0.002, 0.018 and 0.040, respectively, and that LC% was lower in the active group,p = 0.004; however, LC was not different between the two groups (Table 2).

Table 2
Comparison of inflammatory markers in patients with and without active disease

	Inactive group (n = 110)	Active group (n = 40)	p
WBC (× 10^9/L)	5.58 ± 1.72	6.45 ± 1.78	**0.007**
NEUT (× 10^9/L)	3.65 ± 1.43	4.49 ± 1.59	**0.002**
LC (× 10^9/L)	1.36 ± 0.49	1.34 ± 0.46	0.802
NEUT%	64.24 ± 9.48	68.41 ± 9.45	**0.018**
LC%	25.26 ± 8.18	20.99 ± 7.52	**0.004**
CRP (mg/L)	8.90 ± 17.29	18.43 ± 26.57	**0.040**

Based on whether strictures were present, the 158 patients were divided into two groups, a stricture group (n = 87) and non-stricture group (n = 71). After excluding 8 patients treated with azathioprine, there were

82 patients in the stricture group and 68 patients in the non-stricture group. A comparison of the peripheral blood inflammatory indexes of the two groups, revealed that LC and LC% were significantly lower in the stricture group than in the non- stricture group, p was 0.000 and 0.018 (Fig. 1), respectively, but WBC, NEUT, NEUT% and CRP were not significantly different between the two groups. SES-CD and CDAI also showed no significant differences between two groups. Using logistic regression analysis (Backup), we found that LC was an independent risk factor for stricture lesions (OR = 10.526, 95%CI: 1.404–78.905) (Table 3).

Table 3
Logistic regression analysis of stricture lesions in CD patients

	Univariate analysis			Multivariate analysis	
	Stricture group n = 82	Non-stricture group n = 68	p	odds ratio(95%CI)	p
Clinical datas					
Age (years)	34.91 ± 12.23	33.75 ± 15.28	0.602		
Male [n (%)]	63(76.83%)	51(75%)	0.794		
First visit/Subsequent visit [n (%)]	30/52	39/29	0.012	0.955(0.484–1.883)	0.894
Course of disease (years)	3.52 ± 2.99	2.83 ± 2.16	0.020	0.356(0.015–8.653)	0.526
Age at diagnosis	32.70 ± 12.26	32.54 ± 15.56	0.947		
Montreal Classification					
Age [n (%)]			0.696		
A1	4(4.88%)	8(11.76%)			
A2	58(70.73%)	42(61.76%)			
A3	20(24.39%)	18(26.47%)			
Disease location [n (%)]			0.286		
L1	41(48.78%)	29(39.71%)			
L3	37(45.12%)	35(51.47%)			
L1 + L4	3(3.66%)	1(1.47%)			
L3 + L4	1(1.22%)	3(4.41%)			
Disease behavior [n (%)]			0.000	0.000(0.000-0.005)	0.000
B1		67(98.53%)			
B2	79(96.34%)				
B3		1(1.47%)			
B2 + 3	3(3.66%)				
+ P	17(20.73%)	18(26.47%)	0.409		

	Univariate analysis			Multivariate analysis	
Medication of subsequent visit patients [n (%)]			0.249		
no	10(19.23%)	6(20.69%)			
Mesalazine	13(25.00%)	8(27.59%)			
Mesalazine□EN	4(7.69%)	1(3.45%)			
Sulfasalazine		1(3.45%)			
EN	23(44.23%)	11(37.93%)			
Thunder god vine	1(1.92%)				
Infliximab	1(1.92%)	2(6.90%)			
Laboratory tests					
WBC (× 10^9/L)	5.56 ± 1.78	6.11 ± 1.73	0.062	0.899(0.554–1.460)	0.668
NEUT (× 10^9/L)	3.81 ± 1.61	3.95 ± 1.40	0.583		
LC (× 10^9/L)	1.19 ± 0.42	1.55 ± 0.48	0.000	10.526(1.404–78.905)	0.022
NEUT%	66.70 ± 10.86	63.72 ± 7.94	0.061	1.029(0.933–1.135)	0.568
LC%	22.72 ± 8.97	25.81 ± 6.86	0.024	0.985(0.849–1.144)	0.846
CRP (mg/L)	12.03 ± 23.62	10.73 ± 16.19	0.698		
SES-CD (scores)	7.78 ± 3.38	6.57 ± 4.88	0.189		
CDAI (scores)	160.70 ± 69.91	145.35 ± 71.28	0.083	0.996(0.991–1.001)	0.148

To conduct subgroup analysis, 82 patients in the stricture group were divided into two groups. There were 30 cases of strictures with no obstruction (mild stricture group) and 52 cases with obstruction (severe stricture group). A comparison of LC and LC% in the non-stricture group, mild stricture group and severe stricture group, revealed that LC and LC% had differences among groups, p was 0.000 and 0.017, and LC gradually decreased with the stricture extents (Fig. 2A, 2B). There were 24 patients (46.15%) in the severe stricture group, 7 cases (23.33%) in the mild stricture group, and 2 patients (2.94%) in the non-stricture group underwent surgery later, p = 0.000 (Fig. 2C).

A ROC analysis of LC for evaluating stricture lesions in CD patients was performed, and the AUC value was 0.711, with a sensitivity of 73.5% and a specificity of 63.4% (cutoff: 1.245) (Fig. 3).

Discussion

In this study, we found that compared to the non-stricture group, the stricture group had significantly lower LC and LC%, and LC was an independent predictive factor of stricture lesion. In addition, LC notably decreased gradually following with the stricture extents. Furthermore, the AUC of LC for evaluating stricture lesions was 0.711.

CD can affect any part of the gastrointestinal tract and is characterized by mucosal and transmural inflammation of the bowel wall. Chronic inflammation can eventually cause intestinal strictures due to fibrosis involving the full thickness of the bowel wall [12], and no effective medical therapy can reverse these lesions except for surgical resection [2]. When strictures occur, intestinal fibrosis can be diagnosed by different techniques, including intestinal ultrasound (IUS), computed tomography (CT), magnetic resonance (MR) and endoscopy. Therefore, predicting the progression of intestinal strictures formation is important for managing CD. However, currently, there are no biomarkers for predicting the development of intestinal strictures or identifying the progression of fibrosis before clinical obstruction [13]. A reliable, noninvasive and widely available biomarker is required to prevent irreversible strictures.

Intestinal strictures are a consequence of chronic transmural inflammation and an excessive deposition of extracellular matrix components, leading to fibrosis that frequently causes intestinal obstruction [14]. Dysregulation of the intestinal immune system results in mucosal inflammation and participates in the pathogenesis of fibrosis [5]. It was reported that the number of T cells and B cells increases in mucosal and submucosal tissues of the inflamed intestine [15, 16]. Injuries and infections activate both non-immune cell, including myofibroblasts and fibroblasts, and immune cells, including monocytes, macrophages, and LC to enable tissue remodeling and distortion [14]. It has been shown that CD4 + T lymphocyte are involved the initiation, perpetuation and resolution of fibrosis and that fibrosis is regulated by Th2 and Th17 responses and dynamic interactions between fibroblasts and macrophages [7]. Additional, in CD4 + T lymphocyte subsets, the Th1/Th2 balance plays an important regulatory role in the development and progression of many diseases [8–10]. IL-17A is a proinfammatory cytokine that is mainly produced and secreted by Th17 cells. Recently, IL-17A has been shown to be involved in the development of intestinal fibrosis by inducing epithelial–mesenchymal transition [17]. Consequently, Th17 cells are related to intestinal inflammation and fibrosis. In TNBS-induced mouse models of acute and chronic colitis, the administration of vaccine-induced specific antibodies to IL-12 and IL-23 was associated with improvements in intestinal inflammation and fibrosis which is speculated to be due to a reduction in Th1 cells and Th17 cells in the MLNs in acute colitis and chronic colitis, respectively [18]. In summery, several types of LC participate in the development and pathogenesis of fibrosis. However, few studies have investigated the changes in LC of peripheral blood circulation in CD patients with strictures lesions, and the relationship between LC and luminal lesions.

We found that LC was not different between active group and inactive group, however, LC was significantly lower in the stricture group than in the non-stricture group. This result indicated that LC did not correlate with disease activity but correlated with stricture lesions in CD patients. A subsequent

comparison of LC among the non-stricture group, mild stricture group and severe stricture group showed that LC gradually decreased with stricture extent. Furthermore, the rate of surgery was highest in the severe stricture group. These results indicated that LC could reflect the intestinal stricture extents of CD patients and guide treatment, including whether surgical intervention is necessary. The relationship and pathogenesis between LC and stricture lesions in CD patients should be further researched.

However, this study had several limitations. First, more samples are needed for the analysis. Second, this study did not analyze the influence of age, disease duration, medicine and prognosis on LC.

Conclusion

Among CD patients, LC was significantly lower in the stricture group than in the non-stricture group, and was an independent risk factor for stricture lesions. LC gradually deceased with stricture extent and could be a new marker for predicting stricture lesions in CD patients and provide evidence for clinical therapy, including surgery.

Abbreviations

CD: Crohn's disease; LC: lymphocytes; CDAI: CD activity index; SES-CD: Simple Endoscopic Score for CD; CRP: C-reactive protein; LC%: proportion of lymphocytes; AUC: area under the curve; MLNs: mesenteric lymph nodes; WBC: white blood cells; NEUT: neutrophils; NEUT%: proportion of neutrophils; ROC: Receiver operating characteristic; IUS: intestinal ultrasound; CT: computed tomography; MR: magnetic resonance.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

Conception and design of the work: JJZ, ZMW, QRL; the acquisition of data: JJZ, YQD, BLD; analysis of data: JJZ, ZMW; interpretation of data: JJZ, ZG, ZMW; drafting of the work: JJZ, ZG; revision and supervision of the work: ZMW, QRL. All authors have read and approved the manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The protocol for this research project has been approved by the Institutional Ethics Committee of Jinling Hospital.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

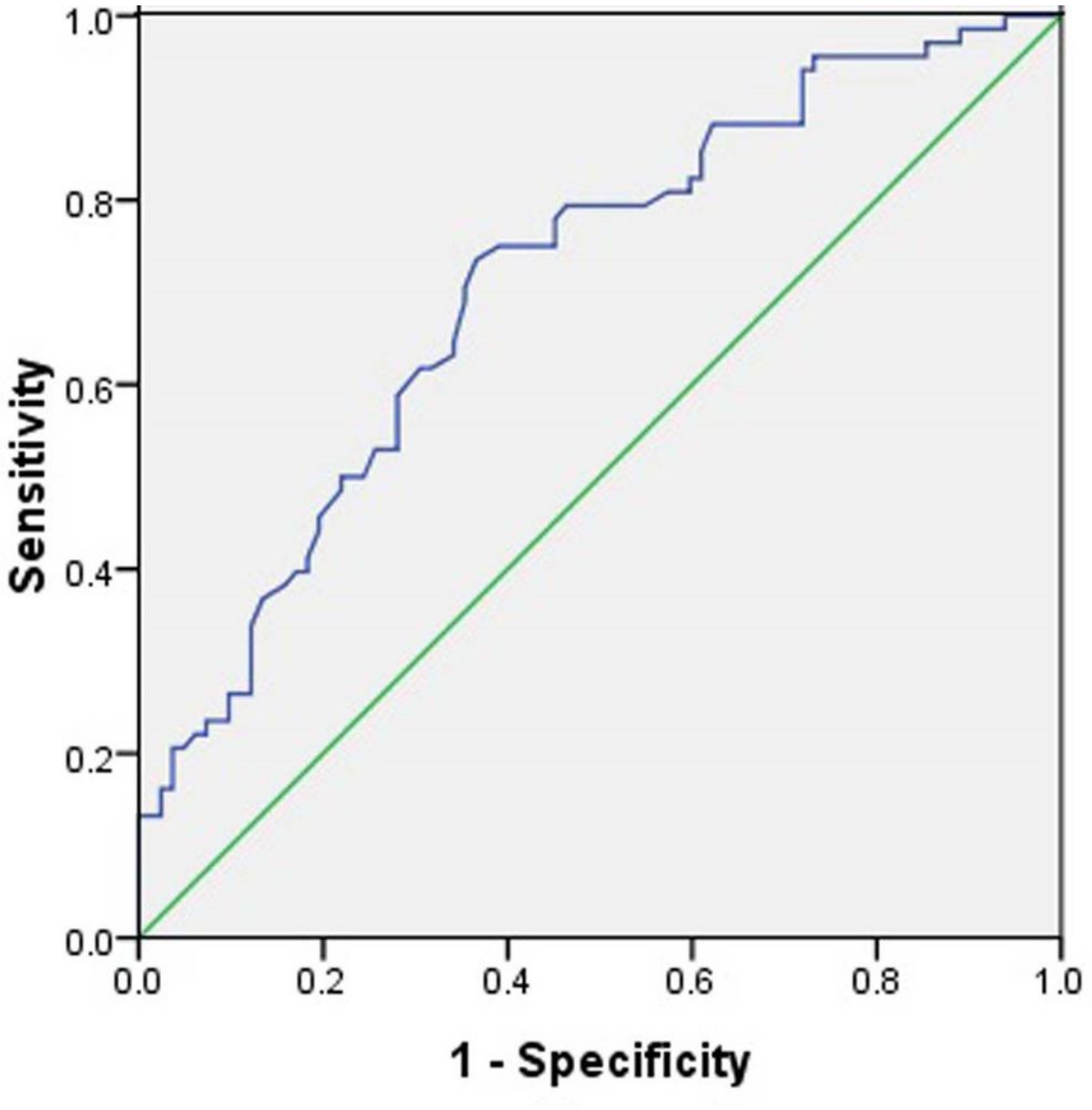

Figure 1

Comparison LC and LC% between stricture group and non-stricture group A Comparison LC between stricture group and non-stricture group, B Comparison LC% between stricture group and non-stricture group, where * indicates $P < 0.05$, *** indicates $P < 0.001$.

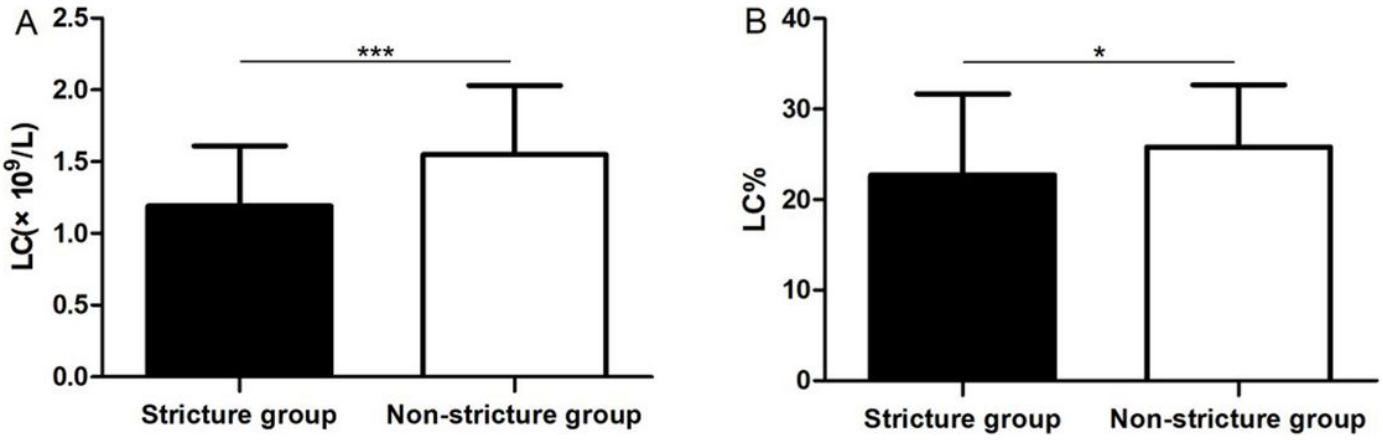

Figure 2

Comparison LC, LC% and the rate of surgery based on stricture extent of CD A Comparison LC based on stricture extent of CD, B Comparison LC% based on stricture extent of CD, C Comparison the rate of surgery based on stricture extent of CD, where * indicates P < 0.05, ** indicates P < 0.01, *** indicates P < 0.001.

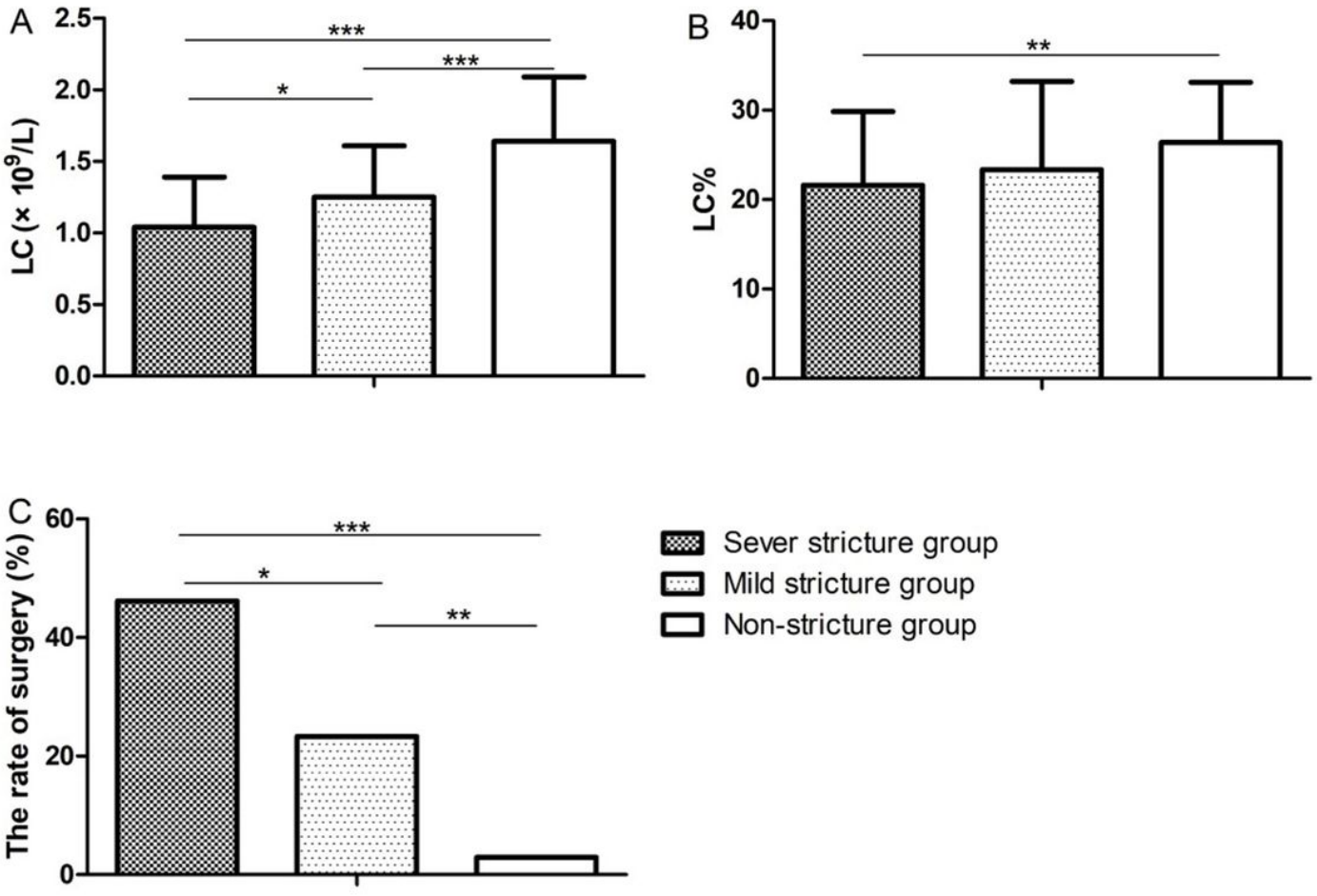

Figure 3

ROC for LC to evaluate stricture lesions of CD