RESEARCH ARTICLE

Novel prognostic biomarkers of pouchitis after ileal pouch-anal anastomosis for ulcerative colitis: Neutrophil-to-lymphocyte ratio

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Abstract

Objectives
Pouchitis is a major complication after restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA) in patients with ulcerative colitis (UC). Although there have been many investigations of the neutrophil-to-lymphocyte ratio (NLR) in various diseases, its role in predicting the development of pouchitis remains unclear. We aimed to evaluate the clinical utility of the NLR for predicting the development of pouchitis after IPAA in UC patients.

Materials and methods
UC patients who underwent IPAA at Osaka City University Hospital between May 2006 and March 2019 were included. The incidence of pouchitis was estimated using the Kaplan-Meier method. Potential preoperative, intraoperative, and postoperative predictors for pouchitis, including various demographic and clinical variables, were analyzed. The combined impact of the NLR and other known prognostic factors were investigated using Cox proportional hazard regression with inverse probability of treatment weighting (IPTW).

Results
Forty-nine patients with UC who underwent IPAA were included. The median follow-up period was 18.3 months (interquartile range: 10.7–47.2 months). Eighteen patients (36.7%) developed pouchitis. The incidence of pouchitis was 19.2%, 32.6%, and 45.9% at 1, 2, and 5 years, respectively. NLR was significantly associated with the development of pouchitis in the univariate Cox regression analysis (hazard ratio (HR), 1.14; 95% confidence interval (CI), 1.01–1.28; P = 0.03). The NLR cutoff value of 2.15 was predictive of the development of pouchitis according to receiver operating characteristic analysis (specificity: 67.7%, sensitivity: 77.8%).
sensitivity: 72.2%). The incidence of pouchitis was significantly lower in the low NLR group than that in the high NLR group (P = 0.01, log-rank test). Cox regression analyses using IPTW also identified NLR as a prognostic factor for the development of pouchitis by statistically adjusting for background factors (HR, 3.60; 95% CI, 1.31–9.89; P = 0.01).

Conclusions

NLR may be a novel and useful indicator for predicting the development of pouchitis after IPAA in UC and should be introduced in clinical practice.

Introduction

Restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA) has become a standard surgical procedure for medically intractable ulcerative colitis (UC), colitis-associated dysplasia, and cancer [1]. The most common long-term complication of this surgery is pouchitis, with a cumulative prevalence of 15%–50% [2–4], which is one of the major factors reducing the postoperative quality of life (QOL) in patients with UC [5, 6].

Although the main cause of pouchitis is unclear, it is more prevalent in patients with UC than in those with familial adenomatous polyposis [1, 7]. Despite conflicting results, the development of pouchitis in patients with UC, as reported in several studies, has been linked to various factors, including primary sclerosing cholangitis [2, 4], other extraintestinal manifestations of inflammatory bowel disease [4, 8, 9], young age at UC diagnosis [4], preoperative terminal ileal inflammation [10, 11], extensive colonic disease [10], presence of interleukin-1 receptor antagonist gene allele 2 [12], total steroid dose of > 10000 mg [13], use of infliximab [14], neutrophil percentage of > 65% [13], and presence of perinuclear antineutrophil cytoplasmic antibodies [15, 16]. Interleukin-1 receptor antagonist gene allele 2 and perinuclear antineutrophil cytoplasmic antibodies have been reported to be useful markers for predicting the development of pouchitis; however, measuring these markers is costly and not covered by medical insurance in Japan. Easily accessible and low-cost markers are required to predict pouchitis in patients with IPAA.

A growing body of evidence has suggested that the neutrophil-to-lymphocyte ratio (NLR) is not only an easily accessible laboratory test, but also a useful predictive parameter for various types of cancer [17–20], rheumatoid arthritis [21], coronavirus disease 2019 [22], and coronary heart disease [23, 24]. Regarding inflammatory bowel disease, several studies have reported an association between the NLR and UC disease activity [25, 26]. We previously reported the utility of NLR as a useful prognostic marker for predicting the long-term outcomes in patients with UC treated with infliximab or tacrolimus therapy [27, 28]. Lorenzo et al. reported that the NLR played a promising role as an early predictor of therapeutic response to anti-tumor necrosis factor (TNF) therapy in patients with UC [29]. However, no studies have tested the value of this parameter in predicting the development of pouchitis after IPAA in patients with UC. Therefore, our study aimed to evaluate the clinical utility of the NLR for predicting the development of pouchitis after IPAA in patients with UC.

Materials and methods

Patients

All patients with UC who underwent IPAA at Osaka City University Hospital between May 2006 and March 2019 were included. In our facility, patients underwent one-, two- or three-stage surgeries considered by the patient’s preoperative conditions, such as massive bleeding,
rectal inflammation, vital signs, anemia, nutritional status, and age. The two-stage operation starts with total proctocolectomy and IPAA, along with a diversion ileostomy construction in the first stage and ends with ileostomy closure in the second stage. The three-stage operation starts with subtotal colectomy, along with ileostomy construction and sigmoid mucous fistula formation, in the first stage, followed by remnant proctocolectomy and IPAA, with a reconstruction of the ileostomy in the second stage. The procedure is completed with ileostomy closure in the third stage [30]. Patients with one-stage surgery were excluded because we analyzed postoperative NLR.

**Evaluation**

All patients were followed up with a physical examination and a blood test. The differential white blood cell (WBC) count was analyzed using an XE-5000 hematology analyzer (Sysmex, Kobe, Japan), as per the manufacturer’s protocol. In patients undergoing one-stage surgery, the NLR was calculated from a blood sample measured before IPAA by dividing the absolute neutrophil count by the absolute lymphocyte count. Patients were followed up from the time of IPAA to the onset of pouchitis, loss to follow-up, or until the end of March 2020. The NLR was calculated from a blood sample measured before stoma closure. Patients were followed up from the time of stoma closure to the onset of pouchitis, loss to follow-up, or until the end of March 2020.

**Diagnosis of pouchitis**

The pouchitis disease activity index (PDAI) is a 19-point index of pouchitis activity based on clinical symptoms as well as endoscopic and histologic findings [31]. In this study, pouchitis was diagnosed based on the modified pouchitis disease activity index (mPDAI) score using a combination of clinical symptoms and endoscopic examination. An mPDAI score of ≥ 5 points was used to define pouchitis in this study [32].

**Study endpoints**

The primary outcome measure of this study was the onset of pouchitis. Potential preoperative, intraoperative, and postoperative predictors for pouchitis, including various demographic and clinical variables, were analyzed.

**Statistical analysis**

Continuous variables are presented as medians and interquartile ranges. The differences in clinical characteristics were compared using either the chi-square test or Fisher’s exact test for categorical variables and the Mann-Whitney U-test for continuous variables. Receiver operating characteristic (ROC) curves were plotted so that the area under the ROC curve could be calculated. Optimal cutoff values were determined according to the Youden criterion, which marks the point on a ROC curve where “sensitivity + specificity− 1” is maximal [33]. The cumulative incidence of pouchitis was illustrated using a Kaplan-Meier plot. Differences in the survival curves were assessed using the log-rank test. Furthermore, continuous values of laboratory data were evaluated using the Cox proportional hazard model. Data are presented as hazard ratios (HRs) with 95% confidence intervals (CIs). An IPTW analysis was applied to each observation in the Cox model to assess the relationship between the NLR and the development of pouchitis. The IPTW analysis was derived using propensity scores on all observations before matching to reduce selection bias by statistically adjusting for background factors [34]. Variables included in the IPTW analysis were age, sex, disease location, and history of anti-TNF therapy.
A P-value of < 0.05 was considered statistically significant. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University), a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0). More precisely, it is a modified version of R commander (version 1.6–3) that includes statistical functions frequently used in biostatistics.

**Ethical considerations**

This study was approved by the Osaka City University Hospital Certified Review Board; (no. 4291), which waived the requirement for written informed consent because the analysis used anonymized clinical data that were retrospectively obtained after each patient agreed to receive the treatment. All data were fully anonymized before we accessed them. Nevertheless, all patients were notified of the content and information of this study and given the opportunity to refuse participation. None of the patients refused participation. This study followed the Ethical Guidelines for Medical and Health Research Involving Human Subjects established by the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labor and Welfare in Japan.

**Results**

**Study subjects**

Overall, we included 79 patients who underwent IPAA for UC during the study period. Twenty-nine patients were excluded due to a lack of data on differential WBC count. One patient with one-stage surgery was excluded. Forty-nine patients were retrospectively reviewed. Of those patients, 30 patients underwent IPAA for disease refractory to medication, 9 patients for dysplasia or cancer, 3 patients for perforation, 3 patients for toxic megacolon, 2 patients for colonic strictures, and 2 patients for massive bleeding.

The median follow-up period was 18.3 months (interquartile range: 10.7–47.2 months). Eighteen patients (36.7%) developed pouchitis. The incidence of pouchitis was 19.2%, 32.6%, and 45.9% at 1, 2, and 5 years, respectively (Fig 1). The demographic characteristics of the patients are summarized in Table 1.

**Risk factors for pouchitis**

NLR was significantly associated with the development of pouchitis according to univariate Cox regression analysis (HR, 1.14; 95% CI, 1.01–1.28; P = 0.03). No other clinical variables such as age, sex, disease duration, age at onset, or disease location have shown a statistically significant association with pouchitis development (Table 2).

When the NLR was examined as a dichotomous variable, a cutoff value of the NLR for the risk of pouchitis was determined using ROC analysis. From the Youden index, the ROC analysis showed that the best cutoff value for the NLR was 2.15 (specificity: 67.7%, sensitivity: 72.2%) (Fig 2). Therefore, a cutoff value of 2.15 was chosen for further study. Twenty-six (53.1%) patients had an NLR of < 2.15 (low NLR group), whereas 23 (46.9%) patients had an NLR of ≥ 2.15 (high NLR group). Table 3 shows a comparison of the baseline characteristics between the low NLR (< 2.15) and high NLR (≥ 2.15) groups. No significant differences were noted in the background characteristics between the two groups. Fig 3 shows a comparison of the cumulative incidence of pouchitis between the low NLR group and the high NLR group. The incidence of pouchitis was significantly lower in the low NLR group than in the high NLR group (P = 0.01, log-rank test). Univariate Cox regression analysis also identified high NLR as a significant risk factor for the onset of pouchitis (unadjusted HR, 3.45; 95% CI, 1.23–9.71; P = 0.02). Therefore, to elucidate the influence of reported risk factors, Cox regression analysis...
Fig 1. Cumulative incidence of pouchitis. The incidence of pouchitis was 19.2%, 32.6%, and 45.9% at 1, 2, and 5 years, respectively.

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Table 1. Baseline characteristics of the study population.

|                                      | all patients |
|--------------------------------------|--------------|
| Number of patients                   | 49           |
| Sex: male/female                     | 25 / 24      |
| Age at diagnosis (years), median     | 32.4 (22.8–44.2) |
| (interquartile range)                |              |
| Age at IPAA (years), median          | 44.3 (32.9–53.6) |
| (interquartile range)                |              |
| Disease duration (years), median     | 4.5 (2.3–10.2) |
| (interquartile range)                |              |
| UC location: Left-sided colitis/Pancolitis | 9 / 40      |
| Concomitant therapies, n (%)         |              |
| Immunomodulator                      | 14 (28.6%)   |
| Corticosteroids                      | 15 (30.6%)   |
| Anti-TNF-α antibody therapy          | 14 (28.6%)   |
| Calcineurin inhibitor therapy        | 16 (32.7%)   |
| Hemoglobin (g/dL), median            | 12.9 (11.9–14.0) |
| (interquartile range)                |              |
| Albumin (g/dL), median               | 4.20 (4.00–4.50) |
| (interquartile range)                |              |
| CRP (mg/dL), median                  | 0.07 (0.04–0.22) |
| (interquartile range)                |              |
| WBC (/μL), median                    | 5900 (5100–7200) |
| (interquartile range)                |              |
| Neutrophil (/μL), median             | 3500 (2900–4600) |
| (interquartile range)                |              |
| Lymphocyte (/μL), median             | 1700 (1400–2000) |
| (interquartile range)                |              |
| NLR, median                          | 2.00 (1.41–2.92) |
| (interquartile range)                |              |
| Pouchitis (+), n (%)                 | 18 (36.7%)   |

IPAA: ileal pouch-anal anastomosis; UC: ulcerative colitis; TNF: tumor necrosis factor; CRP: C-reactive protein; WBC: white blood cell; NLR: neutrophil-to-lymphocyte ratio.

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was performed using the IPTW method to identify factors associated with the development of pouchitis. Variables included in the IPTW analysis were age, sex, disease location, and history of anti-TNF therapy. Cox regression analyses using the IPTW method identified NLR as a prognostic factor for the development of pouchitis by statistically adjusting for background factors (adjusted HR, 3.60; 95% CI, 1.31–9.89; P = 0.01).

As drug discontinuation during the course may occur in a number of situations, including a history of anti-TNF therapy as an adjusting background factor in IPTW analysis might not be appropriate in this study due to several biases. Therefore, we also performed IPTW analysis without a history of anti-TNF-therapy as an adjusting background factor. Cox regression analyses using the IPTW method, excluding the history of anti-TNF therapy as an adjusting background factor, also identified NLR as a prognostic factor for the development of pouchitis (adjusted HR, 3.67; 95% CI, 1.38–9.79; P = 0.01).

**Discussion**

In this study, we investigated the utility of NLR for the development of pouchitis after IPAA in patients with UC. Our results suggest that a high NLR is strongly associated with an increased risk of developing pouchitis, and patients with high NLR should be followed up carefully.

Table 2. Cox regression analysis of risk factors for the development of pouchitis.

| Risk Factor                                      | Unadjusted HR (95% CI) | P-value |
|--------------------------------------------------|------------------------|---------|
| Sex                                              |                        |         |
| Male                                             | 1                      |         |
| Female                                           | 0.76 (0.30–1.95)       | 0.58    |
| Age at diagnosis (continuous)                    | 0.99 (0.95–1.02)       | 0.47    |
| Age at IPAA (continuous)                         | 0.99 (0.95–1.02)       | 0.40    |
| Disease duration (continuous)                    | 0.99 (0.94–1.05)       | 0.81    |
| UC location                                      |                        |         |
| left-sided colitis                               | 1                      |         |
| pan-colitis                                      | 3.98 (0.53–30.0)       | 0.18    |
| Immunomodulators (azathioprine or 6-mercaptopurine) |                        |         |
| No                                               | 1                      |         |
| Yes                                              | 1.29 (0.48–3.45)       | 0.61    |
| Corticosteroids                                  |                        |         |
| No                                               | 1                      |         |
| Yes                                              | 0.65 (0.23–1.79)       | 0.40    |
| Anti-TNF-α antibody therapy                      |                        |         |
| No                                               | 1                      |         |
| Yes                                              | 2.21 (0.77–6.37)       | 0.14    |
| Calcineurin inhibitor therapy                    |                        |         |
| No                                               | 1                      |         |
| Yes                                              | 1.70 (0.66–4.43)       | 0.27    |
| Albumin (continuous)                             | 0.38 (0.11–1.28)       | 0.12    |
| CRP (continuous)                                 | 1.23 (1.02–1.48)       | 0.03    |
| NLR (continuous)                                 | 1.14 (1.01–1.28)       | 0.03    |
| Neutrophil (continuous, per 1000 /μL)            | 1.24 (0.96–1.60)       | 0.01    |
| Lymphocyte (continuous, per 1000 /μL)            | 0.39 (0.17–0.89)       | 0.02    |

HR: hazard ratio; CI: confidential interval; IPAA: ileal pouch-anal anastomosis; UC: ulcerative colitis; TNF: tumor necrosis factor; CRP: C-reactive protein; NLR: neutrophil-to-lymphocyte ratio.

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The mechanism by which the NLR predicts the development of pouchitis is unclear. Regarding neutrophils count, neutrophils are important leukocytes that can cause inflammation in UC [35]. Neutrophil accumulation and abscess formation within the intestinal crypts at the apical epithelial surface are typical pathological features of UC [36]. Patients with high peripheral neutrophil count may be prone to pouchitis. Indeed, patients who had higher neutrophil counts tended to develop pouchitis in this study, in line with a previous report by Koike et al. that identified a neutrophil percentage of > 65% before IPAA surgery as a risk factor for pouchitis [13]. Regarding lymphocyte count, Hirata et al. reported significantly increased numbers of CD19<sup>+</sup>CD138<sup>+</sup> cells in the pouchitis mucosa of patients with UC compared to non-inflamed UC pouches. The proliferation of this cell population suggests the possibility of involvement of a UC-derived abnormality in the pathogenesis of pouchitis [37]. The number of CD19<sup>+</sup>CD20<sup>-</sup>CD138<sup>+</sup> cells, which are an immature subset of IgG-producing plasma cells, was also significantly higher in the peripheral blood and inflamed colon of patients with active UC [38–40]. This cell subset was reported to have a feature of plasma cells; eccentrically located nuclei and abundant rough endoplasmic reticula, by immunoelectron microscopy [39]. Therefore, this cell population could be identified as a highly fluorescent population distinct from lymphocytes by a hematology analyzer, implying that UC patients with abundant CD19<sup>+</sup>CD138<sup>+</sup> cells would have a relatively low percentage of lymphocytes in peripheral blood. This might be one of the possible mechanisms to explain how the NLR could predict the development of pouchitis.

Fig 2. Receiver operating characteristic curve for determining the cutoff value of the neutrophil-to-lymphocyte ratio (NLR) for predicting the development of pouchitis. The optimal cutoff value for the NLR determined by maximal Youden's index was 2.15 (specificity: 67.7%, sensitivity: 72.2%). Area under curve (AUC): 0.68 (95% confidence interval [CI]: 0.52–0.84).

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Table 3. Comparison between the low NLR group and high NLR group.

|                               | Low NLR group | High NLR group | P-value |
|-------------------------------|---------------|----------------|---------|
| Number of patients            | 26            | 23             |         |
| Sex: male/female              | 12 / 14       | 13 / 10        | 0.57    |
| Age at diagnosis (years)      | 36.4 (25.5–49.5) | 29.5 (21.5–43.8) | 0.12    |
| Age at IPAA (years)           | 47.5 (38.6–55.6) | 35.9 (25.0–47.7) | 0.04    |
| Disease duration (years)      | 4.2 (1.7–11.4) | 4.5 (3.1–9.5)   | 0.87    |
| UC location: Left-sided colitis/Pancolitis | 4 / 22 | 5 / 18 | 0.72    |
| Concomitant therapies, n (%)  |               |                |         |
| Immunomodulator               | 6 (23.1%)     | 8 (34.8%)      | 0.53    |
| Corticosteroids               | 7 (26.9%)     | 8 (34.8%)      | 0.76    |
| Anti-TNF-α antibody therapy   | 6 (23.1%)     | 9 (34.8%)      | 0.53    |
| Calcineurin inhibitor therapy | 8 (30.8%)     | 8 (34.8%)      | 1       |
| Hemoglobin (g/dL)             | 12.7 (11.8–13.5) | 12.9 (12.1–14.4) | 0.50    |
| Albumin (g/dL)                | 4.10 (4.00–4.50) | 4.20 (3.90–4.40) | 0.86    |
| CRP (mg/dL)                   | 0.06 (0.04–0.24) | 0.07 (0.04–0.19) | 0.86    |
| WBC (/μL)                     | 5900 (4900–6800) | 5700 (5200–7700) | 0.44    |
| Neutrophil (/μL)              | 5200 (4800–5600) | 4200 (3500–5000) | < 0.01  |
| Lymphocyte (/μL)              | 2000 (1700–2400) | 1400 (1100–1700) | < 0.01  |
| NLR, median (interquartile range) | 1.47 (1.31–1.72) | 2.93 (2.46–4.15) | < 0.01  |
| Pouchitis (+), n (%)          | 5 (19.2%)     | 13 (56.5%)     | < 0.01  |

IPAA: ileal pouch-anal anastomosis; UC: ulcerative colitis; TNF: tumor necrosis factor; CRP: C-reactive protein; WBC: white blood cell; NLR: neutrophil-to-lymphocyte ratio.

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Fig 3. Comparison of cumulative incidence of pouchitis between the high and low NLR groups. The incidence of pouchitis was significantly lower in the low NLR group than in the high NLR group (P = 0.01, log-rank test).

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Another possible mechanism is that peripheral WBCs might affect the gut microbiome. The role of the gut microbiome in pouchitis development is indicated by the effectiveness of antibiotics in the treatment of pouchitis, the use of probiotics for its prevention [41, 42], and the influence of innate lymphoid cells on the microbiome [43]. Taken together, patients with higher NLR might consist of a unique subset whose microbiome predisposes them to pouchitis.

Johnson et al. have reported that fecal calprotectin correlates with the severity of pouchitis and is a useful marker for diagnosing pouchitis [44]. Measuring fecal calprotectin is non-invasive, relatively cheap, and its sensitivity and specificity are very high. Although the fecal calprotectin test is a potentially useful screening tool for estimating the presence and severity of pouch inflammation, no evidence has been reported for its predictive role in inflammation. Regarding NLR, the NLR has been found to predict pouchitis before the operation.

Regarding medications before the operation, only a total steroid dose of >10000 mg [13] and the use of infliximab [14] was reported to be a risk factor for pouchitis, to the best of our knowledge. In this study, although the use of immunomodulators, corticosteroids, anti-TNF-α antibody therapy, and calcineurin inhibitor therapy was not identified a risk factor for pouchitis, we could not say that these medications were not associated with the development of pouchitis because of the relatively small sample size and several biases such as drug discontinuation during the course.

As the sample size of this study was relatively small, we calculated the required sample size. Sample size calculation as a post-hoc analysis indicated that a total of 38 patients were required to detect a significant association between high NHR and the development of pouchitis with the following assumptions: an α level of 0.05, a β level of 0.20, half of the patients were allocated to the high NLR group, the incidences of pouchitis in patients with high and low NLR group were 54% and 19%, registration period was 13 years, and the follow-up period was 1.5 years. Therefore, the sample size in this study was satisfied to examine the association between NLR and the development of pouchitis.

This study has some limitations. First, this was a retrospective study with a relatively small cohort that is susceptible to bias in data selection and analysis. Second, we could not evaluate the predictive value of preoperative NLR since differential WBC count is not routinely measured in all patients just before the operation. Only 39 of 49 patients included in this study had their differential white blood cell count evaluated before the operation. Among these 40 patients, univariate Cox regression analysis did not identify NLR as a predictor of pouchitis development (HR, 0.98; 95% CI, 0.93–1.04; P = 0.56). A possible reason why preoperative NLR did not predict the development of pouchitis is that preoperative NLR would be affected by infection or treatment and, therefore, would not accurately reflect the situation. The other possible reason is that the number of patients analyzed for preoperative NLR was statistically too small. Therefore, we may not be able to conclude that the preoperative NLR is meaningless for pouchitis. Third, we were unable to evaluate the reported pouchitis predictive factors, such as extraintestinal manifestations of inflammatory bowel disease, preoperative terminal ileal inflammation, the presence of interleukin-1 receptor antagonist gene allele 2, total steroid dose of >10000 mg, or presence of perinuclear antineutrophil cytoplasmic antibodies owing to the retrospective design of the study.

Furthermore, NLR can be influenced by concurrent infections and concomitant drugs. Therefore, further large prospective studies will help confirm the NLR as a key predictor for pouchitis after IPAA in patients with UC.

Despite these limitations, our study suggests that the NLR could be associated with the development of pouchitis after IPAA in patients with UC. NLR should, therefore, be introduced in clinical practice.
Supporting information

S1 Dataset. Final analysis data of the study subjects. (CSV)

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References

1. Fazio VW, Ziv Y, Church JM, Oakley JR, Lavery IC, Millsom JW, et al. Ileal pouch-anal anastomoses complications and function in 1005 patients. Annals of surgery. 1995; 222(2):120–7. Epub 1995/08/01. https://doi.org/10.1097/00000658-199508000-00003 PMID: 7639579; PubMed Central PMCID: PMC1234769.

2. Penna C, Dozois R, Tremaine W, Sandborn W, LaRusso N, Schleck C, et al. Pouchitis after ileal pouch-anal anastomosis for ulcerative colitis occurs with increased frequency in patients with associated primary sclerosing cholangitis. Gut. 1996; 38(2):234–9. Epub 1996/02/01. https://doi.org/10.1136/gut.38.2.234 PMID: 8801203; PubMed Central PMCID: PMC1383029.

3. Hurst RD, Molinari M, Chung TP, Rubin M, Michelassi F. Prospective study of the incidence, timing and treatment of pouchitis in 104 consecutive patients after restorative proctocolectomy. Archives of surgery (Chicago, Ill: 1960). 1996; 131(5):497–500; discussion 1–2. Epub 1996/05/01. https://doi.org/10.1001/archsurg.1996.01430170043007 PMID: 8624195.

4. Stahlberg D, Gullberg K, Liljeqvist L, Hellers G, Lofberg R. Pouchitis following pelvic pouch operation for ulcerative colitis. Incidence, cumulative risk, and risk factors. Diseases of the colon and rectum. 1996; 39(9):1012–8. Epub 1996/09/01. https://doi.org/10.1007/BF02054692 PMID: 8797652.

5. Shen B, Fazio VW, Remzi FH, Delaney CP, Bennett AE, Achkar JP, et al. Comprehensive evaluation of inflammatory and noninflammatory sequelae of ileal pouch-anal anastomoses. The American journal of gastroenterology. 2005; 100(1):93–101. Epub 2005/01/19. https://doi.org/10.1111/j.1572-0241.2005.40778.x PMID: 15654787.

6. Coffey JC, Winter DC, Neary P, Murphy A, Redmond HP, Kirwan WO. Quality of life after ileal pouch-anal anastomosis: an evaluation of diet and other factors using the Cleveland Global Quality of Life instrument. Diseases of the colon and rectum. 2002; 45(1):30–8. Epub 2002/01/12. PMID: 11786761.

7. Mahadevan U, Sandborn WJ. Diagnosis and management of pouchitis. Gastroenterology, 2003; 124(6):1636–50. Epub 2003/05/23. https://doi.org/10.1016/s0016-5085(03)00325-1 PMID: 12761722.

8. Lohmuller JL, Pemberton JH, Dozois RR, Listrup D, van Heerden J. Pouchitis and extraintestinal manifestations of inflammatory bowel disease after ileal pouch-anal anastomosis. Annals of surgery. 1990; 211(5):622–7; discussion 7–9. Epub 1990/05/01. PMID: 2339922; PubMed Central PMCID: PMC1358238.

9. Aisenberg J, Wagreich J, Shim J, Almer S, Peen E, Heimann T, et al. Perinuclear anti-neutrophil cytoplasmic antibody and refractory pouchitis. A case-control study. Digestive diseases and sciences. 1995; 40(9):1866–72. Epub 1995/09/01. https://doi.org/10.1007/BF02208648 PMID: 7555435.
10. Schmidt CM, Lazenby AJ, Hendrickson RJ, Sitzmann JV. Preoperative terminal ileal and colonic resection histopathology predicts risk of pouchitis in patients after ileoanal pull-through procedure. Annals of surgery. 1998; 227(5):654–62; discussion 63–5. Epub 1998/05/30. https://doi.org/10.1097/00000658-199805000-00006 PMID: 9605657; PubMed Central PMCID: PMC1191341.

11. Luukkonen P, Jarvinen H, Tankanen M, Kahri A. Pouchitis—recurrence of the inflammatory bowel disease? Gut. 1994; 35(2):243–6. Epub 1994/02/01. https://doi.org/10.1136/gut.35.2.243 PMID: 8307476; PubMed Central PMCID: PMC1374501.

12. Carter MJ, Di Giovenile FS, Cox A, Goodfellow P, Jones S, Shorthouse AJ, et al. The interleukin 1 receptor antagonist gene allele 2 as a predictor of pouchitis following colectomy and IPAA in ulcerative colitis. Gastroenterology. 2001; 121(4):805–11. Epub 2001/10/19. https://doi.org/10.1053/gast.2001.28017 PMID: 11606494.

13. Koike Y, Uchida K, Inoue M, Matsushita K, Okita Y, Toiyama Y, et al. Predictors for Pouchitis After Ileal Pouch-Anal Anastomosis for Pediatric-Onset Ulcerative Colitis. The Journal of surgical research. 2019; 238:72–8. Epub 2019/02/12. https://doi.org/10.1016/j.jss.2019.01.022 PMID: 30743239.

14. Mor IJ, Vogel JD, da Luz Moreira A, Shen B, Hammel J, Remzi FH. Infliximab in ulcerative colitis is associated with an increased risk of postoperative complications after restorative proctocolectomy. Diseases of the colon and rectum. 2008; 51(8):1202–7; discussion 7–10. Epub 2008/06/10. https://doi.org/10.1007/s10350-008-9364-7 PMID: 18536964.

15. Sandborn WJ, Landers CJ, Tremaine WJ, Targan SR. Antineutrophil cytoplasmic antibody correlates with chronic pouchitis after ileal pouch-anal anastomosis. The American journal of gastroenterology. 1995; 90(5):740–7. Epub 1995/05/01. PMID: 7739090.

16. Fleshner PR, Vasiliauskas EA, Kam LY, Fleshner NE, Gaiennie J, Abreu-Martín MT, et al. High level perinuclear antineutrophil cytoplasmic antibody (pANCA) in ulcerative colitis patients before colectomy predicts the development of chronic pouchitis after ileal pouch-anal anastomosis. Gut. 2001; 49(5):671–7. Epub 2001/10/16. https://doi.org/10.1136/gut.49.5.671 PMID: 11600470; PubMed Central PMCID: PMC1728523.

17. Shibutani M, Maeda K, Nagahara H, Noda E, Ohtani H, Nishiguchi Y, et al. A high preoperative neutrophil-to-lymphocyte ratio is associated with poor survival in patients with colorectal cancer. Anticancer research. 2013; 33(8):3291–4. Epub 2013/07/31. PMID: 23898094.

18. Shimada H, Takiguchi N, Kainuma O, Soda H, Ikeda A, Cho A, et al. High preoperative neutrophil-lymphocyte ratio predicts poor survival in patients with gastric cancer. Gastric cancer: official journal of the International Gastric Cancer Association and the Japanese Gastric Cancer Association. 2010; 13(3):170–6. Epub 2010/09/08. https://doi.org/10.1007/s10120-010-0554-3 PMID: 20820986.

19. Mahmoud FA, Rivera NI. The role of C-reactive protein as a prognostic indicator in advanced cancer. Current oncology reports. 2002; 4(3):250–5. Epub 2002/04/09. https://doi.org/10.1007/s11912-002-0023-1 PMID: 11937016.

20. Danese S, Rudzinski J, Brandt W, Dupas JL, Peyrin-Biroulet L, Bouhnik Y, et al. Tralokinumab for moderate-to-severe UC: a randomised, double-blind, placebo-controlled, phase IIa study. Gut. 2015; 64(2):243–9. Epub 2014/10/12. https://doi.org/10.1136/gutjnl-2014-308004 PMID: 25304132.

21. Chandrasekara S, Rajendran A, Bai Jaganath A, Krishnamurthy R. Neutrophil-lymphocyte ratio, pain perception, and disease activity score may serve as important predictive markers for sustained remission in rheumatoid arthritis. Reumatismo. 2015; 67(3):109–15. Epub 2016/02/16. https://doi.org/10.4081/reumatismo.2015.038 PMID: 26876190.

22. Lagunas-Rangel FA. Neutrophil-to-lymphocyte ratio and lymphocyte-to-C-reactive protein ratio in patients with severe coronavirus disease 2019 (COVID-19): A meta-analysis. Journal of medical virology. 2020. Epub 2020/04/04. https://doi.org/10.1002/jmv.25819 PMID: 32242950.

23. Cho KH, Jeong MH, Ahmed K, Hachinohe D, Choi HS, Chang SY, et al. Value of early risk stratification using hemoglobin level and neutrophil-to-lymphocyte ratio in patients with ST-elevation myocardial infarction undergoing primary percutaneous coronary intervention. The American journal of cardiology. 2011; 107(6):849–56. Epub 2011/01/21. https://doi.org/10.1016/j.amjcard.2010.10.067 PMID: 21247535.

24. Nunez J, Nunez E, Bodi V, Sanchis J, Minana G, Mainar L, et al. Usefulness of the neutrophil to lymphocyte ratio in predicting long-term mortality in ST segment elevation myocardial infarction. The American journal of cardiology. 2008; 101(6):747–52. Epub 2008/03/11. https://doi.org/10.1016/j.amjcard.2007.11.004 PMID: 18328833.

25. Demir AK, Demirtas A, Kaya SU, Tastan I, Butun I, Sagcan M, et al. The relationship between the neutrophil-lymphocyte ratio and disease activity in patients with ulcerative colitis. The Kaohsiung journal of medical sciences. 2015; 31(11):585–90. Epub 2015/12/19. https://doi.org/10.1016/j.kjms.2015.10.001 PMID: 26678939.
26. Torun S, Tunc BD, Suvak B, Yildiz H, Tay S, Sayiliar A, et al. Assessment of neutrophil-lymphocyte ratio in ulcerative colitis: a promising marker in predicting disease severity. Clinics and research in hepatology and gastroenterology. 2012; 36(5):491–7. Epub 2012/07/31. https://doi.org/10.1016/j.clinre.2012.06.004 PMID: 22841412.

27. Nishida Y, Hosomi S, Yamagami H, Yukawa T, Otani K, Nagami Y, et al. Neutrophil-to-Lymphocyte Ratio for Predicting Loss of Response to Infliximab in Ulcerative Colitis. PloS one. 2017; 12(1):e0169845. Epub 2017/01/12. https://doi.org/10.1371/journal.pone.0169845 PMID: 28076386; PubMed Central PMCID: PMCP5226844 faculty members of a course sponsored by Eisai Co., Ltd. The other authors declare that they have no conflicts of interest, and that there were no external funding sources for this study. This does not alter the authors’ adherence to PLOS ONE policies on sharing data and materials.

28. Nishida Y, Hosomi S, Yamagami H, Sugita N, Itani S, Yukawa T, et al. Pretreatment neutrophil-to-lymphocyte ratio predicts clinical relapse of ulcerative colitis after tacrolimus induction. PloS one. 2019; 14(3):e0213505. Epub 2019/03/08. https://doi.org/10.1371/journal.pone.0213505 PMID: 30845259; PubMed Central PMCID: PMC6405082 were previously faculty members of a course sponsored by Eisai Co., Ltd, up to last year. They are now no longer faculty members of this course. This does not alter our adherence to PLOS ONE policies on sharing data and materials.

29. Bertani L, Rossari F, Barberio B, Demarzo MG, Tapete G, Albano E, et al. Novel Prognostic Biomarkers of Mucosal Healing in Ulcerative Colitis Patients Treated With Anti-TNF: Neutrophil-to-Lymphocyte Ratio and Platelet-to-Lymphocyte Ratio. Inflammatory bowel diseases. 2020. Epub 2020/04/02. https://doi.org/10.1093/ibd/izaa062 PMID: 32323392.

30. Uchida K, Araki T, Kusunoki M. History of and current issues affecting surgery for pediatric ulcerative colitis. Surgery today. 2013; 43(11):1219–31. Epub 2012/12/04. https://doi.org/10.1007/s00595-012-0434-z PMID: 23203770.

31. Sandborn WJ, Tremaine WJ, Batts KP, Pemberton JH, Phillips SF. Pouchitis after ileal pouch-anal anastomosis: a Pouchitis Disease Activity Index. Mayo Clinic proceedings. 1994; 69(5):409–15. Epub 1994/05/01. https://doi.org/10.1016/s0025-6196(12)61634-6 PMID: 8170189.

32. Shen B, Achkar JP, Connor JT, Ormsby AH, Remzi FH, Bevins CL, et al. Modified pouchitis disease activity index: a simplified approach to the diagnosis of pouchitis. Diseases of the colon and rectum. 2003; 46(6):748–53. Epub 2003/06/10. https://doi.org/10.1016/s0090-1229(03)00476-x PMID: 12794576.

33. Hilden J, Glasziou P. Regret graphs, diagnostic uncertainty and Youden’s Index. Statistics in medicine. 1996; 15(10):969–86. Epub 1996/05/30. https://doi.org/10.1002/(SICI)1097-0258(19960530)15:10<969::AID-SIM211>3.0.CO;2-9 PMID: 8783436.

34. Sugihara M. Survival analysis using inverse probability of treatment weighted methods based on the generalized propensity score. Pharmaceutical statistics. 2010; 9(1):21–34. Epub 2009/02/10. https://doi.org/10.1002/pst.365 PMID: 19199275.

35. Hermanowicz A, Gibson PR, Jewell DP. The role of phagocytes in inflammatory bowel disease. Clinical science (London, England: 1979). 1985; 69(3):241–9. Epub 1985/09/01. https://doi.org/10.1042/ cs0690241 PMID: 3905214.

36. Roche JK, Watkins MH, Cook SL. Inflammatory bowel disease: prevalence and level of activation of circulating T-lymphocyte subpopulations mediating suppressor/cytotoxic and helper function as defined by monoclonal antibodies. Clinical immunology and immunopathology. 1982; 25(3):362–73. Epub 1982/12/01. https://doi.org/10.1016/0090-1229(82)90201-x PMID: 6218846.

37. Hirata N, Oshintani N, Kamata N, Sogawa M, Yamagami H, Watanabe K, et al. Proliferation of immature plasma cells in pouchitis mucosa in patients with ulcerative colitis. Inflammatory bowel diseases. 2008; 14(8):1084–90. Epub 2008/05/03. https://doi.org/10.1016/j.ibd.200447 PMID: 18452202.

38. Hosomi S, Oshintani N, Kamata N, Sogawa M, Okazaki H, Tanigawa T, et al. Increased numbers of immature plasma cells in peripheral blood specifically overexpress chemokine receptor CXCR3 and CXCR4 in patients with ulcerative colitis. Clinical and experimental immunology. 2011; 163(2):215–24. Epub 2010/11/23. https://doi.org/10.1111/j.1365-2499.2010.04290.x PMID: 21067446; PubMed Central PMCID: PMCP3043312.

39. Jinno Y, Ohtani H, Nakamura S, Oki M, Maeda K, Fukushima K, et al. Infiltration of CD19+ plasma cells with frequent labeling of Ki-67 in corticosteroid-resistant active ulcerative colitis. Virchows Archiv; an international journal of pathology. 2006; 448(4):412–21. Epub 2006/01/26. https://doi.org/10.1007/s00428-005-0136-7 PMID: 16435133.

40. Uo M, Hisamatsu T, Miyoshi J, Kaito D, Yoneno K, Kitazume MT, et al. Mucosal CXCR4+ IgG plasma cells contribute to the pathogenesis of human ulcerative colitis through FcγR-mediated CD14 macrophage activation. Gut. 2013; 62(12):1734–44. Epub 2012/09/28. https://doi.org/10.1136/gutjnl-2012-303063 PMID: 23013725.
41. Isaacs KL, Sandler RS, Abreu M, Picco MF, Hanauer SB, Bickston SJ, et al. Rifaximin for the treatment of active pouchitis: a randomized, double-blind, placebo-controlled pilot study. Inflammatory bowel diseases. 2007; 13(10):1250–5. Epub 2007/06/15. https://doi.org/10.1002/ibd.20187 PMID: 17567869.

42. Gionchetti P, Rizzello F, Helwig U, Venturi A, Lammers KM, Brigid P, et al. Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. Gastroenterology. 2003; 124(5):1202–9. Epub 2003/05/06. https://doi.org/10.1016/s0016-5085(03)00171-9 PMID: 12730861.

43. Thaiss CA, Zmora N, Levy M, Elinav E. The microbiome and innate immunity. Nature. 2016; 535(7610):65–74. Epub 2016/07/08. https://doi.org/10.1038/nature18847 PMID: 27383981.

44. Johnson MW, Maestranzi S, Duffy AM, Dewar DH, Forbes A, Bjarnason I, et al. Faecal calprotectin: a noninvasive diagnostic tool and marker of severity in pouchitis. European journal of gastroenterology & hepatology. 2008; 20(3):174–9. Epub 2008/02/28. https://doi.org/10.1097/MEG.0b013e3282f1c9a7 PMID: 18301296.