The Correlation Between Endotoxin, D-Lactate and Diamine Oxidase With Endoscopic Activity in Inflammatory Bowel Disease

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Research article

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Abstract

Background & Objective: The disease activity monitoring of inflammatory bowel disease (IBD) plays a crucial role for making therapeutic strategies. Endoscopy has been recognized as a gold standard for evaluating disease activity of IBD. However, this method is invasive. Currently, a noninvasive biomarker that could replace endoscope is needed in clinical practice. In this study, we examined whether the diamine oxidase (DAO), D-lactate and endotoxin (ETX) could monitor the disease activity and predict endoscopic remission in patient with IBD.

Methods: A total of 149 eligible IBD patients (82CD and 67UC) who had received both endoscopic examination and intestinal barrier function detection in our hospital were enrolled in this study. Endoscopic activity was estimated by the Simple Endoscopic Score (SES-CD) for Crohn's disease, and the ulcerative colitis endoscopic index of severity (UCEIS) for ulcerative colitis. The predictive value and optimal predictive thresholds for those biomarkers were determined by Receiver operating characteristic analysis.

Results: For UC patients, DAO, D-lactate and ETX showed better correlation with UCEIS than ESR and CRP, and exhibited satisfactory predictive value in predicting remission. Among patients with CD, DAO and ETX not only showed a better correlation than ESR and CRP with SES-CD, but also capacity to identify more severe patients.

Conclusion: DAO and ETX could be used to distinguish different endoscopic activity of CD. DAO, D-lactate and ETX could predict UC endoscopic remission.

Introduction

The assessment of disease activity of inflammatory bowel disease (IBD) has crucial implications for making therapeutic strategies and predicting prognosis in clinical practice. Endoscopic remission in patients with IBD is associated with durable remission and is considered the ultimate goal of IBD therapy.^[1] The gold standard of evaluating disease activity of IBD is endoscopic and histopathological examination. However, endoscopy examination with biopsy is an invasive, expensive and time-consuming procedure. In clinical practice, some patients may be reluctant to handle fecal material. Therefore, it is important to find reliable serum biomarkers which can replace endoscopic examination to monitor disease activities or predict endoscopic remission.

The traditional biological activity markers of IBD that are widely used in clinical practice to evaluate disease activity of IBD are C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). However, both of them lack intestinal specificity. Some studies showed that the relationship between CRP and disease activity of CD was weak^[2, 3]. Another study found that there was no relation between serum CRP and endoscopic activity of UC, ESR was the more accurate to assess the endoscopic activity than CRP with low sensitivity and specificity^[4]. The effect of CRP and ESR in evaluating IBD are limited as

previously described^[5, 6]. An accurate and reliable non-invasive serum biomarker for evaluating the endoscopic activity of IBD has not yet been identified.

The barrier function of the intestinal was found to play a significant role in IBD^[7-9]. Previous studies have suggested that monitoring the change of intestinal barrier function may help evaluate disease status of IBD^[10-12]. Diamine oxidase (DAO), D-lactate and endotoxin (ETX) are three biomarkers which are most commonly used to assess the function of intestinal barrier. But until now, there has been no research on the association between DAO, D-lactate and ETX and endoscopic activity of IBD. In this study, we studied the correlation of DAO, D-lactate and ETX levels with endoscopic activity in patients with IBD, aiming to assess whether DAO, D-lactate and ETX could monitor the disease activity of IBD and predict the endoscopic remission.

Patients And Methods

Patients

A total of 149 IBD patients (82 CD, 67 UC) who have undergone both endoscopic examination and intestinal barrier function detection in the Department of Gastroenterology of Zhongnan Hospital of Wuhan University between December 2016 and October 2017 were included in this study. The diagnosis of IBD was made bases on a combined assessment of clinical features, endoscopic findings, histopathological findings and results of follow-up evaluation, including response to medical therapy, such as corticosteroids, immunomodulators, and/or anti-tumor necrosis factor- α (TNF- α) biologics. All patients were informed and consented to the study. All research processes were carried out in accordance with relevant guidelines and ethics regulations and with approval from Zhongnan Hospital of Wuhan University.

Inclusion and Exclusion Criteria

The inclusion criteria were as follows: 1) Diagnosed with IBD; 2) Patients have undergone both endoscopic examination and intestinal barrier function detection; 3) The interval between endoscopic examination and intestinal barrier function was less than 3 weeks; The exclusion criteria were as follows: 1) patients who had previous surgery of bowel resection; 2) Patients who had taken non-steroidal anti-inflammatory drug (NSAID) in the previous 3 weeks; 3) Patients with specific pathogens infection, renal failure, chronic liver, colorectal cancer, connective tissue diseases or pancreatitis.

Protocol

Endoscopic disease activity was respectively scored by experienced endoscopist using the Simple Endoscopic Score for Crohn's Disease (SES-CD) or the ulcerative colitis endoscopic index of severity (UCEIS)^[13, 14].

The concentration of D-lactate, the activity of DAO and ETX in the blood were detected by Diamine oxidase, Lactic acid, Bacterial Endotoxin Assay kit (Enzyme), respectively. All the reagents were

purchased from Beijing Zhongsheng Jinyu diagnostic technology Ltd, China. The concentration of D-lactate and DAO was detected with spectrophotometry, and the concentration of ETX was tested by chromogenic method.

Definitions of Variables

The “Big Ulcer” were defined as their longest diameter more than 20 mm, the “Small ulcers” were defined as whose longest diameter less than 5 mm. The calculation of SES-CD was based on four endoscopic variables (presence and size of ulcers, proportion of surface covered by ulcers, proportion of surface affected by disease, and presence and severity of stenosis) and assessed in 5 ileocolonic segments. Vascular pattern, bleeding, and erosions or ulcerations were evaluated in the most inflamed colonic segment to calculate the UCEIS. The SES-CD ≤ 2 or UCEIS ≤ 1 were defined as remission. For active CD, we defined SES-CD 3–6 as mild activity, 7–15 as moderate activity, and score ≥ 16 as severe activity. For active UC patients, we defined UCEIS 2–6 as mild and moderate activity, 7–8 as severe activity.

Statistical Analysis

Statistical analysis was performed using the SPSS 21.0 software (SPSS, Chicago, IL, USA) and GraphPad Prism 5.0 software (GraphPad Software Inc., La Jolla, CA, USA). Correlation between categorical variables was determined by Pearson correlation coefficient r . Receiver operating characteristic (ROC) analysis was used to find the optimal thresholds of the DAO, D-lactate and ETX for discriminating endoscopic activity in patients with IBD. The optimal diagnostic thresholds for a test were determined by plotting Youden’s index ($YI = [\text{sensitivity} + \text{specificity}] - 1$). The area under the curve (AUC) was used to compare accuracies of biomarkers in predicting different endoscopic severity. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy (proportion of correct diagnoses) were calculated to determine the reliability of each of the biomarkers to predict endoscopic severity in patients with IBD.

Results

Patient Baseline Characteristics

A total of 149 eligible patients (82CD, 67UC) were enrolled, and 134 patients (73CD, 61UC) were in active, 15 patients were in remission. The baseline characteristics of all patients were shown in **Table 1**. The median disease duration of all patients was 3 years (interquartile range [IQR]:1.0-4.0yr). The patients with CD were younger than UC (Median age 28 .0 vs 46.0 years, $P < 0.001$). There was no statistical difference in gender and smoking history between patients with UC and CD. Based on the Montreal classification, most of the patients with CD (55.65%) had an ileocolonic disease (L3) and 64.18% of the patients with UC had an extended colitis (E3).

Correlation Between Biomarkers and Endoscopic Score in CD Patients

Correlation coefficient r between biomarkers and endoscopic score of disease severity of CD were shown in **Table 2**. As shown in the table, DAO and ETX were demonstrated to have a better correlation with SES-

CD ($r=0.532$ and $r=0.468$, respectively; $P<0.0001$ for both) compared to WBC, ESR and CRP ($r=0.146$, $P=0.191$; $r=0.346$, $P=0.0015$; $r=0.250$, $P=0.023$), while there was no correlation between D-lactate and SES-CD ($r=0.167$, $P=0.133$).

Correlation Between Biomarkers and Endoscopic Score in UC Patients

Correlation coefficient r between biomarkers and endoscopic score of disease severity of UC were shown in **Table 3**. Similar to CD, DAO and ETX were demonstrated to have better correlation with UCEIS ($r=0.600$ and $r=0.505$, respectively; $P<0.0001$ for both) compared with WBC, ESR and CRP ($r=0.151$, $P>0.05$; $r=0.285$, $P=0.021$; $r=0.334$, $P=0.006$). There was significant but weak correlation between D-lactate and UCEIS ($r=0.407$, $P=0.0006$). The relationship of DAO, D-lactate and ETX with endoscopic score of UC were shown to be better than those of CD.

Value of Intestinal Barrier Marker in Predicting Endoscopic Severity in CD

As was shown in **Table 4** and **Figure 1**, the DAO levels of severe groups were higher than those of moderate groups ($P<0.05$, Figure 1A), and the DAO levels of moderate groups were also higher than those of mild groups ($P<0.001$, Figure 1A). The ETX levels of severe groups were higher than those of moderate groups ($P<0.05$, Figure 1B), and the ETX levels of moderate groups were also higher than those of mild group ($P<0.01$, Figure 1B). In brief, as severity of endoscopy increased, the levels of DAO and ETX increased. There was no difference in D-lactate levels among three groups of active CD patients (**Figure 1C**).

The DAO and ETX had diagnostic utility in differentiating CD patients with different endoscopic activities (**Figure 2** and **Table 4**). For DAO, the optimum discriminative cutoff threshold for distinguishing endoscopic mild disease was 18.54u/L , with an AUC of 0.80 , $P<0.001$ (sensitivity 71.93% , specificity 71.93% , PPV 87.2% , NPV 54.3% and accuracy 71.95%). For ETX, the optimum discriminative cutoff threshold for distinguishing endoscopic mild disease was 10.51u/L , AUC was 0.76 ($P<0.001$), sensitivity was 70.18% , specificity was 80.0% , corresponding PPV, NPV and accuracy were 88.9% , 54.1% and 73.3% , respectively. Further research has found that the optimum discriminative cutoff of DAO threshold for severe disease in CD ($\text{SES-CD} \geq 16$) was 18.63 U/L (AUC= 0.75 , $P=0.001$). The test parameters of DAO were as follows: sensitivity 89.47% , specificity 53.79% , PPV 37.0% , NPV 94.4% , and accuracy 62.2% . For ETX, the optimum discriminative cutoff for severe disease was 22.38 U/L (sensitivity 57.89% , specificity 82.54% , PPV 50.0% , NPV 86.7% , and accuracy 76.83% , respectively), the AUC was 0.70 , $P=0.010$ ($\text{SES-CD} \geq 16$). The D-lactate, as well as WBC, ESR or CRP, failed to assess endoscopic severity in CD ($P>0.05$ for all).

Value of intestinal barrier marker in Predicting Endoscopic Remission in CD

Although we have demonstrated that DAO and ETX were positively correlated with SES-CD, there was no significant difference in level of DAO, D-lactate and ETX between active and inactive groups in CD patients ($P>0.05$, respectively; **Table 5**). The multivariate analyses demonstrated that no optimal

threshold could be identified that distinguished active CD from inactive CD, as the NPV of test was not satisfied (Figure 3 and Table 6).

Value of intestinal barrier marker in Predicting Endoscopic Severity in UC

In active UC patients, mild and moderate activity was merged into one group. There was no significant difference in DAO and D-lactate between mild-moderate and severe patients ($P > 0.05$ for both, Figure 4), only levels of ETX for severe group were higher than mild-moderate group ($P < 0.05$, Figure 4). The result is quite different with CD. ROC curves analysis was shown in Figure 5. No endoscopic disease category was individually distinguishable by DAO, D-lactate, ETX, WBC, ESR and CRP. Although the levels of ETX of severe group were higher than mild-moderate group, we have not found an optimum discriminative cutoff of ETX to distinguish the severity of endoscopy.

Value of intestinal barrier marker in Predicting Endoscopic Remission in UC

Significant differences were found in DAO, D-lactate and ETX levels between active and inactive UC patients ($P = 0.004$, $P = 0.008$ and $P = 0.003$; Table 8). On the whole, the levels of DAO, D-lactate and ETX of the active group were higher than that of the inactive group ($\text{UCEIS} \leq 1$). ROC analysis shown that DAO, D-lactate and ETX have a certain diagnostic utility in predicting endoscopic remission (Table 9 and Figure 6). The AUC of DAO for endoscopic disease thresholds of $\text{UCEIS} \leq 1$ was 0.86, $P < 0.001$. The optimum discriminative cutoff of DAO threshold was 15.97 U/L, predicting endoscopic remission with 83.93% sensitivity and 90.91% specificity, the PPV, NPV and accuracy of this threshold were 97.9%, 52.6% and 85.07%. The AUC for D-lactate and ETX for endoscopic disease thresholds of $\text{UCEIS} \leq 1$ was 0.80, $P = 0.002$ and 0.80, $P = 0.002$, the corresponding cutoff of D-lactate and ETX are 36.53 mg/l and 17.55 U/L. The sensitivity, specificity, PPV, NPV and accuracy of D-lactate in predicting remission of this thresholds are 75%, 81.82%, 95.5%, 39.1% and 76.12%. The sensitivity, specificity, PPV, NPV and accuracy of ETX in predicting remission of this thresholds are 62.5%, 92.91%, 97.2%, 32.3% and 67.16%.

We further repeated the experiment, and the results showed that if $\text{UCEIS} = 0$ was defined as remission, there was no statistically significant difference in the level of DAO d-lactic acid and ETX of UC patients with active patients (both $P > 0.05$). ROC curve analysis also showed that DAO, D-lactate and ETX could not predict endoscopic UC remission when $\text{UCEIS} = 0$ was defined as remission.

Discussion

The activity of IBD is closely related to readmission rates and prognosis of patients and leading to significant increases in morbidity and mortality. The endoscopic evaluation of IBD activity is of great significance for the development of treatment strategies and the assessment of prognosis in clinical practice. It's quite a difficult task to find an accurate, reliable and reproducible non-invasive biomarker to replace endoscope. Currently, DAO, D-lactate and ETX are used to evaluate the function of the intestinal barrier which plays an important role in the development of IBD by assessing intestinal permeability changes, intestinal bacterial migration, and intestinal injury. In this study, we evaluated the correlation

between DAO, D-lactate, ETX and IBD endoscopic score, as well as their value as alternative markers in predicting IBD endoscopic activity and remission. Our results showed that DAO and ETX are positively correlated with SES-CD in CD patients, but D-lactate is not significantly correlated with SES-CD. In UC, DAO, D-lactate and ETX are also positively correlated with UCEIS. In addition, we found that DAO, D-lactate and ETX could be used to distinguish endoscopic severity of CD patients to some degree, as well as that DAO, D-lactate and ETX could be used to distinguish endoscopic remission in UC patients.

Diamine oxidase (DAO) is an enzyme mainly located in villus tip enterocytes of humans and other mammals, which mainly expressed in intestinal mucosal. The level of DAO in peripheral blood is usually very low. The DAO would release into the circulation when the intestinal mucosal cells were damaged, resulting in the increase of DAO in blood. Several previous researches of humans and rats have suggested that its activity reflected the integrity of intestinal mucosa^[15], as well as injury and recovery^[16, 17]. In humans, D-lactate is mainly produced by metabolism of indigenous bacteria in the gastrointestinal tract. Research shows that Mammals are not equipped with the enzyme systems to metabolize it quickly^[18, 19]. As a consequence, baseline levels of D-lactate in serum of healthy subjects are very low. When the intestinal mucosa barrier was destroyed, D-lactate could be released to the blood through the dysfunctional intestinal barrier, leading to the increase of serum D-lactate. Endotoxin (ETX) is the outer membrane of gram-negative bacteria cell walls of lipopolysaccharide (LPS), usually be released after the death of a bacterium, as well as in the metabolic process. Intestinal tract is the body's largest bacterial endotoxin storage, and endotoxin enters the blood circulation with unknown mechanism through the damaged intestinal mucosa barrier. A study of 18 CD patients suggested that CD patients had elevated levels of ETX during the acute exacerbation^[20], and several other studies have found similar results^[21].

A recent study demonstrated that the levels of serum DAO and D-lactate are higher in IBD patients than those in healthy people, and serum DAO and D-lactate could be decreased after treatment^[22], suggesting that IBD patients suffer intestinal barrier dysfunction. In this study, DAO and ETX were found to be associated with CD endoscopy score (SES-CD) in patients with CD, and the correlation coefficient was superior to traditional inflammatory markers (WBC, ESR and CRP). DAO, D-lactate and ETX were significantly correlated with UCEIS in UC patients, and the correlation coefficient was also superior to traditional inflammatory markers. Our hypothesis was proved that DAO, D-lactate and ETX showed significant intestinal specificity compared with traditional inflammatory markers (WBC, ESR and CRP). The verification could explain that why DAO, D-lactate and ETX were more correlated with IBD endoscopic score than traditional inflammatory markers. At the same time, our results showed that DAO, D-lactate and ETX were more correlated with UC endoscopic score than CD. However, it is worth noting that our results showed no significant correlation between D-lactate and SES-CD. In addition, due to the limitation of retrospective study, changes in DAO, D-lactate and ETX levels and their correlation with endoscopic scores after treatment have not been studied, which requires further prospective studies.

In the study of patients with different endoscopic activity levels (severity), our results showed that in patients with CD, DAO level in patients with severe activity is higher than that in patients with moderate

activity, and DAO level in patients with moderate activity is also higher than that in patients with mild activity. The same was true for the analysis in EXT. Overall, DAO and ETX levels showed an upward trend with the increase of endoscopic activity. However, there were no statistically significant differences in D-lactate levels among CD patients with mild, moderate and severe activity, which was consistent with the correlation analysis results, suggesting that CD patients with high levels of DAO and ETX tend to be with more severe endoscopic activities.

Further analysis suggested that DAO and ETX have certain diagnostic value in distinguishing CD patients with different endoscopic activity. ROC curves showed that DAO and ETX could be used to distinguish moderate and severe endoscopic activity. The thresholds for identifying endoscopic moderately active patients with mild activity were 18.54 U/L (sensitivity 71.93%, specificity 71.93%) and 10.51 U/L (sensitivity 70.18%, specificity 80.0%). The best discriminant threshold for identifying DAO thresholds for patients with severe activity was 18.63 U/L (sensitivity 89.47%, specificity 53.79%). For ETX, the threshold for predicting patients with severe activity was 22.38 U/L (sensitivity was 57.89%, specificity was 82.54%). D-lactate was unable to assess the different activities of endoscopy in the CD, which was consistent with the results of the correlation analysis. Salim^[12] believed that intestinal mucosal barrier is severely damaged during acute IBD attack, resulting in structural and functional destruction, and subsequently increased permeability and tissue damage. A study of 18 CD patients showed elevated levels of ETX during acute episodes in CD patients^[20]. Our research confirmed this point, it also indicated that patients with high levels of DAO and ETX are more prone to have more serious endoscopic activities.

Although DAO and ETX are proved to be positively correlated with SES-CD in this study. When $SES-CD \leq 2$ was defined as endoscopic remission, the difference between DAO and ETX among patients with activity and remission was not statistically significant. ROC curve analysis also showed that DAO and ETX could not identify remission and activity ($P > 0.05$). There was no statistical difference in D-lactate level between patients with activity and remission, ROC curve analysis also showed that D-lactate has no diagnostic value in predicting endoscopic remission at $SES-CD \leq 2$. Thus, no threshold was found for the best intestinal barrier marker to distinguish CD activity from remission. Pastor Rojo^[21] found that the serum endotoxin level increased in IBD patients, although decreased after treatment, but did not fully return to normal levels in CD patients. The reason may be related to the recovery degree of intestinal barrier function in patients with remission. Some studies suggest that some patients in remission stage also have increased intestinal permeability^[23]. Combined with our study, the possible cause is that the intestinal barrier function of CD patients is still not fully recovered, although the intestinal mucosa heals after treatment. Jeff Chang^[24] found that the degree of intestinal permeability damage is related to the severity of diarrhea, so not only mucosal healing should be included in the final treatment goal of IBD, but also the recovery of intestinal barrier function. Some studies have shown that CD patients with increased intestinal permeability in remission stage also have an increased risk of disease recurrence^[25]. Although we found that DAO and ETX are not very valuable in predicting endoscopic remission of CD, our study showed that even CD patients with endoscopic remission stage still need recovery treatment for intestinal

barrier. This may be helpful to reduce the recurrence of CD patients, so that patients get more lasting relief.

However, in UC patients with different endoscopic activity, there was no significant difference in DAO and D-lactate between the mild moderate activity group and the severe group, only patients with severe activity had higher ETX levels than the mild-moderate group. Although high levels of ETX seem to imply more serious endoscopic activity, we have not found the best ETX discriminant threshold. Although correlation analysis showed that DAO, D-lactate and ETX were correlated with UCEIS to some extent, with the increase of endoscopic severity, the levels of DAO, D-lactate and ETX increased not as obvious as CD. This may be related to the fact that UC is a lesion limited to submucosa and CD is a transmural lesion of whole mucosa.

However, when distinguishing endoscopic relief from activity, ROC curve analysis showed that DAO, D-lactate and ETX could predict endoscopic UC relief ($UCEIS \leq 1$). There were significant differences in DAO, D-lactate and ETX levels between UC patients with activity ($UCEIS > 1$) and remission ($UCEIS \leq 1$) ($P = 0.004$, $P = 0.008$ and $P = 0.003$), and DAO, D-lactate and ETX levels in the active group were significantly higher than those in the remission group. The best prediction boundary of DAO between activity ($UCEIS > 1$) and remission ($UCEIS \leq 1$) was 15.97 U/L, with a sensitivity of 83.93% and specificity of 90.91% for predicting endoscopic remission, and an accuracy of 85.07%. The best predictive values of D-lactate and ETX for activity ($UCEIS > 1$) and remission ($UCEIS \leq 1$) were 36.53 mg/l (sensitivity 75%, specificity 81.82%) and 17.55U/ l (sensitivity 62.5%, specificity 92.91%), with accuracy of 76.12% and 67.16%, respectively. Further repeated analysis was performed at $UCEIS = 0$ and $UCEIS \leq 2$, and no better results were obtained.

Combined with correlation analysis and detection analysis results of different activity levels, although DAO, D-lactate and ETX could not distinguish UC endoscopic activity level, our study showed that UC patients with low level DAO, D-lactate and ETX were more inclined to endoscopic remission. Pastor Rojo^[21] found that endotoxin levels were reduced in patients with IBD after treatment, but CD patients did not fully return to normal levels. Part of the description showed that compared with CD, UC patients are more likely to achieve recovery of intestinal barrier function during remission, which is subject to further prospective trials. Our research showed that DAO, D-lactate and ETX have good predictive value of endoscopic remission in UC, and have potential application value in clinical.

To sum up, biomarker is an important aspect of IBD assessment, and endoscopic examination is obviously important for IBD. Our study found that DAO, D-lactate and ETX had a certain correlation with UCEIS score in UC patients, and the correlation coefficient was better than traditional inflammatory markers ESR and CRP. We also found that in patients with CD, DAO and ETX not only showed better correlation with SES-CD than ESR and CRP, but also could distinguish different endoscopic activities. Due to the influence of sample size and the nature of retrospective study, our study still has some limitations. Further prospective trials are necessary in the future. It is of great significance for clinical practice to develop treatment strategies and predict prognosis.

Declarations

CONSENT FOR PUBLICATION

Not applicable

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

FUNDING

Not applicable.

AUTHORS' CONTRIBUTIONS

Min Chen contributed to the conception of the study; Qi Zhang performed the data collection and analyses and wrote the manuscript; Xi Chen helped perform the analysis with constructive discussions. All authors reviewed the manuscript.

References

1. Baert, F., et al., *Mucosal healing predicts sustained clinical remission in patients with early-stage Crohn's disease*. Gastroenterology, 2010. **138**(2): p. 463-8; quiz e10-1.
2. Boirivant, M., et al., *The clinical significance of serum C reactive protein levels in Crohn's disease. Results of a prospective longitudinal study*. J Clin Gastroenterol, 1988. **10**(4): p. 401-5.
3. Niederau, C., et al., *Inflammatory mediators and acute phase proteins in patients with Crohn's disease and ulcerative colitis*. Hepatogastroenterology, 1997. **44**(13): p. 90-107.
4. Miranda-Garcia, P., M. Chaparro, and J.P. Gisbert, *Correlation between serological biomarkers and endoscopic activity in patients with inflammatory bowel disease*. Gastroenterol Hepatol, 2016. **39**(8): p. 508-15.
5. Yoon, J.Y., et al., *Correlations of C-reactive protein levels and erythrocyte sedimentation rates with endoscopic activity indices in patients with ulcerative colitis*. Dig Dis Sci, 2014. **59**(4): p. 829-37.
6. Schoepfer, A.M., et al., *Monitoring inflammatory bowel disease activity: clinical activity is judged to be more relevant than endoscopic severity or biomarkers*. J Crohns Colitis, 2012. **6**(4): p. 412-8.
7. Bjarnason, I., A. MacPherson, and D. Hollander, *Intestinal permeability: an overview*. Gastroenterology, 1995. **108**(5): p. 1566-81.
8. Scaldaferri, F., et al., *The gut barrier: new acquisitions and therapeutic approaches*. J Clin Gastroenterol, 2012. **46 Suppl**: p. S12-7.
9. Antoni, L., et al., *Intestinal barrier in inflammatory bowel disease*. World J Gastroenterol, 2014. **20**(5): p. 1165-79.

10. Wyatt, J., et al., *Intestinal permeability and the prediction of relapse in Crohn's disease*. Lancet, 1993. **341**(8858): p. 1437-9.
11. Vogelsang, H., *Do changes in intestinal permeability predict disease relapse in Crohn's disease?* Inflamm Bowel Dis, 2008. **14 Suppl 2**: p. S162-3.
12. Salim, S.Y. and J.D. Soderholm, *Importance of disrupted intestinal barrier in inflammatory bowel diseases*. Inflamm Bowel Dis, 2011. **17**(1): p. 362-81.
13. Travis, S.P., et al., *Developing an instrument to assess the endoscopic severity of ulcerative colitis: the Ulcerative Colitis Endoscopic Index of Severity (UCEIS)*. Gut, 2012. **61**(4): p. 535-42.
14. Daperno, M., et al., *Development and validation of a new, simplified endoscopic activity score for Crohn's disease: the SES-CD*. Gastrointest Endosc, 2004. **60**(4): p. 505-12.
15. Luk, G.D., T.M. Bayless, and S.B. Baylin, *Diamine oxidase (histaminase). A circulating marker for rat intestinal mucosal maturation and integrity*. J Clin Invest, 1980. **66**(1): p. 66-70.
16. Luk, G.D., et al., *Diamine oxidase as a plasma marker of rat intestinal mucosal injury and regeneration after administration of 1-beta-D-arabinofuranosylcytosine*. Cancer Res, 1981. **41**(6): p. 2334-7.
17. D'Agostino, L., et al., *Postheparin plasma diamine oxidase values in the follow up of patients with small bowel Crohn's disease*. Gut, 1991. **32**(8): p. 932-5.
18. Smith, S.M., et al., *D-lactic acid measurements in the diagnosis of bacterial infections*. J Clin Microbiol, 1989. **27**(3): p. 385-8.
19. Nielsen, C., et al., *L- and D-lactate as biomarkers of arterial-induced intestinal ischemia: an experimental study in pigs*. Int J Surg, 2012. **10**(6): p. 296-300.
20. Wellmann, W., et al., *Endotoxaemia in active Crohn's disease. Treatment with whole gut irrigation and 5-aminosalicylic acid*. Gut, 1986. **27**(7): p. 814-20.
21. Pastor Rojo, O., et al., *Serum lipopolysaccharide-binding protein in endotoxemic patients with inflammatory bowel disease*. Inflamm Bowel Dis, 2007. **13**(3): p. 269-77.
22. Song, W.B., et al., *Soluble intercellular adhesion molecule-1, D-lactate and diamine oxidase in patients with inflammatory bowel disease*. World J Gastroenterol, 2009. **15**(31): p. 3916-9.
23. Vivinus-Nebot, M., et al., *Functional bowel symptoms in quiescent inflammatory bowel diseases: role of epithelial barrier disruption and low-grade inflammation*. Gut, 2014. **63**(5): p. 744-52.
24. Chang, J., et al., *Impaired Intestinal Permeability Contributes to Ongoing Bowel Symptoms in Patients With Inflammatory Bowel Disease and Mucosal Healing*. Gastroenterology, 2017. **153**(3): p. 723-731 e1.
25. Arnott, I.D., K. Kingstone, and S. Ghosh, *Abnormal intestinal permeability predicts relapse in inactive Crohn disease*. Scand J Gastroenterol, 2000. **35**(11): p. 1163-9.

Tables

Table 1. Baseline characteristics of patients with CD and UC			
	Total (n =149)	CD (n =82)	UC (n =67)
Median age (years, IQR)	31.5 (24.0-47.3)	28.0 (21.0-33.0)	46.0 (29.0-49.0)
Median disease duration (years, IQR)	3.0 (1.0-4.0)	3.0(1.0-4.0)	3.0 (0.5-4.0)
Gender (%)			
male	100 (67.11)	56 (68.29)	44 (65.67)
female	49 (32.89)	26 (31.71)	23 (34.33)
Smoking history (%)			
Yes	10 (6.71)	3 (3.66)	7 (10.45)
No	139 (93.29)	79 (96.34)	60 (89.55)
CD location (%)			
Ileal (L1)	19 (12.67)	19 (22.90)	—
Colonic (L2)	18 (12.00)	18 (21.69)	—
Ileocolonic (L3)	44 (29.53)	44 (53.65)	—
Upper gastrointestinal involvement (L4)	1(0.67)	1(1.22)	—
UC extent (Montreal) (%)			
Proctitis (E1)	9 (6.00)	—	9 (13.43)
Left-sided (E2)	15 (10.00)	—	15 (22.39)
Extensive (E3)	43 (28.67)	—	43 (64.18)
medication			
None	12 (8.00)	9 (10.84)	3 (4.48)
5-ASA	96 (64.43)	34 (41.46)	62 (92.54)
Steroids	32 (21.33)	14 (16.87)	18 (26.87)
Thiopurine	15 (10)	13 (15.67)	2 (2.99)
Anti-TNF	46 (30.67)	43 (51.80)	3 (4.48)

Table 2. Coefficient of correlation between intestinal barrier index, inflammatory markers, and endoscopic scores of disease severity in CD patients.		
	Pearson' r	<i>P</i>
White blood cells	0.146	0.191
Erythrocyte sedimentation rate	0.346	0.0015
C reactive protein	0.250	0.023
Diamine oxidase	0.532	<0.0001
D-lactate	0.167	0.133
Endotoxin	0.468	<0.0001

Table 3. Coefficient of correlation between intestinal barrier index, inflammatory markers and endoscopic scores of disease severity in UC patients.		
	Pearson' r	<i>P</i>
White blood cells	0.151	0.222
Erythrocyte sedimentation rate	0.285	0.021
C reactive protein	0.334	0.006
Diamine oxidase	0.600	<0.0001
D-lactate	0.407	0.0006
Endotoxin	0.505	<0.0001

Table 4. ROC analysis results of intestinal barrier markers and traditional inflammatory markers at different CD endoscopy activity thresholds.

	SES-CD	
	≤6	<16
DAO	0.80(0.69-0.90)	0.75(0.62-0.87)
<i>P</i>	<0.001***	0.001**
D-lactate	0.60(0.46-0.74)	0.55(0.39-0.71)
<i>P</i>	0.15	0.492
ETX	0.76(0.65-0.86)	0.70(0.54-0.85)
<i>P</i>	<0.001***	0.010*
WBC	0.62(0.49-0.74)	0.53(0.39-0.68)
<i>P</i>	0.094	0.656
ESR	0.69(0.57-0.81)	0.66(0.51-0.80)
<i>P</i>	0.005**	0.041*
CRP	0.69(0.56-0.81)	0.69(0.55-0.80)
<i>P</i>	0.008**	0.020*
AUC, 95% Confidence Interval		

Table 5. Intestinal barrier markers and inflammatory markers in Remission and Active CD patients.

	Remission	Active	<i>P</i>
	☐SES-CD≤2☐	☐SES-CD>2☐	
DAO (U/L)	16.06±12.46	28.63±20.35	0.075
D-lactate (mg/l)	37.78±22.94	37.82±19.84	0.997
ETX (U/L)	9.37±5.51	16.46±13.05	0.113
WBC (10 ⁹ /L)	5.21 ±1.48	6.62 ±2.55	0.109
ESR (mm/h)	9.67±14.92	26.39±26.34	0.066
CRP (mg/L)	9.87±12.41	28.55±30.17	0.071

Table 6. ROC analysis of intestinal barrier markers and traditional inflammatory markers predicting endoscopic remission in CD.

	SES-CD	
	≤2	≤3
DAO	0.68(0.51-0.86)	0.72(0.58-0.86)
<i>P</i>	0.074	0.008**
D-lactate	0.49(0.26-0.71)	0.52(0.34-0.71)
<i>P</i>	0.894	0.77
ETX	0.67(0.53-0.80)	0.67(0.55-0.80)
<i>P</i>	0.106	0.04*
WBC	0.67(0.52-0.83)	0.71(0.59-0.84)
<i>P</i>	0.091	0.010*
ESR	0.81(0.65-0.97)	0.70(0.56-0.84)
<i>P</i>	0.003**	0.015*
CRP	0.71(0.57-0.85)	0.76(0.64-0.88)
<i>P</i>	0.042*	0.002**
AUC, 95% Confidence Interval		

Table 7. ROC analysis results of intestinal barrier markers and traditional inflammatory markers at different UC endoscopy activity thresholds

	UCEIS≤6	<i>P</i>
DAO	0.73(0.58-0.89)	0.034*
D-lactate	0.74(0.59-0.89)	0.029*
ETX	0.78(0.66-0.90)	0.011*
WBC	0.40(0.18-0.62)	0.353
ESR	0.64(0.41-0.86)	0.216
CRP	0.67(0.41-0.93)	0.124
AUC, 95% Confidence Interval		

Table 8. Intestinal barrier markers and inflammatory markers in Remission and Active UC patients.

	Remission (UCEIS $\leq$ 1)	Active [UCEIS $\geq$ 1]	<i>P</i>
DAO (U/L)	13.52 $\pm$ 4.29	38.92 $\pm$ 24.61	0.004**
D-lactate (mg/l)	31.41 $\pm$ 5.62	51.22 $\pm$ 19.78	0.008**
ETX (U/L)	8.47 $\pm$ 2.68	20.67 $\pm$ 10.73	0.003**
WBC (10 ⁹ /L)	5.74 $\pm$ 0.43	6.99 $\pm$ 2.44	0.107
ESR (mm/h)	9.73 $\pm$ 4.77	19.38 $\pm$ 18.57	0.100
CRP (mg/L)	4.27 $\pm$ 1.55	23.85 $\pm$ 41.18	0.117

Table 9. ROC analysis of intestinal barrier markers and traditional inflammatory markers predicting endoscopic remission in UC.

	UCEIS $\leq$ 1	<i>P</i>
DAO	0.86(0.74-0.98)	<0.001***
D-□□	0.80(0.63-0.96)	0.002**
ETX	0.80(0.66-0.95)	0.002**
WBC	0.64(0.49-0.80)	0.136
ESR	0.72(0.57-0.89)	0.018*
CRP	0.70(0.55-0.84)	0.041*
AUC, 95% Confidence Interval		

Figures

Figure 1

In active CD patients (SES-CD>2), the DAO levels of severe groups were higher than moderate groups ($P<0.05$, Figure 1A), moderate groups were higher than mild groups ($P<0.001$, Figure 1A), severe groups were significantly higher than mild groups ($P<0.001$, Figure 1A). The ETX levels of severe groups were higher than moderate groups ($P<0.05$, Figure 1B), moderate groups were higher than mild group ($P<0.01$, Figure 1B), severe groups were significantly higher than mild groups ($P<0.01$, Figure 1B). There was no difference in D-lactate level between each group in active CD patients ($P>0.05$ for all, Figure 1C).

Figure 2

Receiver operator curves (ROCs) of DAO, D-lactate, ETX and inflammatory markers in detecting IBD severity corresponding to different endoscopic scoring thresholds. The results showed that DAO and ETX are valuable in differentiating CD patients with different endoscopic activity levels. The ROC curve at SES-CD ≤ 6 is shown in Figure A, the diagnostic value of DAO is better than ETX. As well as the ROC curve at SES-CD < 16 is shown in Figure B, and the diagnostic value of DAO is better than ETX. D-lactate and WBC, ESR and CRP failed to predict CD endoscopy severity.

Figure 3

ROC curve of DAO, D-lactate, ETX and inflammatory markers in predicting CD endoscopic remission. Figure A: ROC curve at SES-CD ≤ 2 . Studies showed that DAO, D-lactate and ETX values at SES-CD ≤ 2 could not predict the endoscopic remission of CD ($P > 0.05$), nor could WBC, ESR or CRP. Figure B: ROC curve at SES-CD ≤ 3 . DAO and ETX could predict endoscopic remission of CD with AUC of 0.72 ($P = 0.008$) and 0.67 ($P = 0.04$), respectively, while D-lactate failed to predict remission ($P > 0.05$).

Figure 4

In active UC patients, there was no significant difference in DAO and D-lactate between mild-moderate and severe patients ($P > 0.05$ for both), while ETX levels of severe group were higher than mild-moderate group ($P < 0.05$).

Figure 5

The ROC curves of DAO, D-lactate, ETX and inflammatory markers for detecting the severity of UC endoscopy showed that the above indicators failed to distinguish the endoscopic severity of UC.

Figure 6

ROC curve of DAO, D-lactate, ETX and inflammatory markers for UC endoscopic remission ($\text{UCEIS} \leq 1$). The results showed that DAO, D-lactate and ETX had diagnostic value in differentiating remission ($\text{UCEIS} \leq 1$) from activity ($\text{UCEIS} > 1$) of UC patients.