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Leucine-Rich Alpha-2 Glycoprotein in Monitoring Disease Activity and Intestinal Stenosis in Inflammatory Bowel Disease

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Leucine-rich alpha-2 glycoprotein (LRG) is a novel biomarker for monitoring disease activity in inflammatory bowel disease (IBD). The aim of this study was to evaluate its utility in monitoring disease activity. In this retrospective study based on case records between August 2020 and July 2021 at our two centers, we examined the correlation between serum levels of LRG and C-reactive protein (CRP) with disease activity in IBD patients. Background factors related to serum LRG levels were also analyzed. Overall, 47 Crohn's disease (CD) and 123 ulcerative colitis (UC) patients were evaluated. In patients with CD, LRG and CRP levels correlated with Harvey-Bradshaw Index (HBI) and Simple Endoscopic Score for CD (SES-CD) (LRG and HBI, r = 0.397; LRG and SES-CD, r = 0.637; CRP and HBI, r = 0.253; CRP and SES-CD, r = 0.332). In patients with UC, LRG and CRP significantly correlated with the partial Mayo score (PMS) and Mayo endoscopic subscore (MES) (LRG and PMS, r = 0.3; CRP and PMS, r = 0.282; LRG and MES, r = 0.424; CRP and MES, r = 0.459). In CD patients with normal CRP, serum LRG level was significantly higher in those with mucosal inflammation than in those with mucosal healing (16.4 vs. 10.7 μg/ mL). Stenosis was associated with serum LRG levels in CD group using multiple regression analysis. Therefore, LRG is a useful biomarker for monitoring disease activity and mucosal inflammation, and indicates the status of intestinal stenosis in IBD patients.

Keywords: biomarker; disease activity; inflammatory bowel disease; leucine-rich alpha-2 glycoprotein; stenosis
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Introduction

The incidence of inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is increasing globally (Ng et al. 2017; Murakami et al. 2019). Recent therapeutic advances have contributed significantly towards the optimization of treatment for these patients (Verstockt et al. 2018). Consequently, achieving mucosal healing (MH) has been reported to decrease recurrence and malignant transformation rates (Neurath and Travis 2012; Ungaro et al. 2020). According to the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE)-II program recommended by the International Organization for the Study of IBD (IOIBD) in 2021, MH should be a long-term goal in the treatment of IBD (Turner et al. 2021). Endoscopy is the gold standard for evaluating MH (Magro et al. 2017). However, frequent endoscopic examinations are expensive and discomforting, and entail a potential risk of pretreatment-induced exacerbation.

Biomarkers are minimally invasive, relatively inexpensive, and useful predictors of recurrence and long-term prognosis (Poncin et al. 2014; Rokkas et al. 2018; Haisma et al. 2019). Leucine-rich alpha-2 glycoprotein (LRG) is a glycoprotein with a 50-kDa leucine-rich repeat domain, which was discovered by Haupt and Baudner (1977). Serada et al. (2010) reported that LRG as a serum biomarker correlated with clinical disease activity in patients with rheumatoid arthritis and CD, and LRG elevation implied active CD despite normal C-reactive protein (CRP) levels. Further, Serada et al. (2012) reported a good correlation between LRG and clinical activity index (r = 0.731, p

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< 0.00001), and significantly higher serum LRG levels in patients with endoscopically active UC (Matt's grade 3-4) compared to those in remission (Matt's grade 1-2). Similar results were reported by Shinzaki et al. (2017), who also reported, using a receiver operating characteristics (ROC) curve and area under the curve (AUC) analysis, that the discriminatory ability of LRG for detecting mucosal healing (MH) in patients with UC was better than that of CRP. Recently, a good relationship between LRG and disease activity and a high predictive value of LRG for the modified Simple Endoscopic Score for Crohn's Disease (SES-CD) of 0 has been reported in patients with CD (Shinzaki et al. 2021; Yasutomi et al. 2021).

In STRIDE-II, normalization of the serum level of CRP is a short-term goal since CRP has been a validated biological marker for IBD (Turner et al. 2021). However, inflammation is occasionally observed endoscopically in patients with normal CRP levels (Vermeire et al. 2006; Sands 2015), because CRP is produced under the influence of IL-6 (Mitsuyama et al. 1991). Since LRG production does not depend on IL-6 alone, its serum level has been reported to reflect disease activity, even if that of CRP is normal (Serada et al. 2010).

Despite serum LRG test in IBD patients getting covered by insurance since June 2020 in Japan, the clinical significance of LRG remains unclear. This study aimed to examine the usefulness of LRG in the clinical domain and other background factors associated with a high serum level of LRG.

Materials and Methods

Patients

Patients with IBD in whom serum LRG and CRP levels were assessed at Akita University Hospital and Omagari Kosei Medical Center between August 2020 and July 2021 were enrolled in this study. Diagnosis of CD or UC was proven clinically, endoscopically, and histologically. Patients with colon cancer or confirmed inflammation other than IBD were excluded from the analysis. In total, 170 patients (CD, n = 47; UC, n = 123) with 384 LRG measurements (CD, n = 110; UC, n = 274) were evaluated. The background data are listed in Table 1. There were more

Table 1. Patients' background.

	Crohn's disease	Ulcerative colitis
n (patients/LRG measurement)	47/110	123/274
Sex (male/female)	35/12	70/53
Age (years), median (IQR)	33 (25, 41)	43 (31, 58)
Duration (year), median (IQR)	3 (1, 13.5)	7 (2, 16.25)
Disease location		
Ileitis/Colitis/Ileocolitis	16/3/28	NA
Proctitis/Left-sided/Total	NA	13/29/76
Medication, n (%)		
5-ASA	37 (86.0)	113 (91.9)
Immunomodulator	11 (28.9)	24 (19.5)
Corticosterid	6 (16.7)	13 (10.6)
Monoclonal antibodies	20 (57.1)	33 (26.8)
IFX/ADA/GLM	8/3/0	15/3/5
UST/VED	8/1	6/4
Tofacitinib	0	4
History of surgery, n (%)	20 (42.6)	7 (5.7)
Presence of stenosis, n (%)	17 (36.7)	3 (2.4)
LRG (μg/mL), median (IQR)	16.4 (12, 21)	11.8 (10.2, 15.6)
CRP (mg/dL), median (IQR)	0.12 (0.06, 0.3)	0.05 (0.02, 0.17)
Disease activity		
HBI, n = 68, median (IQR)	2 (0, 3)	NA
SES-CD, n = 29, median (IQR)	6 (2, 8.5)	NA
PMS, n = 252, median (IQR)	NA	0 (0, 2)
MES, n = 93, median (IQR)	NA	1 (0, 1)

LRG, leucine-rich alpha-2 glycoprotein; IQR, interquartile range; NA, not applicable; 5-ASA, 5-aminosalicylate acid; HBI, Harvey-Bradshaw Index; SES-CD, Simple Endoscopic Score for Crohn's Disease; PMS, partial Mayo score; MES, Mayo endoscopic subscore; IFX, infliximab; ADA, adalimumab; GLM, golimumab; UST, ustekinumab; VED, vedolizumab.

males in both CD (74.5%) and UC (57%) groups. In the CD group, ileocolitis type was present in approximately 60% of patients; in the UC group, total colitis type was present in approximately 65% of patients. Stenosis was observed in 17 patients with CD and in 3 patients with UC. Stenosis in the 3 UC patients was caused by active ulcer and inflammation in two patients, and fibrotic stenosis in the third patient. The majority of patients had clinical remission (81.7% had HBI ≤ 3 in the CD group, 93% had PMS ≤ 2 in the UC group).

Study design

In this retrospective observational study, we examined the following points: (1) correlation of LRG and CRP levels with clinical/endoscopic disease activity in CD and UC; (2) ability of LRG and CRP to detect MH in CD and UC, respectively; (3) differences in LRG levels between the MH and non-MH (NMH) groups in the CRP-normal subgroup (patients who had normal serum CRP values); and (4) background factors related to serum LRG levels using multiple regression analysis. Age (in years), sex (female compared with male), disease duration (in years), history of surgery (having history of surgery compared with none), serum CRP levels and current stenosis (presence of stenosis compared with absence) were included as covariates. All LRG data were evaluated as mentioned in points (1), (2), and (3) above. For multivariate analysis of background factors [(4) above], the LRG measurement on the day nearest to the endoscopic examination was considered.

Data collection

Patient's background: Data regarding age, sex, disease duration, disease location, use of 5-aminosalicylic acid (5-ASA), immunomodulators, corticosteroids, therapeutic monoclonal antibodies (mAbs) (infliximab, adalimumab, golimumab, Ustekinumab, and vedolizumab), Janus kinase (JAK) inhibitor (tofacitinib), history of surgery, and presence of intestinal stenosis, as on the day when serum LRG and CRP levels were assessed for the first time, and were collected from the medical records. Nanopia LRG® (Sekisui Medical, Tokyo, Japan) was used for LRG measurements. Stenosis was defined as intestinal constriction with insufficient luminal space to pass the endoscope, or stenosis with intestinal lumen of one centimeter or less which is detected radiologically.

Clinical activity: To assess the clinical disease activity in CD, the Harvey-Bradshaw Index (HBI) was used, which is derived from the general condition, degree of abdominal pain, number of aqueous stools, presence of abdominal mass, and complications, and in UC, partial Mayo score (PMS), which is calculated by the number of defecations, degree of bloody stool, and physician's global assessment, was used. Cases wherein it was difficult to obtain medical records or evaluate the clinical symptoms necessary for calculation due to extensive intestinal resection or colostomy (CD 42 measurement, UC 22 measurement) were excluded from the examination using clinical disease activity. Clinical remission was defined as an HBI of 0-3 in CD and a PMS of 0-2 in UC.

Endoscopic activity: SES-CD data in CD and Mayo endoscopic subscore (MES) in UC patients were collected. Endoscopic disease activity was analyzed in this study only in patients in whom total colonoscopy was performed within 1 month of LRG measurement, without changes in clinical symptoms and treatment (CD, n = 29; UC, n = 93). Total colonoscopy was performed after sufficient pretreatment with intestinal cleaning agents. Scoring was independently performed by two endoscopists and reviewed if the scores were different. Endoscopic disease activity was not calculated in patients for whom it was difficult to calculate the score due to intestinal resection. MH was defined as an SES-CD of 0-2 in CD and MES of 0 or 1 in UC.

Statistical analysis

Statistical analysis was performed using Easy R (version 1.54, Jichi Medical University Saitama Medical Center, Saitama, Japan) (Kanda 2013). The correlation between biomarker levels and disease activity was analyzed using Spearman's rank correlation coefficient. Receiver operating characteristic (ROC) curve analysis was used to assess the discriminatory performance of LRG and CRP for detecting MH, and the area under the curve (AUC) was compared using Delong's test in patients with CD and UC, respectively. The Mann-Whitney U test was performed to test the differences in continuous variables between the two groups, and multivariate analysis using multiple regression analysis was performed to extract background factors. CRP-normal was defined as serum CRP level lower than 0.2 mg/dL in both CD and UC. In each test, $p < 0.05$ was defined as statistically significant.

Ethical considerations

Appropriate consent was obtained to notify and publish research information on the research subjects. Information about this study was published on the websites of Akita University School of Medicine, Department of Gastroenterology and Neurology (https://www.med.akita-u.ac.jp/~naika1/) and Akita University Hospital Clinical Research Support Center (https://www2.hos.akita-u.ac.jp/chiken/info/index.html). Patients who did not participate in the study were excluded.

The protocol for this study was examined and approved by the ethics committee of the Akita University School of Medicine (approval number: 2693).

Results

Correlations between biomarkers and clinical and endoscopic activity

First, correlations between biomarkers and clinical activity were examined. The results are shown in Fig. 1. In patients with CD, correlations were observed between serum LRG or CRP levels, and HBI (LRG and HBI, r =

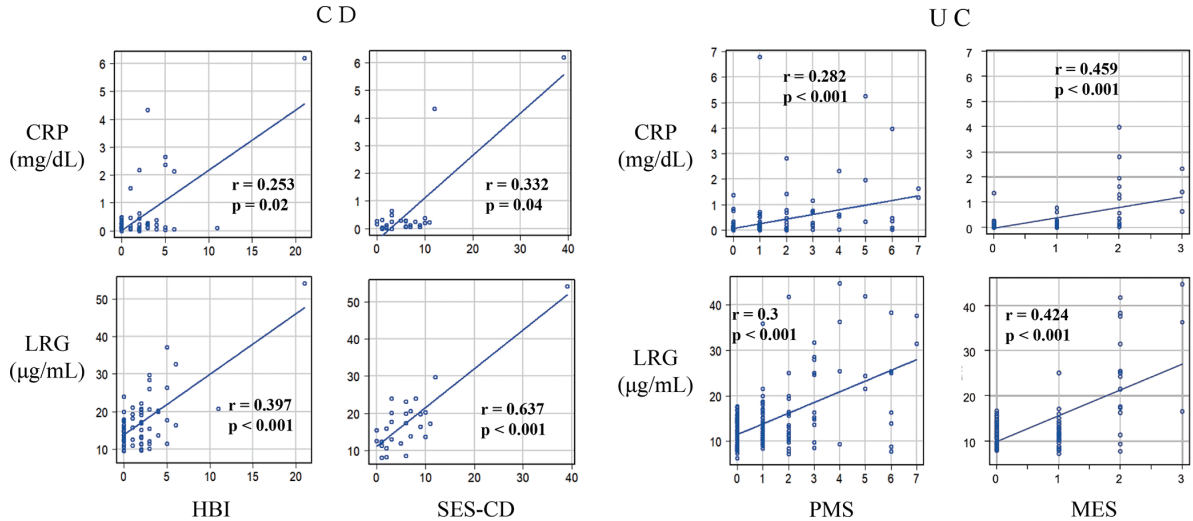
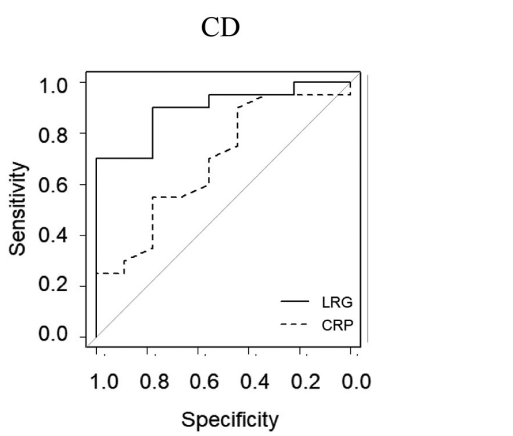
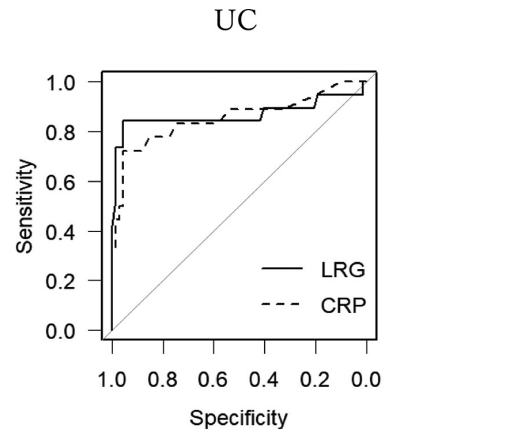


Fig. 1. Correlations between biomarkers and clinical/endoscopic activity in inflammatory bowel disease (IBD).



	AUC	p-value	Sensitivity	Specificity	Cut-off
LRG	0.894	0.038	0.778	0.9	12.6 μg/mL
CRP	0.689		0.778	0.55	0.21 mg/dL



	AUC	p-value	Sensitivity	Specificity	Cut-off
LRG	0.874	0.672	0.944	0.818	16.3 μg/mL
CRP	0.853		0.873	0.762	0.18 mg/dL

Fig. 2. Receiver operating characteristic (ROC) curve analysis to assess the discriminatory performance of leucine-rich alpha-2 glycoprotein (LRG) and C-reactive protein (CRP) for detecting mucosal healing (MH).
ROC, receiver operating characteristics; AUC, area under the curve.

0.397, $p < 0.001$; CRP and HBI, $r = 0.253$, $p = 0.02$). In patients with UC, the correlations between serum LRG or CRP levels, and PMS were significant (LRG and PMS, $r = 0.3$, $p < 0.001$; CRP and PMS, $r = 0.282$, $p < 0.001$, respectively). Second, the correlations between biomarkers and endoscopic activity were examined. Serum LRG or CRP levels, and SES-CD were significantly correlated (LRG and SES-CD, $r = 0.637$, $p < 0.001$; CRP and SES-CD, $r = 0.332$, $p = 0.04$). The correlations between serum LRG or CRP levels, and MES were also significant (LRG and MES, $r = 0.424$, $p < 0.001$; CRP and MES, $r = 0.459$, $p < 0.001$).

The discriminatory ability of LRG and CRP for detecting MH

To assess the discriminatory ability of LRG and CRP in detecting MH, ROC analysis was performed, and the AUC was calculated. The results are shown in Fig. 2. In patients with CD, the AUC was 0.894 for LRG and 0.689 for CRP ($p = 0.038$). The AUC of LRG was significantly higher than that of CRP. The cut-off level of LRG and CRP was 12.6 μg/mL and 0.21 mg/dL, respectively. In patients with UC, the AUC was 0.874 for LRG and 0.853 for CRP ($p = 0.672$). A good AUC was demonstrated for both LRG and CRP levels. Cut-off levels of LRG and CRP were 16.3 μg/mL and 0.18 mg/dL, respectively.

LRG levels between MH and NMH groups in the CRP-normal subgroup

In the CRP-normal subgroup, we evaluated whether serum LRG levels reflected proven disease activity. The results are shown in Fig. 3. In the CD group, serum LRG levels were higher in the NMH group than in the MH group

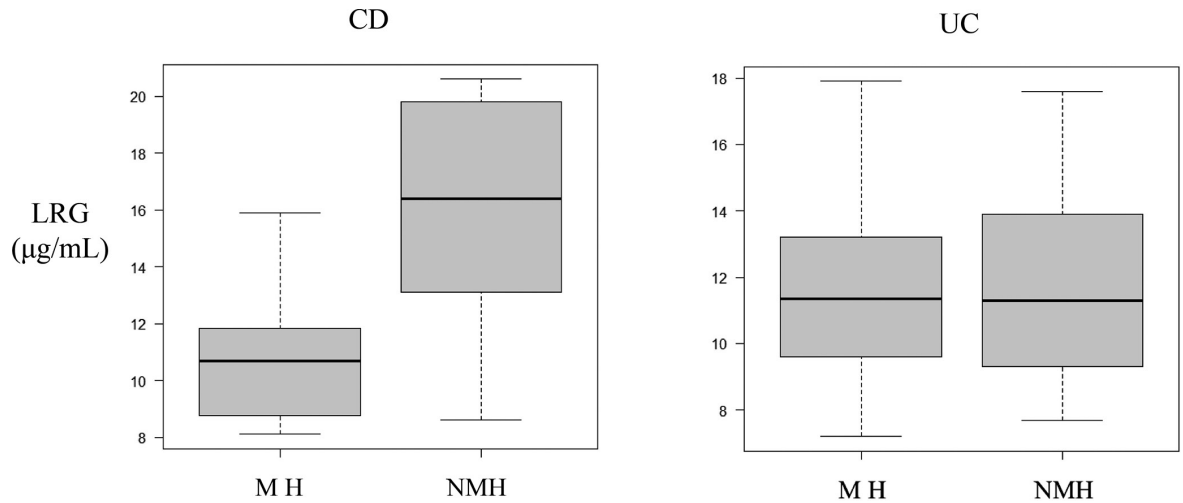


Fig. 3. Difference of LRG level between mucosal healing (MH) group and non-mucosal healing (NMH) group in CRP-normal subgroup.
In the CD patients, LRG level in NMH group was significantly higher than in MH group ($p < 0.05$).

Table 2. Multiple regression analysis of patients' background correlated with serum leucine-rich alpha-2 glycoprotein (LRG) levels.

	Crohn's disease				Ulcerative colitis			
	Estimate	SE	t-value	p-value	Estimate	SE	t-value	p-value
Age	0.04	0.07	0.53	0.60	0.02	0.04	0.57	0.57
Sex	2.65	1.78	1.48	0.15	1.79	1.14	1.57	0.12
Disease duration	−0.07	0.12	−0.63	0.53	0.05	0.08	0.55	0.59
Serum CRP	5.98	0.62	9.72	< 0.01	9.82	1.32	7.46	< 0.01
History of surgery	3.53	2.00	1.76	0.09	1.46	1.81	0.80	0.42
Current stenosis	4.23	1.66	2.55	0.016	4.29	2.84	1.51	0.14

Estimate, estimated regression coefficient; SE, standard error; CRP, C-reactive protein.

[16.4 (13.1-19.8) vs. 10.7 (8.75-11.85) μg/mL, respectively, $p < 0.05$]. However, there was no significant difference in the serum LRG values between the two groups in the UC group [12.6 (9.8-16.5) vs. 11.4 (9.6-13.2) μg/mL, respectively, $p = 0.495$].

Background factors associated with serum LRG levels

Multiple regression analysis was performed to evaluate the background factors associated with serum LRG levels. Table 2 presents the results. Intestinal stenosis was significantly related to serum LRG levels in the CD group (estimated regression coefficient 4.23, standard error 1.66, t-value 2.55, $p = 0.016$), while age, sex, disease duration, history of surgery were not. Serum CRP level was significantly related to serum LRG level in both CD group and UC group.

Discussion

LRG is a glycoprotein expressed in hepatocytes, neutrophils (O'Donnell et al. 2002; Shirai et al. 2009), and focal inflamed intestinal cells in patients with IBD (Serada et al. 2012). LRG levels disease activity and mucosal inflammation in patients with UC (Serada et al. 2012; Shinzaki et al. 2017) and CD (Shinzaki et al. 2021; Yasutomi et al. 2021). However, evidence regarding LRG application in clinical practice is still scarce.

We examined the application of serum LRG and CRP levels as validated, independent serum biomarkers for disease activity in IBD. In patients with CD, the correlation between disease activity and both serum LRG and CRP levels was statistically significant; however, LRG tended to be better than CRP. CRP has been reported to be a useful biomarker that correlates well with mucosal inflammation and recurrence in CD; however, the correlation between serum CRP levels and mucosal inflammation in the small intestine has been reported to be weak (Arai et al. 2017). In the current study, more than 90% of patients with CD had small intestinal lesions (34% of ileitis type and 59.6% of ileocolitis type), which might explain the poor correlation between colonic mucosal inflammation and serum CRP levels, since we did not evaluate small intestinal mucosal inflammation. The correlation between serum LRG levels and small-intestinal inflammation requires further investigation. In UC patients, both serum LRG and CRP levels correlated signifi-

cantly with disease activity. Serum LRG and CRP are considered useful biomarkers in patients with UC. Additionally, we compared the discriminatory ability of LRG and CRP in detecting MH using ROC curve analysis (Fig. 2). In patients with CD, the AUC of LRG was better than that of CRP (0.894 vs. 0.689, p = 0.038). In patients with UC, a good AUC was demonstrated for both LRG and CRP levels (0.874 vs. 0.853, p = 0.672). Similar to previous reports, LRG is a useful biomarker that corresponds with endoscopic disease activity in patients with IBD. Cut-off levels of LRG and CRP in CD were 12.6 μg/mL and 0.21 mg/dL, and 16.3 μg/mL and 0.18 mg/dL in UC, respectively. In patients with CD, the cut-off level of LRG was lower than the recommended level of 16 μg/mL. This result suggests that a lower LRG cut-off level is more useful for the detection of mucosal healing in patients with CD.

Serum LRG levels were assessed in the CRP-normal subgroup. In patients with CD, serum LRG levels were found to be higher in the presence of mucosal inflammation. CRP has been reported as a useful biomarker in patients with IBD. It is produced in hepatocytes under the influence of interleukin (IL)-6 (Mitsuyama et al. 1991). However, in patients with IBD, complicated mucosal inflammation leads to elaboration of various cytokines, such as IL-6, tumor necrosis factor-α (TNF-α), IL-22, and others. Hence, serum CRP levels, mediated by IL-6, may not increase even if the disease is in the active phase (Vermeire et al. 2006; Sands 2015). Therefore, serum LRG could reflect inflammation in patients with IBD better than CRP as it is not mediated by IL-6 alone (Serada et al. 2012). However, there was no significant difference in the serum LRG values between the MH and NMH groups in the CRP-normal subgroup with UC. The following points may have affected the results. First, a good AUC for both LRG and CRP was obtained in patients with UC. It has been reported that associated cytokines differ between CD and UC. CD is associated with Th1 mediated response, characterized by enhanced production of IFN-γ and TNF-α. IL-12 and IL-23 govern Th1 differentiation, which, in combination with IL-15, IL-18 and IL-21, induces the stabilization of polarized Th1. In contrast, in UC, the local immune response is less polarized, but it is characterized by CD1 reactive natural killer T cell production of IL-13 and Th2 cytokines (Sanchez-Muñoz et al. 2008). These differences in cytokine profiles may affect the differences in the serum levels of LRG and CRP between CD and UC. Second, in this study, CD patients were treated more with biologics than UC patients (57.1% vs. 26.1%), which may have suppressed CRP production. Finally, the presence of inflammation in the small intestine was not assessed in our study, and this may have impacted the biomarker values.

Factors associated with elevated serum LRG levels were analyzed using a multiple regression analysis. In the CD group, only the presence of intestinal stenosis was significantly associated with a serum LRG level, even if a serum CRP level was included as a covariate. This result indicates that high serum levels of LRG are associated not only with the inflammation or ulceration aspects of the stenosis but also with fibrotic stenosis, which represents a reparative process. Previously, LRG was reported to control transforming growth factor (TGF)-β signaling in fibroblasts in a mouse model of pulmonary fibrosis, and LRG knockout mice demonstrated suppressing pulmonary fibrosis (Honda et al. 2017). The physiological role of LRG in IBD remains unknown; however, it may be part of the chemical mediators cascade responsible for recruiting fibroblasts, resulting in fibrosis and thereby causing stenosis.

This study had several limitations. First, since this was a retrospective study, there were some variations in the patients' backgrounds. Increased LRG levels have been reported in patients with IBD as well as other inflammatory diseases, such as rheumatic diseases (Serada et al. 2010), adult-onset Still's disease (Ha et al. 2015), systemic juvenile idiopathic arthritis (Shimizu et al. 2017), psoriasis (Nakajima et al. 2017), and gastric and colorectal neoplasms associated with UC (Yamamoto et al. 2017; Shinozaki et al. 2018). These comorbidities, if present, may have affected LRG levels. In this study, two patients with UC were excluded because one had no mucosal inflammation but had advanced colonic cancer with elevated serum LRG level (36.1 μg/mL) (CRP 2.6 mg/dL), and the other was affected with acute gastroenteritis on the day of LRG measurement with elevated serum LRG level (29.9 μg/mL) (CRP 2.81 mg/dL), which decreased to the normal level when gastroenteritis was healed. Second, we could not perform a time series analysis because only a few cases could be followed up from the start of treatment to the remission stage. Future studies on a larger sample size and complete follow-up monitoring changes in LRG level, disease activity, and endoscopic activity are needed. Finally, we did not evaluate small intestinal mucosal inflammation in this study. In patients with CD, active inflammation of the small intestine may affect LRG levels. Hence, evaluation of the small intestine has to be included in the studies as well.

In conclusion, serum LRG level is a useful biomarker for monitoring disease activity and mucosal inflammation in patients with IBD and appears to be more reliable than CRP. A high serum LRG level also correlates well with the status of intestinal stenosis. However, further studies are needed to establish LRG as a routine investigation in monitoring IBD.

Author Contributions

Conceptualization: Yosuke Shimodaira. Methodology: Yosuke Shimodaira. Data collection: Tatsuki Yoshida, Yosuke Shimodaira, Sho Fukuda, Noboru Watanabe, Shigeto Koizumi, Tamotsu Matsuhashi, and Kengo Onochi. Formal analysis and investigation: Tatsuki Yoshida. Writing - original draft preparation: Tatsuki Yoshida, and Yosuke Shimodaira. Writing - review and editing: Katsunori Iijima.

Conflict of Interest

The authors declare no conflict of interest.

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