Leucine-rich α2-glycoprotein as a potential biomarker for immune-related colitis after anti-PD-1 therapy: A report of case series

Leucine-rich α_2-glycoprotein as a potential biomarker for immune-related colitis after anti-PD-1 therapy: A report of case series

Katsuhiro Yoshimura, MD[1, 2], Aiko Saku, MD, PhD[1,3], Masato Karayama, MD, PhD[1], Naoki Inui, MD, PhD[1,4], Haruhiko Sugimura, MD, PhD[2], Takafumi Suda, MD, PhD[1]

[1] Second Division, Department of Internal Medicine, Hamamatsu University School of Medicine, Hamamatsu, Japan

[2] Department of Tumor Pathology, Hamamatsu University School of Medicine, Hamamatsu, Japan

[3] Department of Allergy and Clinical Immunology, Graduate School of Medicine, Chiba University, Chiba, Japan

[4] Department of Clinical Pharmacology and Therapeutics, Hamamatsu University School of Medicine, Hamamatsu, Japan

Corresponding author: Katsuhiro Yoshimura, MD.

Address: 1-20-1 Handayama Higashi-ku, Hamamatsu, Shizuoka 431-3129 Japan

Tel: +81-53-435-2263

Fax: +81-53-435-2354

E-mail: ky@hama-med.ac.jp

Role of funding source: The funders had no role in study design, data collection and

analysis, decision to publish, or preparation of the manuscript.

Conflicts of interest: The authors have declared that no competing interests exist.

Author contribution: Katsuhiro Yoshimura: Conceptualization, Methodology, Investigation, Resources, Data curation, Visualization, Writing–Original Draft, **Aiko Saku**: Investigation, Resources, Data curation, Writing–Review & Editing, **Masato Karayama**: Resources, Data curation, Writing - Review & Editing, **Naoki Inui**: Writing–Review & Editing, **Haruhiko Sugimura**: Writing–Review & Editing, **Takafumi Suda**: Project administration, Writing–Review & Editing, Supervision.

Acknowledgments: None

Key words: immune-related adverse event (irAE), colitis, leucine-rich a_2-glycoprotein (LRG), immune checkpoint inhibitor, non-small cell lung cancer

Word count: 1351 words

Figure and Table: 2 Figures, and 1 Table.

Introduction

The use of immune checkpoint inhibitors (ICIs) targeting the PD-1/PD-L1 axis is one of the main therapies for advanced non-small-cell lung cancer (NSCLC). With the increasing use of ICIs, immune-related adverse events (irAEs) are also been increasing reported.[1] Immune-related colitis is an important irAE.[2] Grade 3 immune-related colitis develops in 1%–2% of the patients after therapy with ICIs and can lead to further deterioration, occasionally resulting in fatal outcomes.[1] Similar to inflammatory bowel diseases (IBDs) cases, some immune-related colitis patients show resistance to steroid therapy and, therefore, need further medications, including infliximab. Early diagnosis and optimal management are crucial for immune-related colitis; therefore, identifying a biomarker specific to immune-related colitis is important. Recently, serum leucine-rich a_2-glycoprotein (LRG) has been found to be associated with disease severity and endoscopic findings of inflammatory bowel diseases (IBDs) such as ulcerative colitis (UC)[3]. Immune-related colitis frequently shows UC-like endoscopic and pathological findings. Here, we evaluated the changes in serum LRG levels during ICI therapy in three patients with immune-related colitis.

Case series

Case 1 (**Figure 1A**): A 64-year-old female patient with metastatic adenocarcinoma (cT4N3M1c stage IV; EGFR wild type; ALK and ROS-1 negative; PD-L1 expression [clone22C3] was 100%; **Figure 2A**) was treated with cisplatin (75 mg/m^2), pemetrexed (500 mg/m^2), and pembrolizumab (200 mg). After one week, diarrhea, abdominal pain, and vomiting were reported. Computed tomography (CT) scans showed thickening of the enteric wall in the rectum. Colonoscopy showed erythematous mucosa and erosive

patches in the colon and rectum, which on histological studies were found to be prominent inflammatory cell infiltration and crypt abscesses, respectively (**Figure 2D and 2G**). After ruling out other infectious diseases, the patient was diagnosed with immune-related colitis and started on systemic steroids (oral prednisolone 50 mg; 1 mg/kg body weight). However, a sufficient response was not obtained even after increasing the steroid dose (intravenous methylprednisolone 100 mg; 2 mg/kg body weight). Single administration of infliximab (5 mg/kg) could not sufficiently control the symptoms. Simultaneously, her CMV antigenemia level had increased significantly, and another colonoscopy revealed ulcerative changes and easy bleeding erosion; immunohistochemical analysis confirmed CMV infection. Treatment with ganciclovir was started, and the CMV antigenemia level decreased significantly. However, her diarrhea did not improve. Again, colonoscopy was performed; the CMV-induced ulcerative changes had improved, but the erythematous mucosa and erosions had worsened. Infliximab (5 mg/kg) was additionally administered, which alleviated the severity of diarrhea. Therefore, immune-related colitis was completely controlled with the administration of infliximab on three occasions.

Case 2 (**Figure 1B**): A 77-year-old female patient was diagnosed with advanced adenocarcinoma (cT2N3M1a stage IV; EGFR wild type; ALK and ROS-1 negative; PD-L1 was expressed in more than 50% of the tumors; **Figure 2B**). The patient also had right carcinomatous pleurisy, so we carried out pleural drainage and pleurodesis after starting pembrolizumab monotherapy (200 mg). The tumor had shrunk slightly, and CT evaluation showed no further worsening of the condition. In approximately six weeks, three cycles of pembrolizumab had been administered. However, the patient developed persistent diarrhea. She was admitted because the diarrhea progressively worsened up to

grade 3, and endoscopic examination revealed discontinuous redness and edematous mucosa (**Figure 2E**); she was diagnosed with immune-related colitis. Her meals were stopped, and anti-diarrheic drugs were started; however, the symptoms did not improve sufficiently. When we administered oral prednisolone at a dose of 60 mg (1 mg/kg body weight), the diarrhea improved immediately.

Case 3 (**Figure 1C**): A 73-year-old male with metastatic adenocarcinoma (cT3N3M1b stage IV; EGFR wild type; ALK and ROS-1 negative; positive PD-L1 expression was 85%; **Figure 2C**) was initially treated with pembrolizumab monotherapy (200 mg). After four cycles of pembrolizumab, he developed diarrhea. He was admitted for appetite loss. Colonoscopy showed edematous redness mucosal changes mainly in the rectum, which histology showed the monocytic infiltration in the lamina propria (**Figure 2F and 2H**); he was diagnosed with grade 2 immune-related colitis. He was started on anti-diarrheic drugs and probiotics and pembrolizumab was halted. His symptoms gradually improved.

Changes in LRG levels in the three cases

The patients' characteristics are summarized in **Table 1**. All were diagnosed with metastatic lung adenocarcinoma and administered pembrolizumab-based therapies. Among the three patients, two developed grade 3 immune-related colitis, and one, grade 2. The former required systemic treatment with steroid alone or with steroid plus infliximab. In these severe cases, colonoscopy showed erythematous mucosa and erosion in the colon and rectum, similar to UC findings. The condition of the patient with grade 2 immune-related colitis improved when ICI treatment was simply discontinued. We assessed serum LRG levels using the ELISA method (IBL, Fujioka, Japan) at the

following timepoints: before ICI, at the onset of immune-related colitis, and after improvement of colitis (if present) (**Figure 3**). Serum evaluation was performed in the study on serum biomarkers for ICIs, which was approved by the appropriate ethics review board (#19-225). Additionally, five patients who was treated with pembrolizumab but did not show ir-AEs including immune-related colitis were evaluated as control cases (**Table 1**). The serum LRG levels in patients with immune-related colitis significantly increased than those in control cases (**Figure 3A and 3B**). LRG change ratio from baseline also demonstrated that the two patients with severe immune-related colitis showed significantly increased serum LRG levels, while the control case and the patient with mild immune-related colitis did not (**Figure 3C**). In contrast, serum CRP did not reflect the clinical course more precisely than serum LRG (**Figure 3D**). UC-like colonoscopy findings had also diminished in line with the changes in serum LRG levels (**Figure 3E**).

Discussion

We presented three cases of immune-related colitis, with the diagnosis based on colonoscopy findings. All patients showed improvement with or without treatment. During the treatment courses, the corresponding changes in serum LRG levels were observed. To the best of our knowledge, this is the first report on the use of serum LRG level as a biomarker for immune-related colitis.

C-reactive protein (CRP) is widely used in the evaluation of inflammatory diseases, including IBDs. However, CRP levels can be affected by several inflammatory processes. Serum LRG level has been reported to be associated with disease severity in rheumatoid arthritis (RA) and IBDs such as ulcerative colitis (UC)[3]. LRG is mainly produced by intestinal epithelial cells and has attracted attention as an intestine-specific

biomarker.[4] LRG production is induced by TNF-α, IL-22, and IL1-β, independently of IL-6.[4,5] In fact, a marked increase in the levels of various cytokines including TNF produced by hyper-activated effector T cells is considered one of the main causes of immune-related colitis.[6] LRG levels reflect disease severity in rheumatoid colitis and IBD, regardless of CRP levels.[3] Moreover, serum LRG levels significantly reflect the colonoscopy findings of UC.[3] Case 2 demonstrated increased CRP levels even after improvement in immune-related colitis. Cancer itself could cause an increase in CRP levels, possibly mimicking immune-related colitis activity. The endoscopic findings of Case 1 showed similar deterioration, but they improved in line with the changes in LRG levels. In particular, with Case 1, it was difficult to distinguish between CMV colitis and immune-related colitis during the treatment course. The CRP level did not significantly change, but the LRG level changed with persistent symptoms of immune-related colitis. Similarly, previous studies have mentioned the importance of considering opportunistic infections, such as CMV, because we frequently use prednisolone and other immunosuppressants for irAEs.[7] Therefore, serum LRG level could be useful in distinguishing between different forms of colitis such as CMV colitis and drug-induced colitis. Furthermore, LRG levels might help distinguish between inflammatory and non-inflammatory diseases, which could be useful in treatment decisions for RA patients.[8]

Previous evidence has demonstrated that LRG regulates TGF-β signaling, associated with angiogenesis and cellular proliferation.[9,10] Regarding NSCLC, in-vivo and in-vitro experiments with LRG showed cell-growth inhibition via TGF-β modulation.[10] Proteomic studies also demonstrated that serum LRG level could be one of the prognostic biomarkers for NSCLC.[11] Of note, LRG itself has some biological properties that play a role in lung cancer pathogenesis. Namely, during the management

of immune-related colitis, changes in serum LRG levels could be affected by the NSCLC treatment course. We must consider these points to understand the importance of LRG levels in patients with NSCLC.

Indeed, this small case numbers could not conclude the utility of seum LRG level for immune-related colitis. Also, LRG changes at multiple-time points according to clinical course, in particular case 1, is desirable. Moreover, validation of evaluation method for LRG have been still immature for use in the clinical setting. Therefore, further studies are required to confirm the study finding. However, our findings indicate that LRG level may have potential as a specific biomarker for immune-related colitis.

Conclusion

The serum LRG levels of the three immune-related colitis patients showed changes according to the clinical course of ICI therapy. Serum LRG level could be a specific biomarker for immune-related colitis, but further case studies and research are needed to confirm this.

REFERENCES

1. Martins F, Sofiya L, Sykiotis GP, et al. Adverse effects of immune-checkpoint inhibitors: epidemiology, management and surveillance. *Nat Rev Clin Oncol*. 2019. doi:10.1038/s41571-019-0218-0
2. Soularue E, Lepage P, Colombel JF, et al. Enterocolitis due to immune checkpoint inhibitors: A systematic review. *Gut*. 2018;67(11):2056-2067. doi:10.1136/gutjnl-2018-316948
3. Naka T, Fujimoto M. LRG is a novel inflammatory marker clinically useful for the evaluation of disease activity in rheumatoid arthritis and inflammatory bowel disease. *Immunol Med*. 2018;41(2):62-67. doi:10.1080/13497413.2018.1481582
4. Serada S, Fujimoto M, Terabe F, et al. Serum leucine-rich alpha-2 glycoprotein is a disease activity biomarker in ulcerative colitis. *Inflamm Bowel Dis*. 2012;18(11):2169-2179. doi:10.1002/ibd.22936
5. Serada S, Fujimoto M, Ogata A, et al. iTRAQ-based proteomic identification of leucine-rich α-2 glycoprotein as a novel infl ammatory biomarker in autoimmune diseases. *Ann Rheum Dis*. 2010. doi:10.1136/ard.2009.118919
6. Samaan MA, Pavlidis P, Papa S, Powell N, Irving PM. Gastrointestinal toxicity of immune checkpoint inhibitors: From mechanisms to management. *Nat Rev Gastroenterol Hepatol*. 2018. doi:10.1038/nrgastro.2018.14
7. Lankes K, Hundorfean G, Harrer T, et al. Anti-TNF-refractory colitis after checkpoint inhibitor therapy: Possible role of CMV-mediated immunopathogenesis. *Oncoimmunology*. 2016. doi:10.1080/2162402X.2015.1128611
8. Fujimoto M, Serada S, Suzuki K, et al. Brief Report: Leucine-Rich α2-

Glycoprotein as a Potential Biomarker for Joint Inflammation During Anti–Interleukin-6 Biologic Therapy in Rheumatoid Arthritis. *Arthritis Rheumatol*. 2015;67(8):2056-2060. doi:10.1002/art.39164

9. Wang X, Abraham S, McKenzie JAG, et al. LRG1 promotes angiogenesis by modulating endothelial TGF-β signalling. *Nature*. 2013. doi:10.1038/nature12345

10. Takemoto N, Serada S, Fujimoto M, et al. Leucine-rich α-2-glycoprotein promotes TGFβ1-mediated growth suppression in the Lewis lung carcinoma cell lines. *Oncotarget*. 2015. doi:10.18632/oncotarget.3557

11. Walker MJ, Zhou C, Backen A, et al. Discovery and Validation of Predictive Biomarkers of Survival for Non-small Cell Lung Cancer Patients Undergoing Radical Radiotherapy: Two Proteins With Predictive Value. *EBioMedicine*. 2015. doi:10.1016/j.ebiom.2015.06.013

LEGENDS

Figure 1. The detailed clinical courses of the patients with immune-related colitis

The clinical courses of individual cases: case 1 (A), case 2 (B), and case 3 (C). All three patients were treated pembrolizumab, and two patients manifested Grade 3 colitis symptoms, who were then treated with steroid with/without infliximab. The other patient showed grade 2 colitis, which improved with the halting of ICI. In the line graph, the Y axis represents the frequency of diarrhea per day, and in the bar graph, the second Y axis represents the incidence of CMV antigenemia (for only case 1), and the X axis represents the number of days from ICI administration.

Figure 2. Pathologic images of lung cancer and colitis, and colonoscopic findings of each case.

The top row of the images shows pathologic findings of the primary tumors in each case; (A) low grade adenocarcinoma with acinar subtype in case 1, (B) low grade adenocarcinoma with papillary subtype, and (C) low grade adenocarcinoma with acinar subtype.

The second row of the images shows colonoscopic images in each case; (D) case 1 shows discontinuous erythematous mucosa and erosive patches in the both colon and rectum, and (E-F) case 2 and 3 shows edematous mucosa, erythema, loss of vascular marking in the colon and rectum.

The bottom row of the images shows colon pathologic images in each case; (G) case 1 shows inflammatory infiltration with lymphocytes, plasma cells and neutrophils in the lamina and crypt abscess formation, and (H) case 3 shows monocytic infiltration in the lamina propria. These colonoscopic and pathogical findings corresponds with immune-

related colitis.

Figure 3. Serum marker changes during the treatment course for immune-related colitis.

(A) The measured values of serum LRG are shown in the patients with immune-related colitis and control cases. The values are represented at two time points; 0 week as the baseline, and 8–10 weeks as the onset colitis for the patients with immune-related colitis or the date after the ICI therapy for the control cases. (B) The degree of serum LRG change from baseline are shown. Error bars represent mean ± standard deviation. The *P* value was determined by t-test. The mean serum LRG levels among patient with colitis significantly increased than those among the control cases.

(C–D) LRG change ratio and CRP values changes from baseline in the patients with immune-related colitis and the control cases are shown. The time points when serum LRG levels were assessed were described in **Table 1**.

(C) Colonoscopy findings of Case 1 are shown at the onset of colitis and after improvement.

Table 1. Characteristics and serum LRG levels of immune-related colitis patients and the control cases.

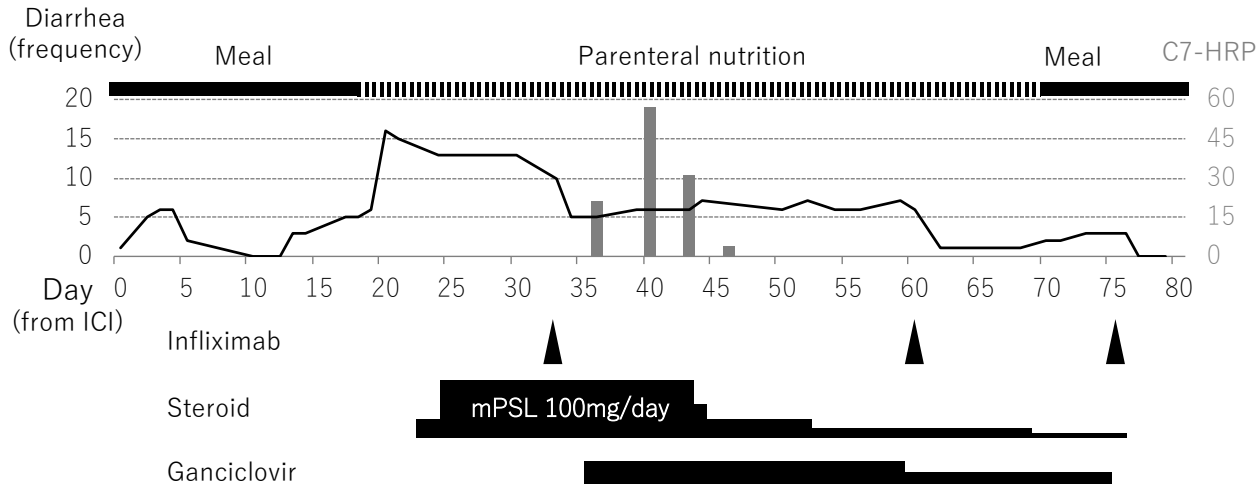
Table 1. The characteristics of the patients with immune-related colitis and one control patient.

Case	Age	Sex	Histology	Stage	Smoking status	Regimen	Onset of colitis	Severity of dirrhea	Treatmnent	ICI withholding	Response to colitis	PD-L1 TPS (%)	Stage
1	63	F	ADC	IV	Never	CDDP + Pemetrexed + Pembrolizumab	2 weeks	Grade 3	Steroid + infliximab	Yes	Improved	100	cT2aN3M1a
2	78	F	ADC	IV	Current	Pebmrolizumab	6 weeks	Grade 3	Steroid	Yes	Improved	60	cT3N3M1b
3	73	M	ADC	IV	Never	Pebmrolizumab	12 weeks	Grade 2	None	Yes	Improved	85	cT4N3M1c
Ctrl	65	F	ADC	IV	Ex	CDDP + Pemetrexed + Pembrolizumab	—	—	—	—	—	75	cT4N3M1c

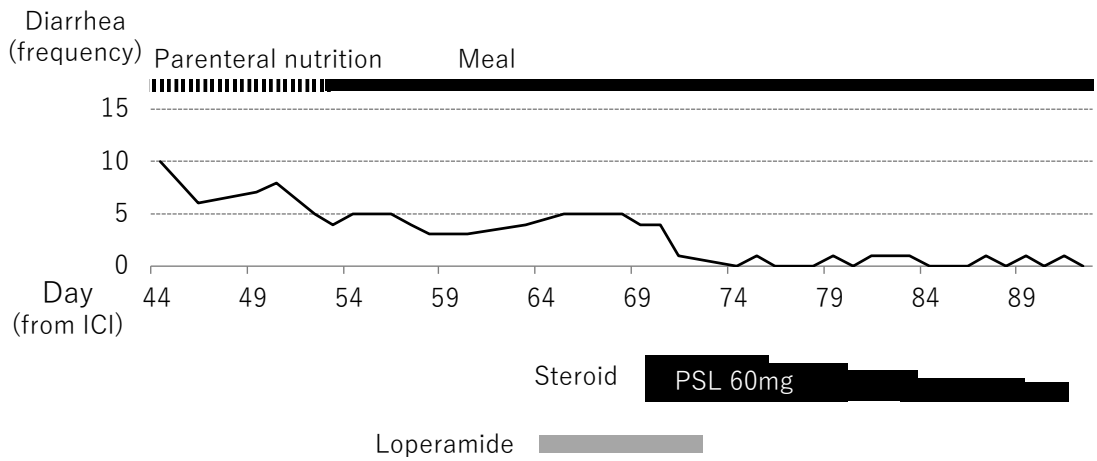
Abbreviations: ADC: adenocarcinoma, CDDP: cisplatin, Ctrl: control

FIgure 1

Case 1 (Grade 3, colitis)



Case 2 (Grade 3, colitis)



Case 3 (Grade 2, colitis)

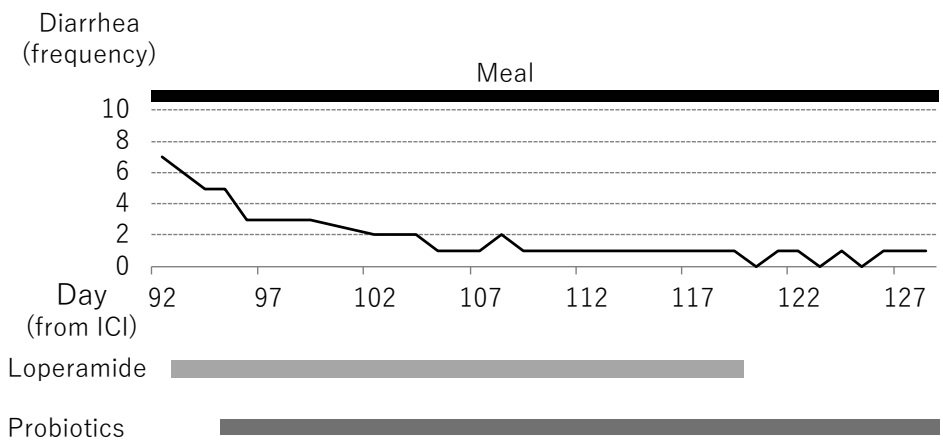


FIgure 2

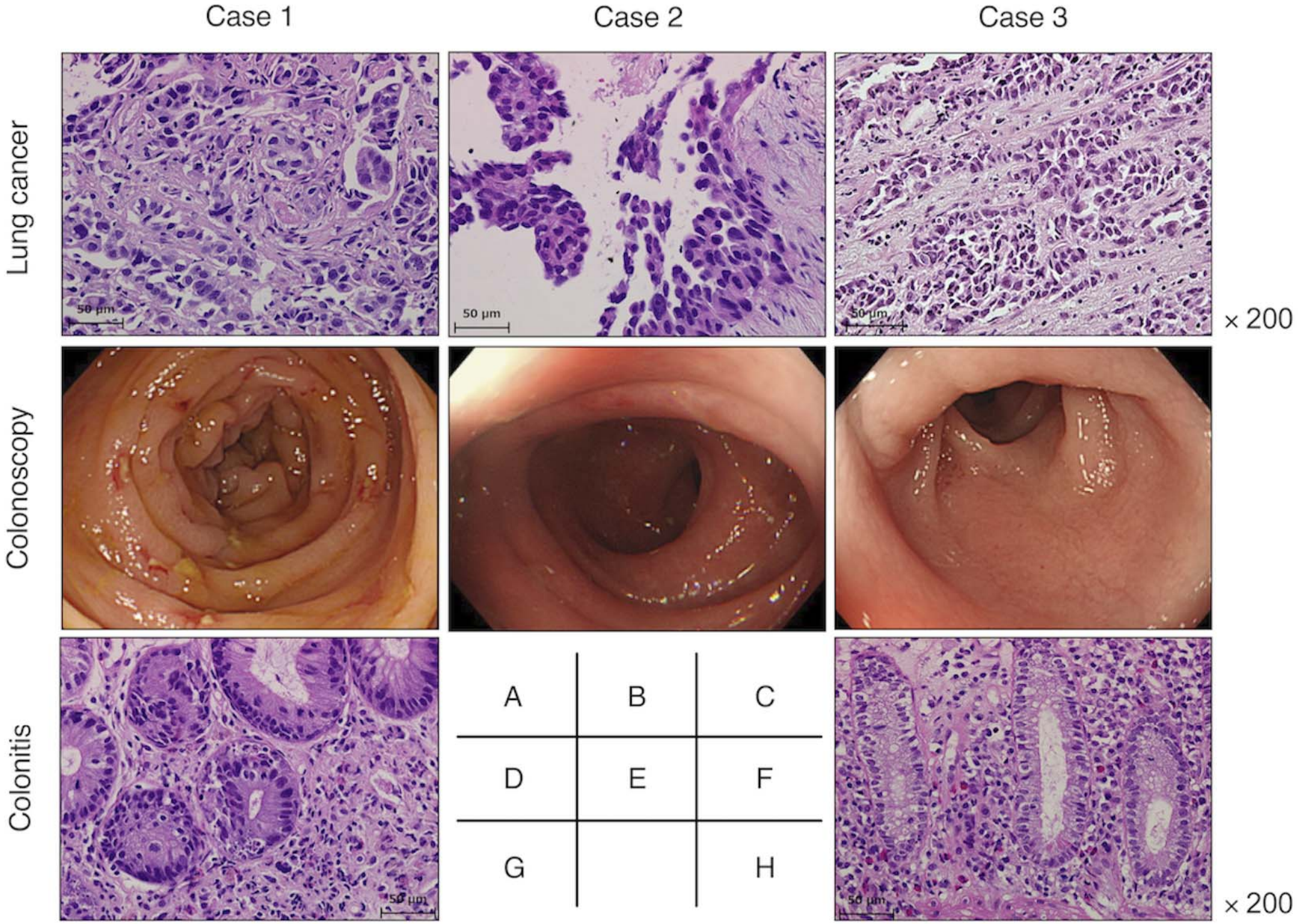
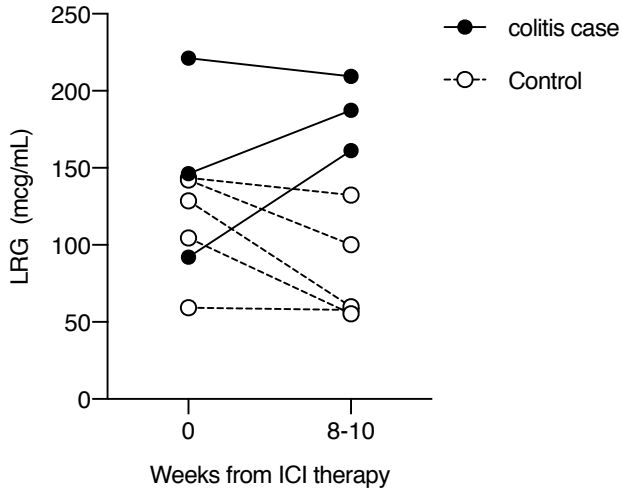
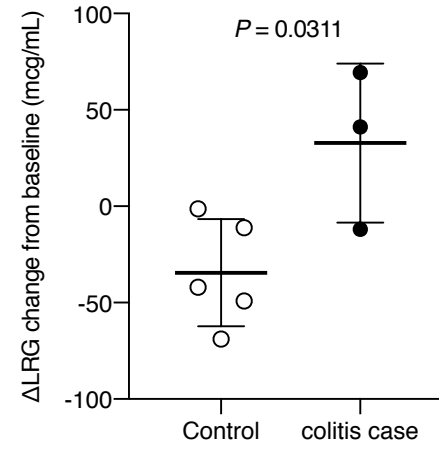

Case 1
Case 2
Case 3
Lung cancer
Colonoscopy
Colonitis
50 μm
× 200
× 200
A
B
C
D
E
F
G
H

FIgure 3

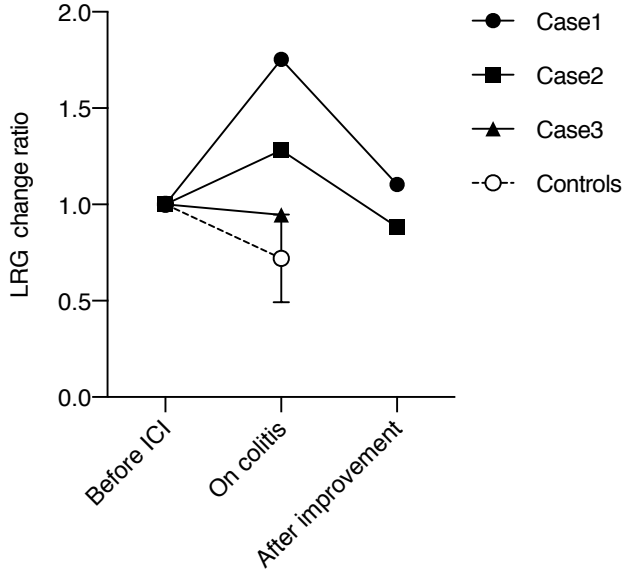
A



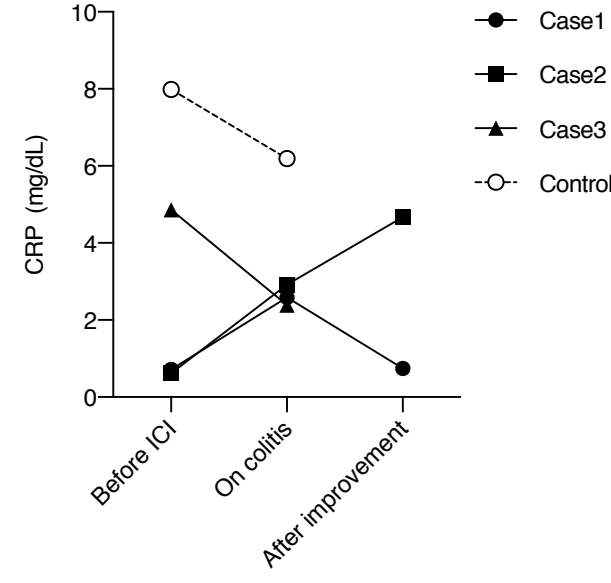
B



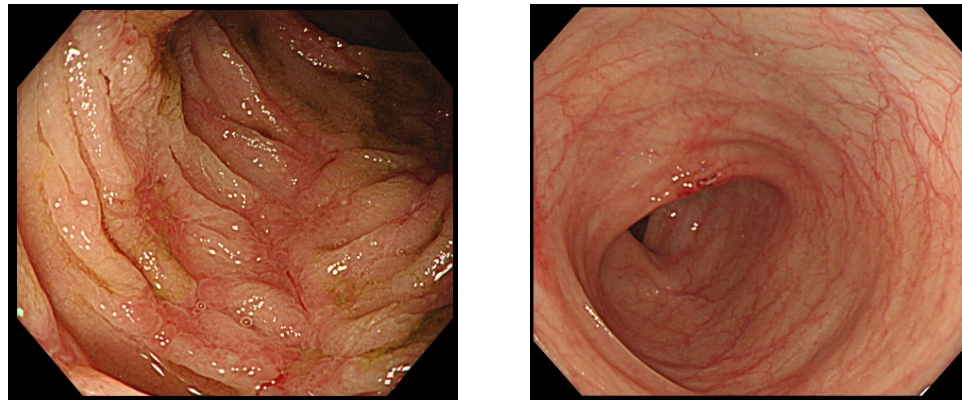
C



D



E



On colites

After improvement