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Serum Periostin is Able to Stratify Type 2-Dominant Ulcerative Colitis

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Background: Ulcerative colitis (UC) is a heterogeneous disease composed of different endotypes. It is important to develop useful biomarkers for endotyping UC; however, available biomarkers are insufficient. We have already established that periostin is a surrogate biomarker of type 2 inflammation. In this study, we examined the usefulness of periostin as a biomarker of UC and the role of periostin in its pathogenesis.

Methods: We examined periostin expression in the colons of UC patients. We next investigated serum periostin in UC patients and its correlation with eosinophilic infiltration in their colons. We then examined whether serum periostin could predict the efficacy of oral prednisolone. Finally, we investigated the role of periostin in UC pathogenesis by creating its genetic deficiency using dextran sulfate sodium (DSS)-treated mice.

Results: Periostin expression and serum periostin were significantly high in UC patients compared to healthy controls; however, both were diverse, showing heterogeneity of the underlying mechanism of UC. Both serum periostin and tissue periostin expression, but not blood eosinophils, were significantly associated with eosinophil infiltration. Type 2-dominant UC patients as defined by serum periostin showed significantly higher clinical remission rates for the treatment with oral prednisolone. Genetic deficiency in periostin improved colonic inflammation in a DSS-treated mouse model.

Conclusions: Periostin can be a useful biomarker to stratify type 2-dominant UC patients, thereby predicting the efficacy of oral prednisolone. Moreover, periostin plays an important role in the setting of type 2-dominant UC.

Lay Summary

In this study, we demonstrated that serum periostin can be a useful biomarker for stratifying type 2-dominant ulcerative colitis patients. We also showed that periostin plays an important role in the pathogenesis of chronic colitis model mice.

Key Words: biomarker, periostin, ulcerative colitis

Introduction

Ulcerative colitis (UC), one of the two major forms of inflammatory bowel disease, is a chronic inflammatory disorder of the colon characterized by repeated cycles of relapse and remission.[1,2] Ulcerative colitis shows diffuse and superficial inflammation of the mucosa and submucosa restricted to the colon, whereas Crohn's disease, the other major form, shows patchy and transmural inflammation that can occur anywhere in the alimentary tract.[1,2] The prevalence of UC worldwide has increased during the past two decades and is estimated to exceed 400 per 100 000 population in Western countries.[2] Several molecularly targeted drugs for UC have been developed that show good efficacy; however, remission rates do not surpass 20%-30% in clinical trials, and 30%-60% of patients in a real-life setting so that some patients still require surgery.[1] Therefore, it is important to develop novel agents for patients resistant against the present treatments for UC.

It is widely accepted that many factors—genetic predisposition, environmental factors, dysbiosis of intestinal microflora, and dysregulated immune responses—are involved in the setting of UC, although the precise mechanism still remains

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Key Messages

What is Already Known?

Ulcerative colitis (UC) is a heterogeneous disease with different cytokine profiles, and developing effective biomarkers for endotype-based stratification is crucial for selecting optimal treatment.

What is New Here?

Periostin would be a useful biomarker for stratifying type 2-dominant UC patients and predicting the efficacy of oral prednisolone.

How Can This Study Help Patient Care?

This study suggests that stratifying type 2-dominant UC patients with serum periostin is helpful for selecting optimal treatments.

unclear.[1,3,4] The pathogenesis of UC is multi-factorial, a mixture of NF-κB–related, type 1, type 2, and type 17 inflammations, based on the expression profiles of cytokines.[3,4] It is of note that it depends on the stage of UC and that UC patients have high heterogeneity.[5,6] Accordingly, several mouse models of colitis show various cytokine profiles.[3] This complexity and heterogeneity of UC pathogenesis make one-size-fits-all treatment difficult. Therefore, it is important to be able to stratify UC patients by endotypes in order to select a suitable treatment for each endotype.

To do so, the development of useful biomarkers is essential. Right now, many biomarkers—fecal calprotectin, fecal immunochemical test, C-reactive protein (CRP), leucine-rich α2-glycoprotein (LRG), and prostaglandin E-major urinary metabolite (PGE-MUM)—have been developed and are used in the management of UC patients.[7–9] Most of these biomarkers are aimed at estimating and monitoring the disease activity of UC. These biomarkers are noninvasive, frequently used, and have become essential tools for monitoring UC patients.[7] However, the biomarkers available now are not enough to stratify UC patients based on endotypes. Therefore, it is important to develop useful biomarkers to reflect UC endotypes.

Periostin is a 90-kD matricellular protein that acts by binding to its receptor, $\alpha_V\beta_3$ integrin, on target cells.[10] We found that IL-4 and IL-13, signature cytokines of type 2 inflammation, induce periostin production so that periostin can be a surrogate biomarker of type 2 inflammation. Actually, we and others have already demonstrated that periostin is a useful biomarker for allergic diseases in which type 2 inflammation is dominant.[11] Moreover, we have shown that periostin links type 2 inflammation as a downstream molecule of IL-4/IL-13 with NF-κB–related inflammation in the pathogenesis of atopic dermatitis, suggesting that periostin acts as an accelerator of type 2 inflammation.[12,13] It has been reported that periostin expression is higher in inflamed sites of UC patients than in healthy controls[14,15] and that genetic deficiency of periostin improves colitis in UC model mice.[15,16] However, neither the usefulness of periostin as a biomarker nor its role in the pathogenesis of UC has been firmly established.

In this study, we aimed to reveal the role of periostin in the pathogenesis of UC and to examine its usefulness as a biomarker for the treatment of UC patients.

Materials and Methods

Subjects

We collected surgical specimens from 6 UC patients and 3 patients with early-stage sigmoid colon cancer. Clinical backgrounds of the UC and control patients are shown in Table S1. We analyzed sigmoid colon surgical specimens without neoplasia from UC patients and normal surgical margin areas of patients with early-stage sigmoid colon cancer.

We enrolled 208 UC patients who visited our hospital from April 2022 to December 2023. The flow chart for patient inclusion and exclusion in the present study is shown in Figure 1. We excluded 97 patients having a history of intestinal surgery or other diseases that could affect serum periostin levels, such as asthma, interstitial pneumonia, atopic dermatitis, allergic conjunctivitis, allergic rhinitis, chronic sinusitis, or malignancy. In total, we measured serum periostin in 111 patients. Clinical backgrounds of the patients from whom serum was collected are shown in Table 1. According to the Montreal classification,[17] disease extent is classified into 3 types: E1 (proctitis: inflammation limited to the rectum), E2 (left-sided; distal: inflammation limited to the splenic flexure), and E3 (pancolitis: inflammation extends to the proximal splenic flexure). According to the Japanese evidence-based clinical practice guidelines for inflammatory bowel disease,[18] clinical courses are classified into 4 types according to treatment course: first attack type (only one attack, but there is a high possibility of relapse in the future), relapse-remitting type (symptoms worsen and improve repeatedly), chronic continuous type (symptoms continue for more than 6 months), acute fulminating type (extremely severe symptoms, and often involves complications such as toxic megacolon, perforation, and sepsis). In addition, we recruited 13 healthy volunteer controls (male 7 and female 6, age 22-58, median: 33.5), applying the above exclusion criteria.

We also analyzed endoscopic findings from 66 UC patients who underwent colonoscopy during the period. In addition, we investigated histological findings of the 54 of these 66 patients who underwent biopsy.

We further enrolled 14 patients who were newly treated with more than 25 mg/day of oral prednisolone for their active disease in order to investigate the usefulness of serum periostin in predicting the efficacy of oral prednisolone for ulcerative colitis. The clinical remission rate and the clinical response rate at 2 weeks after induction were 42.9% (6/14) and 85.7% (12/14), respectively. Clinical characteristics of the patients are shown in Table S2.

Histology and Immunohistochemistry

We prepared paraffin-embedded colonic tissue sections and performed Hematoxylin–eosin (H&E) staining and Masson trichrome staining for histological analysis. For periostin expression analysis, colonic sections were subjected to immunostaining using mouse anti-periostin monoclonal antibody (Ab, 1:500; SS19C) for human tissue or rabbit anti-periostin polyclonal Ab for mouse tissue, as we previously reported.[13]

Assessment of Histological Findings

We evaluated scores of inflammation by H&E staining, fibrosis by Masson trichrome staining, and periostin expression

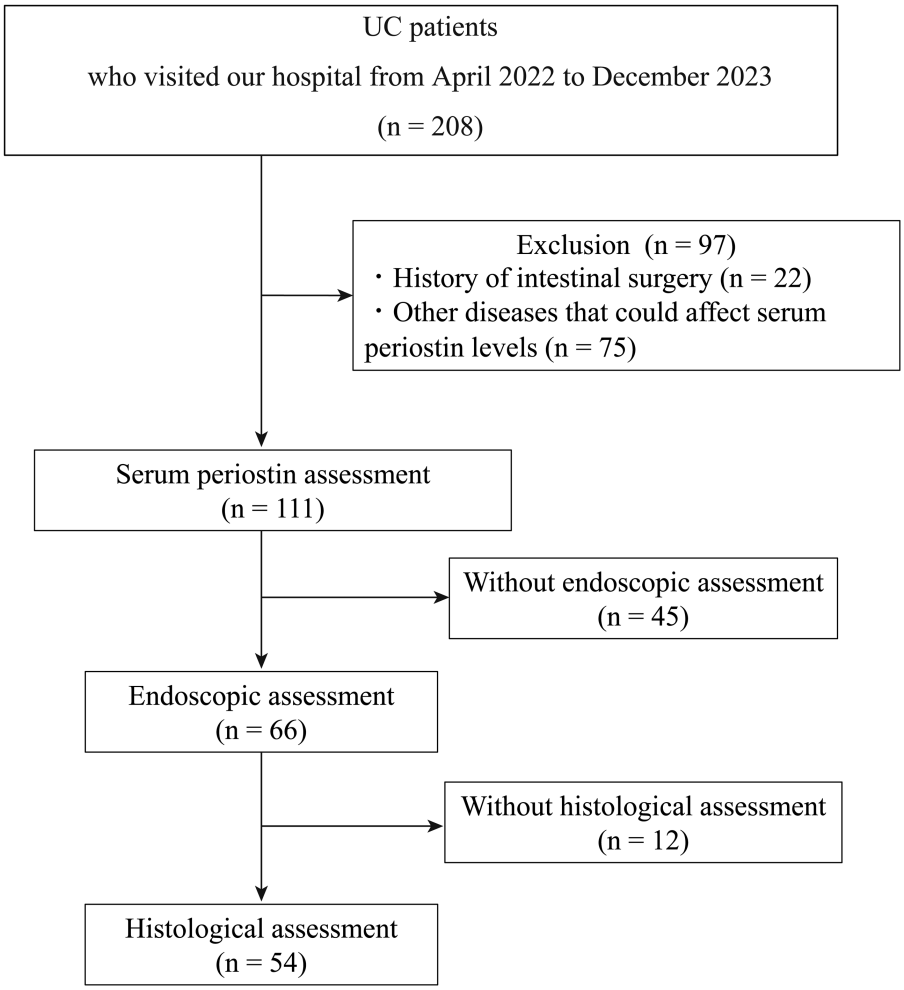

Figure 1. Flow chart for patient inclusion and exclusion in the present study. We enrolled 208 ulcerative colitis (UC) patients who visited our hospital from April 2022 to December 2023. We excluded 97 patients having a history of intestinal surgery or other diseases that could affect serum periostin levels, such as asthma, interstitial pneumonia, atopic dermatitis, allergic conjunctivitis, allergic rhinitis, chronic sinusitis, or malignancy. First, we evaluated the clinical characteristics and blood test data of 111 patients. Next, we assessed the endoscopic findings of 66 UC patients who underwent colonoscopy among these patients. In addition, we investigated the histological findings of 54 of these 66 patients who underwent biopsy.

by immunohistochemistry in surgical specimens into five grades (0: no findings, 1: trace, 2: mild, 3: moderate, 4: severe) by a certified pathologist.

We measured the number of eosinophils in 2 high-power fields (400×) with image-viewing software (NDP.view2, Hamamatsu Photonics, Shizuoka, Japan).

We quantitatively analyzed periostin expression using an image analysis platform (HALO version 2.3.2089.34; Indica Labs, Corrales, NM, USA). We annotated the edges of the entire tissue and automatically measured the area and the degree of expression (strong, moderate, and weak). We calculated the scores using the following formula; 3 × {strong area (%)} + 2 × {moderate area (%)} + {weak area (%)}.

Measurement of Serum Periostin

We obtained serum samples from subjects and then stored the samples at – 80 °C until measurement. We measured serum periostin using a periostin detection kit composed of 2 monoclonal anti-periostin Abs (SS17B and SS18B), as we previously reported.[11]

Assessment of Clinical, Endoscopic, and Histological Disease Activity of UC Patients

We evaluated the clinical disease activity of UC patients using a partial Mayo score calculated by the sum of each parameter: stool frequency (0: normal number for this patient, 1: 1-2 stools more than normal, 2: 3-4 stools more than normal, and 3: ≥5 stools more than normal), rectal bleeding (0: no blood seen, 1: streaks of blood with stool less than half the time, 2: obvious blood with stool most of the time, and 3: blood alone passes) and physician's global assessment (0: normal, 1: mild disease, 2: moderate disease, and 3: severe disease) with the range from 0 to 9.[1,2] Clinical remission was defined as 0 or 1 of partial Mayo score.

We assessed the efficacy of oral prednisolone therapy by the ratio of patients who achieved clinical remission (partial Mayo score < 2) and clinical response (decrease of ≥ 2 of partial Mayo score from baseline) at week 2 after induction.

We assessed the endoscopic activity of the UC patients according to the Mayo Endoscopic Subscore (MES).[1,2] Mayo Endoscopic Subscore is a 4-point scale (0-3), and we defined endoscopic remission as MES 0 or 1. All examinations were

Table 1. Clinical characteristics of the UC patients.

	All patients (*N* = 111)	Endoscopic group (*N* = 66)
Sex, male, *N* (%)	67 (60.4)	41 (62.1)
Age, median (IQR) (years)	51 (37-62)	51.5 (36.8-61.3)
Disease duration, median (IQR) (years)	9 (4-15)	8.5 (3-15)
Disease location, *N* (%) E1, E2, E3	7(6.3)/32 (28.8)/72 (64.9)	44 (66.7)/19 (28.8)/3 (4.5)
Clinical course, *N* (%) Relapse-remitting, chronic continuous, acute fulminating, first attack	80 (72.1)/8 (7.2)/0/23 (20.7)	53 (80.3)/4 (6.1)/0/9 (13.6)
Treatment		
5-Aminosalicycic acid, *N* (%)	92 (82.9)	52 (78.8)
Corticosteroids, *N* (%)	12 (10.8)	6 (9.1)
Immunomodulators, *N* (%)	23 (20.7)	16 (24.2)
Biologic agents, *N* (%)[a]	24 (21.6)	15 (22.7)
JAK inhibitors, *N* (%)[b]	12 (10.8)	8 (12.1)
Calcineurin inhibitors, *N* (%)	1 (0.9)	0
Partial Mayo score, *N* (%) 0/1/2/3/4-6/7-9	68 (61.3)/13 (11.7)/14 (12.6)/7 (6.3)/5 (4.5)/4 (3.6)	36 (54.5)/6 (9.1)/13 (19.7)/4 (6.1)/3 (4.5)/4 (6.1)
Mayo endoscopic subscore, *N* (%) 0/1/2/3		24 (36.4)/25 (37.9)/12 (18.2)/5 (7.6)
Nancy histological index, *N* (%)[c] 0/1/2/3/4		9 (16.7)/9 (16.7)/12 (22.2)/17 (31.5)/7 (12.9)

[a]Biologic agents included Infliximab (*n* = 6), Adalimumab (*n* = 9), Golimumab (*n* = 1), Ustekinumab (*n* = 3), Vedolizumab (*n* = 5).
[b]JAK inhibitors included Tofacitinib (*n* = 9), Filgotinib (*n* = 3).
[c]Histological examination was performed in 54 patients.

performed by expert endoscopists who were blinded to the results of serum biomarkers, including periostin.

A certified pathologist evaluated histological activity by the Nancy histological index (NHI).[2] Nancy histological index is a 5-point scale (0-4), and we defined histological remission as NHI 0 or 1. For cases with biopsies from multiple colonic sites, histological activity was assessed with the biopsy taken from the maximum endoscopic activity, while it was analyzed with the biopsy from the rectum in remission cases.

Dextran Sodium Sulfate (DSS)-Induced Colitis Model

We purchased C57BL/6 wild-type (WT) mice from Japan SLC (Hamamatsu, Japan) and prepared periostin-deficient (*Postn*$^{-/-}$) mice as previously described.[13] We used sex-matched mice aged 8-10 weeks in the experiments.

We generated chronic DSS-induced colitis mice by modifying the protocol of the previous report[19] as shown in Figure 6A. We fed mice 1% DSS (molecular weight: 36 000–50 000, MP Biomedicals) ad libitum for 7 days in the first cycle and 2% DSS for 7 days, both in the second and third cycles, separating each cycle by 14 days. Control mice received the same drinking water without DSS. The mice were weighed twice a week and visually inspected for diarrhea and rectal bleeding.

We calculated the disease activity index (DAI) by summing the following three parameters: weight loss (0 point: none, 1 point: 1%-5% weight loss, 2 point: 6%-10% weight loss, 3 point: 11%-18% weight loss, and 4 point: more than 18% weight loss), stool consistency/diarrhea (0 point: normal, 1 point: soft but still formed, 2 point: soft, 3 point: very soft, and 4 point: watery diarrhea), and bleeding (0 point: no bleeding, 2 point: slight bleeding, and 4 point: gross bleeding). The total DAI score ranged from 0 (unaffected) to 12 (most severe colitis).[19]

We calculated the histological activity score by summing the following two parameters: inflammatory cell infiltrate (0 point: normal, 1 point: mild inflammatory cell infiltration into mucosa, 2 point: moderate inflammatory cell infiltration into mucosa and submucosa, and 3 point: marked inflammatory cell infiltration into transmural) and intestinal architecture (0 point: normal, 1 point: focal erosion, 2 point: focal ulcerations, and 3 point: extensive ulcerations). The total histological activity score ranged from 0 (unaffected) to 6 (most severe colitis).[20]

Statistical Analysis

The data shown are the mean ± standard deviation (SD) or standard error of the mean (SEM). The statistical analyses were performed with Prism 9.0 software (GraphPad Software, La Jolla, CA, USA) using the paired and unpaired *t* test, Wilcoxon test, Tukey's multiple comparison test, Spearman correlation coefficient test, and chi-square test. *P* values less than .05 were considered to indicate statistically significant differences.

Results

Heterogeneity of Periostin Expression in the Colons of UC Patients

Several studies have reported that periostin is highly expressed in the colonic mucosa of UC patients.[14,15] We first examined its expression in the colons of 6 UC patients and 3 controls who had undergone colectomy as shown in Table S1. All UC patients showed the E3 (pancolitis type) and underwent total colectomy due to medical resistance (*n* = 2) or development of UC-associated neoplasia (UCAN, *n* = 4). We evaluated the degree and the area of inflammation (Figure 2A), fibrosis (Figure 2B), and periostin expression (Figure 2C), respectively, and calculated these scores (Figure 2D and E, Table S1). Periostin was overall highly expressed in the colons of UC patients and high expression of periostin was observed in the lamina propria of the subepithelium in the UC patients, as previously reported.[14,15] However, expression levels of periostin were diverse, and half of the patients (*n* = 3) showed only score 2, comparable to those of control specimens, although the scores of periostin expression were statistically higher in the UC patients than in the control patients. The same was true of inflammation and fibrosis. These results suggest that the underlying inflammation of UC is heterogeneous, so expression levels of periostin in UC patients would be diverse. Moreover, the 3 parameters—inflammation, fibrosis, and periostin expression—were not correlated (data not shown). Even between the medically refractory patients and the UCAN patients, the former of which was higher in the preoperative endoscopic

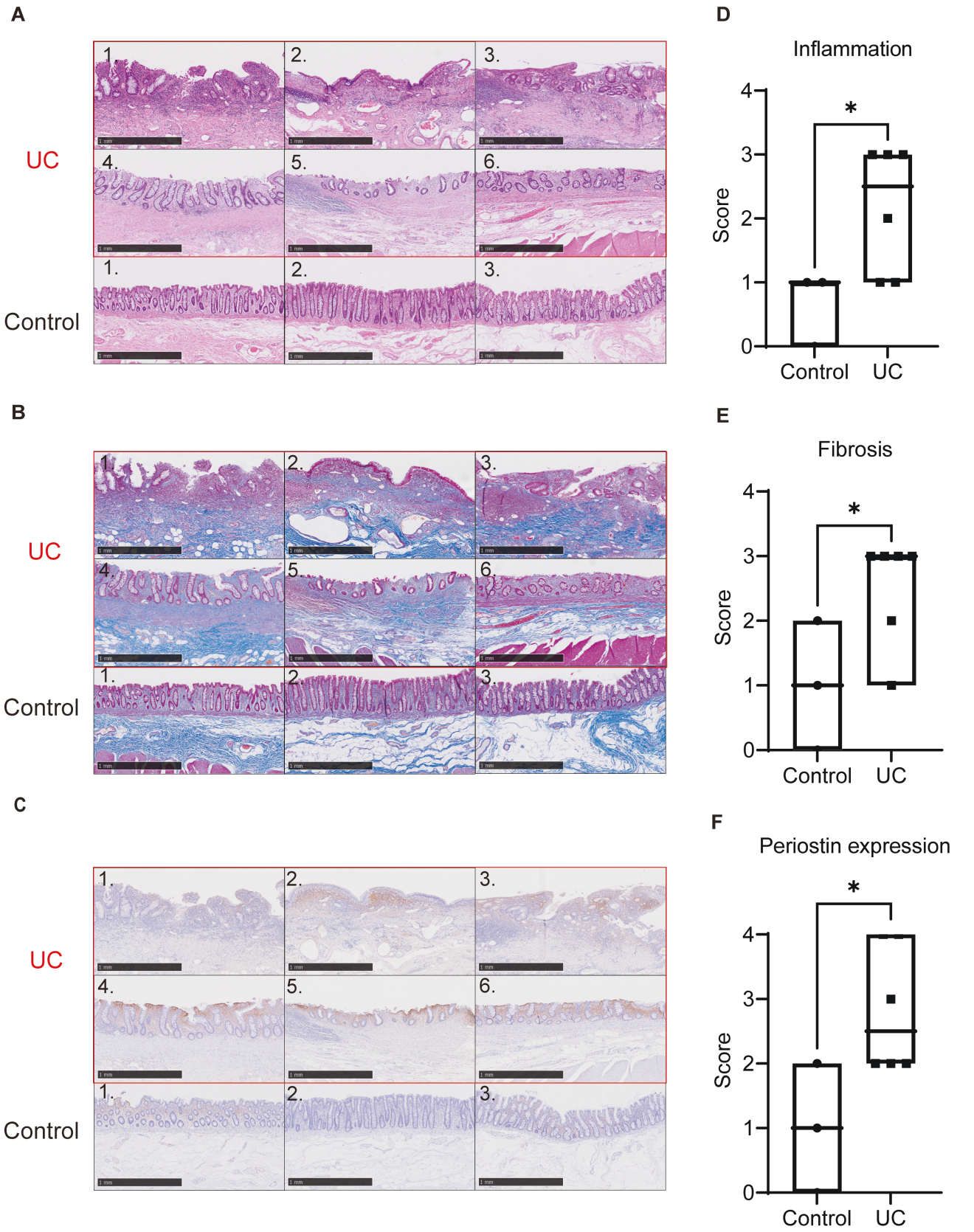

Figure 2. Heterogeneity of periostin expression in the colons of ulcerative colitis (UC) patients. A–C, H&E staining (A), Masson trichrome staining (B), and immunostaining of periostin (C) of surgical specimens of UC patients ($n = 6$) and control subjects ($n = 3$). D–F, Scores of the degree of inflammation (D), fibrosis (E), and periostin expression (F). The data are shown in boxplots, with minimum, maximum, and median. Statistical analysis was performed using the Wilcoxon test. *$P < .05$. Scale bar: 1 mm.

activity score, the tissue inflammation score, and the fibrosis score, the periostin expression score was comparable (data not shown). These results suggest that periostin expression does not simply reflect the degree of inflammation nor of fibrosis, probably because many factors other than type 2 inflammation can contribute to the inflammation and fibrosis in UC patients.

Heterogeneity of Serum Periostin in UC Patients

We next examined whether serum periostin reflected topical periostin levels in the inflamed sites of UC patients, as shown in Table 1. Serum periostin was significantly higher in the UC patients than in healthy donors (91.7 ± 34.1 vs. 72.7 ± 10.3 ng/mL, $P = 0.048$) (Figure 3A). However, serum periostin levels in the UC patients were diverse; 31.5% showed below 72.7 ng/mL, the mean value of the healthy donors. We then examined the association between serum periostin and clinical characteristics—age, disease duration, body mass index, disease course, disease location, disease activity, white blood cell, eosinophil, CRP, hemoglobin, albumin, and LRG. However, no significant association was

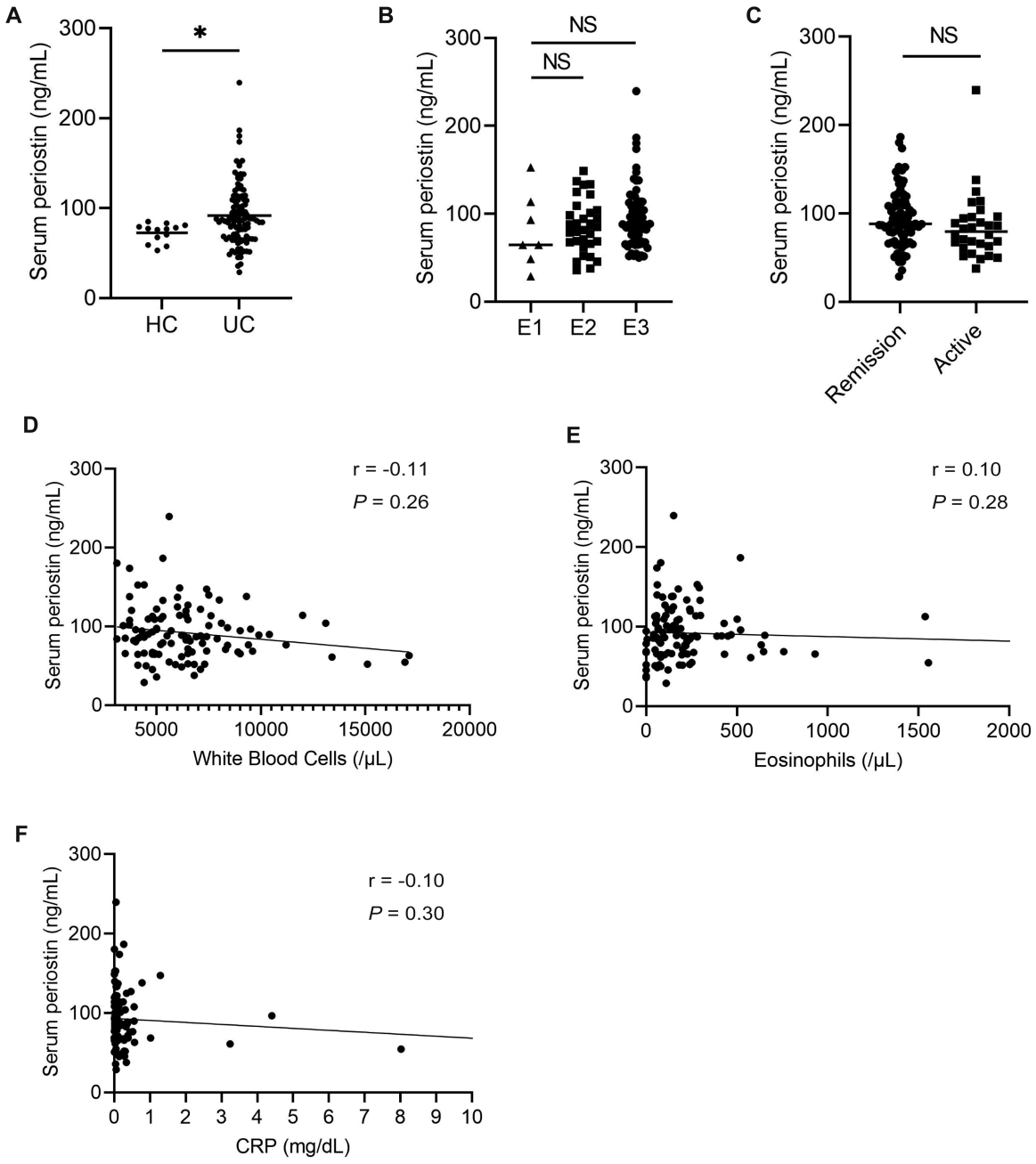

Figure 3. Heterogeneity of serum periostin levels in ulcerative colitis (UC) patients. A, Serum periostin in UC patients ($n = 111$) and in healthy controls (HC, $n = 13$). B, Serum periostin in UC patients with E1 (proctitis type, $n = 7$), E2 (left-sided colitis type, $n = 32$), or E3 (pancolitis type, $n = 72$) is shown. C, Serum periostin in clinical remission (partial Mayo score < 2, $n = 81$) and in active (partial Mayo score ≥ 2, $n = 30$) stages. D–F, Scatter plots show the correlation of serum periostin with the number of white blood cells (D), blood eosinophils (E), and serum C-reactive protein (F). Statistical analysis was performed using an unpaired *t* test for 2 groups comparision, The Tukey's multiple comparison test, or the Spearman correlation coefficient test. *$P < .05$. Abbreviation: NS, not significant.

found (Figure 3B–F, Figure S1A–H). Similarly, no significant difference in the serum periostin level was found based on the types of medical treatments (Figure S1I). These results, again, suggest that the underlying inflammation of UC is heterogeneous and that many factors other than type 2 inflammation could generate clinical characteristics.

High Potency of Serum Periostin to Stratify Type 2-Dominant UC

We have already established that periostin is a surrogate biomarker of type 2 inflammation.[11,21] Given that the analyses of periostin expression in the colons and serum periostin in UC patients suggested heterogeneity of the underlying inflammation of UC, we explored the possibility that serum periostin could stratify "type 2-dominant" from the whole group of UC patients. It is generally considered that eosinophil infiltration in the colon is a hallmark of type 2-dominant UC.[22,23] Therefore, we next compared eosinophilic infiltration and serum periostin in UC patients who had undergone endoscopy. The endoscopic activity is shown in Table 1.

There was no association between serum periostin and endoscopic activities (Figure 4A, Figure S2A) or histological activities (Figure 4B, Figure S2B), suggesting again that clinical or histological activity would be regulated by a mixture of type 2 and non-type 2 inflammations. However, both serum periostin and tissue periostin expression in biopsied tissues showed significant associations with eosinophil infiltration (Figure 4C and D, Figure S2C). In contrast, blood eosinophil counts were not associated with mucosal eosinophil infiltration (Figure 4E), demonstrating that serum periostin, but not blood eosinophil, could be a biomarker to stratify type 2-dominant UC. We then assessed the potency of serum periostin to detect dense eosinophil infiltration (≥20 eosinophils/high-power field). A receiver operating characteristic (ROC) curve analysis showed 0.875 of the area under the curve (AUC) and 85.7% of sensitivity and 87.2% of specificity in case of 97.8 ng/mL of the cutoff level (Figure 4F). In contrast, the mucosal periostin scores were not associated with any clinical characteristic (age, disease duration, body mass index, disease course, disease location, disease activity, white blood cell, eosinophil, CRP, hemoglobin, albumin, LRG, and medical treatments) except negative association with steroid treatment (Figure S2D). These data show that serum periostin has a high potency to stratify type 2-dominant UC.

Usefulness of Serum Periostin to Predict the Efficacy of Oral Prednisolone for UC

It has been reported that responsiveness to oral prednisolone is characteristic of type 2-dominant UC.[17,23] Therefore, we examined whether the efficacy of oral prednisolone was different between type 2-dominant and non-type 2-dominant UC patients, as defined by serum periostin. We enrolled 14 patients who were newly treated with more than 25 mg/day of oral prednisolone for their active disease, as shown in Table S2. Serum periostin at the start of prednisolone administration was significantly higher in remitting than in non-remitting patients (Figure 5A) and tended to be high in responsive compared to nonresponsive patients, although statistically not significant (Figure 5B). We defined type 2-dominant and non-type 2–dominant UC patients as ≥80 ng/mL and <80 ng/mL of serum periostin, respectively. Although there was no difference in baseline clinical activity between these 2 groups (Figure 5C), the clinical remission rate was significantly higher in the type 2-dominant type than the non-type 2–dominant type (80.0% vs 22.2%, respectively) (Figure 5D). Moreover, serum periostin levels were significantly reduced by prednisolone treatment in the type 2-dominant, but not in the non-type 2–dominant patients (Figure 5E). Taken together, stratification of UC into type 2-dominant and non-type 2–dominant based on serum periostin would be useful to predict the efficacy of oral prednisolone in UC patients.

Significance of Periostin in a DSS-Inducing Mouse Model of UC

Given that serum periostin is a signature biomarker of type 2-dominant UC and that DSS-treated mice are considered to be a mouse model of type 2-dominant UC,[24,25] we investigated the role of periostin in the pathogenesis of type 2-dominant UC by creating a genetic deficiency of periostin in DSS-treated mice. The protocol of induction of chronic DSS colitis is shown in Figure 6A. DSS treatment significantly caused body weight loss, elevated DAI score, and colon length shortening in WT mice (Figure 6B–D). In contrast, genetic deficiency of periostin improved these inflammatory parameters, although the weight loss was not statistically significant. Histological analyses showed that DSS treatment enhanced histological activity score and induced eosinophil recruitment in WT mice, whereas both were decreased in periostin-deficient mice (Figure 6E, G, and H). We confirmed that periostin is highly and widely expressed throughout transmural layers of the colon in the DSS-treated WT mice (Figure 6F and I). These data suggest that periostin acts as an accelerator in the setting of colitis in DSS-inducing mouse model of UC.

Discussion

In this study, we first confirmed the heterogeneity of the underlying mechanism of UC by periostin expression and serum periostin (Figures 2 and 3). It is generally known that cytokine expression profiles of UC patients differ according to disease stage and that UC patients have high heterogeneity.[5,6,26] For example, mucosal and systemic immune profiles have been reported to differ between early and late stages in patients with active UC. Expression of type 1-related genes—*TNF* and *SOCS1*—is increased during the early stage, while expression of the type 2-related genes—*IL4R*, *GFI1*, *IL1RL1*, *PPARG*, and *IL5*—is increased during the late stage.[5] Accordingly, a mouse study shows that the cytokine profile of DSS colitis changes from type 1- and type 17-dominant inflammation at the acute stage to type 2-dominant inflammation at the chronic stage.[24] Expression of cytokines and transcription factors related to type 1 (TNF-α, IFN-γ, IL-12, and T-bet), type 2 (IL-13, IL-33, and GATA3), type 17 (IL-17A, IL-17F, IL-21, IL-22, IL-23, and IL-6), and Treg (TGF-β and Foxp3) are high in inflamed mucosa compared to noninflamed mucosa and controls, and their expression levels vary widely among UC patients.[27] In particular, expression of IL-13, a signature cytokine of type 2 inflammation, is diverse among UC patients, tending to be high in young and extensive-type patients.[6] Therefore, it is reasonable that the expression of periostin, a mediator of type 2 inflammation, in the inflamed sites and serum was heterogeneous among UC patients.

We then showed the high potency of periostin to stratify type 2-dominant UC (Figure 4). Although it has been reported

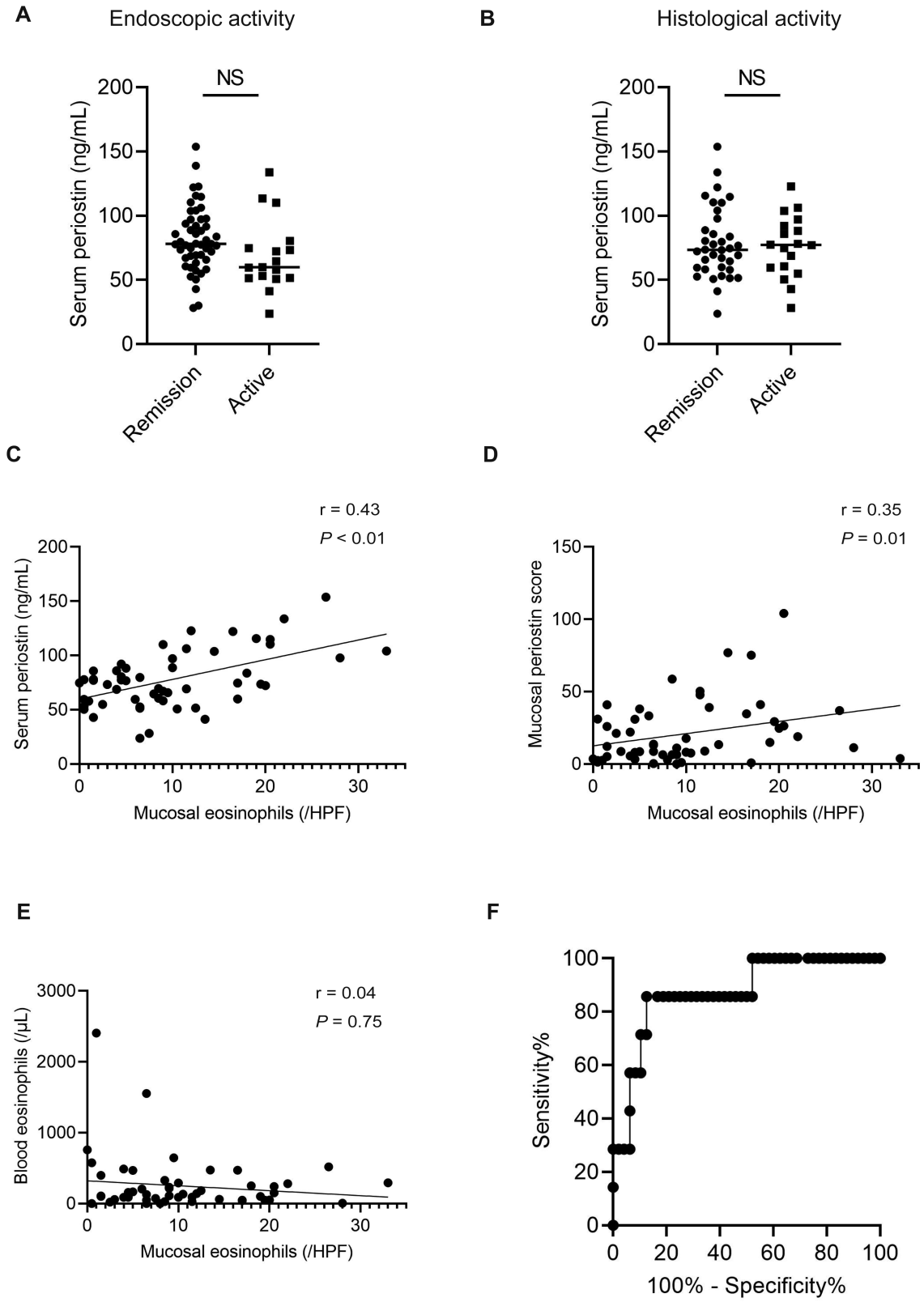

Figure 4. High potency of serum periostin to stratify type 2-dominant ulcerative colitis (UC). A, Serum periostin in UC patients in remission (Mayo Endoscopic Subscore (MES) of 0 or 1, $n = 49$) and in active (MES of 2 or 3, $n = 17$) stages as estimated by endoscopic findings. B, Serum periostin in UC patients in remission (Nancy histological index (NHI) of 0 or 1, $n = 18$) and in active (NHI of 2–4, $n = 36$) stages as estimated by histological findings. C–E, Scatter plots showing the correlation of the number of mucosal eosinophils with serum periostin (C), mucosal periostin expression scores (D), and blood eosinophils (E). F, Receiver operating characteristic curve for serum periostin to discriminate the dense eosinophil infiltration (≥20 eosinophils/high-power field). Statistical analysis was performed using an unpaired *t* test for 2 groups comparison, and the Spearman correlation coefficient test. Abbreviation: NS, not significant.

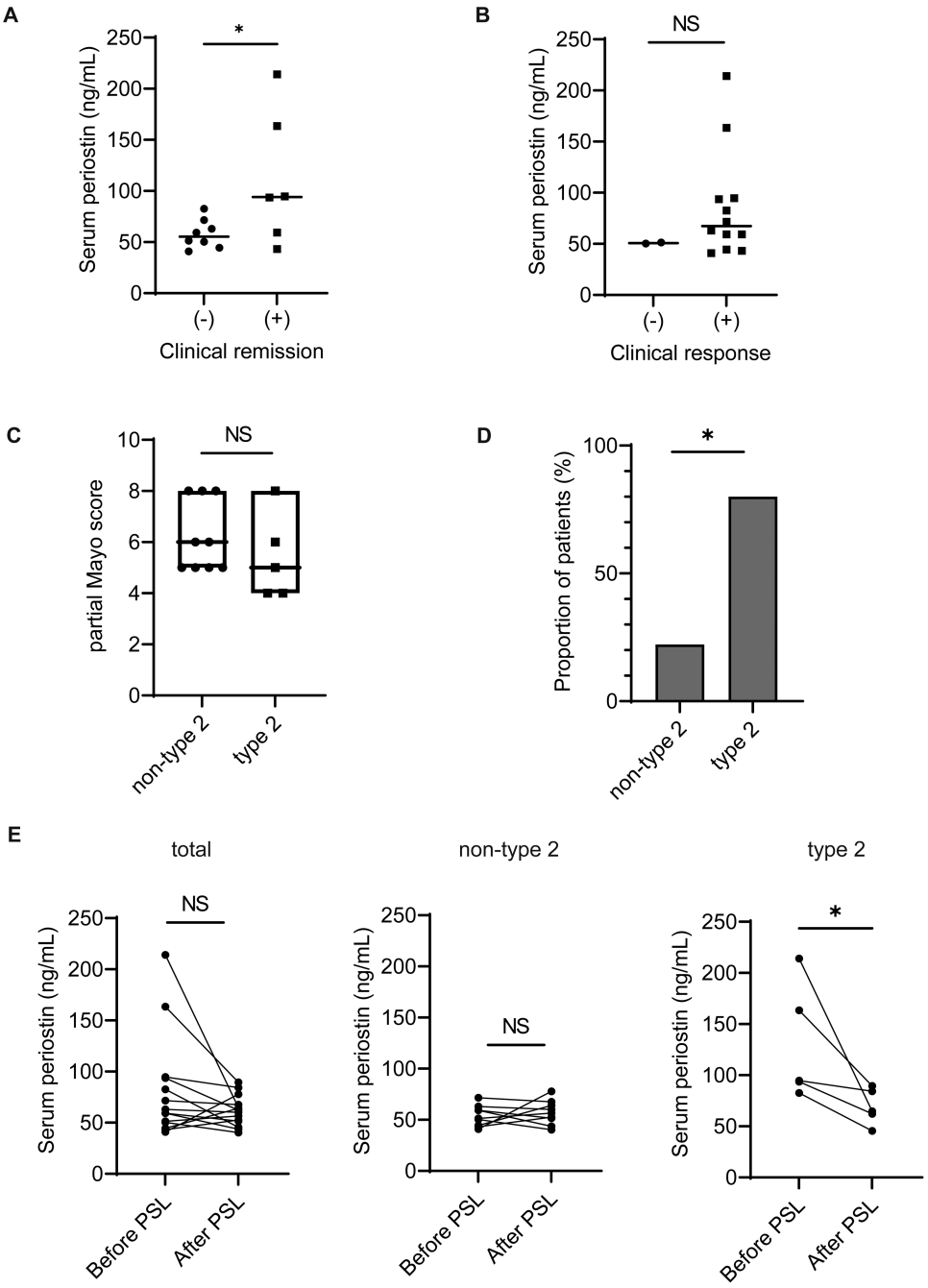

Figure 5. Usefulness of serum periostin to predict efficacy of oral prednisolone for ulcerative colitis (UC). A and B, Serum periostin before prednisolone (PSL) administration in non-remission (partial Mayo score ≥ 2, $n = 8$) and in clinical remission (partial Mayo score < 2, $n = 6$) (A) and without clinical response (partial Mayo decrease from baseline < 2, $n = 2$) and with clinical response (partial Mayo decrease from baseline ≥ 2, $n = 12$) (B) at 2 weeks after the start of administration. C, Partial Mayo score at the beginning of PSL treatment in type 2-dominant (serum periostin ≥ 80 ng/mL) and in non-type 2-dominant type (serum periostin < 80 ng/mL). D, Clinical remission rates after 2 weeks of PSL induction in type 2-dominant and non-type 2–dominant types. E, Serum periostin of the total (left), non-type 2–dominant type (middle), and type 2-dominant type (right) of UC patients before and after 2 weeks of PSL treatment. Statistical analysis was performed using an unpaired *t* test (A–C), chi-square test (D), and paired *t* test (E); **P* < .05. Abbreviation: NS, not significant.

that patients with Crohn's disease show high levels of serum periostin,[14,28] to our knowledge, there is no report showing high serum periostin levels in patients with UC alone. Several biomarkers—fecal calprotectin,[29] fecal immunochemical test,[30] CRP,[31] LRG,[8] and PGE-MUM[9]—are now available for the management of UC patients. These biomarkers are useful for monitoring intestinal inflammation; however, there is no biomarker clinically available for endotyping of UC. Although peripheral blood eosinophils might be a candidate biomarker reflecting type 2 inflammation in UC, it has been reported that tissue eosinophil counts are not correlated with peripheral blood eosinophil counts in UC patients,[32] in agreement with our present study (Figure 4E). Therefore, our present finding that serum periostin has a high potency to stratify type 2-dominant UC highlights the significance of serum periostin as a biomarker for treating UC patients.

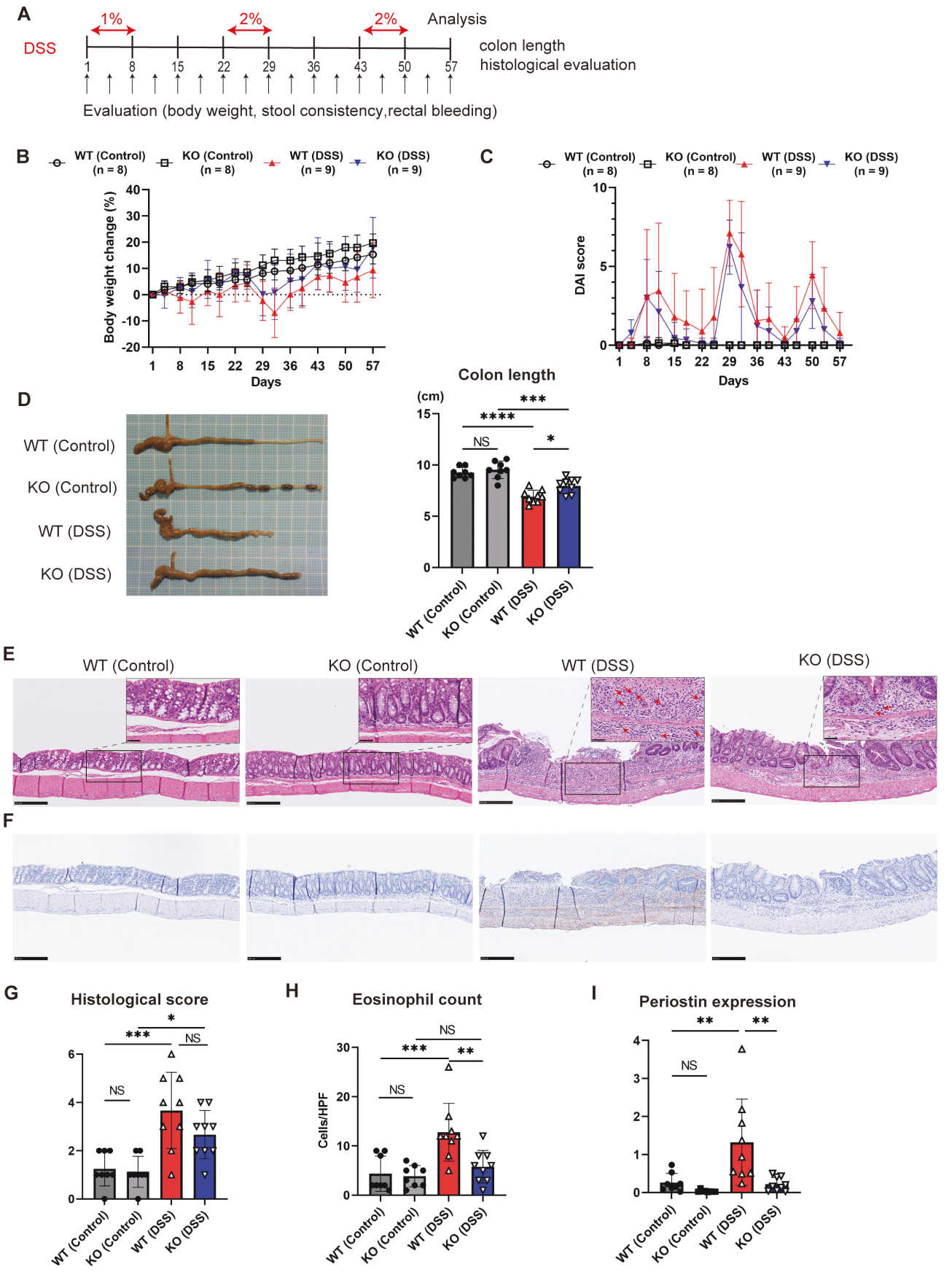

Figure 6. Significance of periostin in a dextran sulfate sodium (DSS)-inducing mouse model of ulcerative colitis. A, The protocol of DSS administration into mice. B–I, Change in %body weight compared to initial body weight (B), disease activity index score (C), colon length (D), H&E staining (E), immunostaining of periostin (F), histological score (G), eosinophil number (H), and periostin expression (I) of distal colons of control (drinking water) or DSS-treated wild-type or *Postn*$^{-/-}$ (KO) mice (n = 8-9 for each group). The data shown are the mean ± SEM. Statistical analysis was performed using the Tukey's multiple comparison test. Scale bars: 250 μm. Arrows in panel E indicate infiltrated eosinophils.*P < .05, **P < .01, ***P < .001. Abbreviation: NS, not significant.

Furthermore, we found that a high potency to predict the efficacy of oral prednisolone in UC patients is another characteristic of serum periostin as a biomarker (Figure 5). Oral prednisolone therapy is recommended to induce remission in moderately to severely active UC.[1,2,18] However, it has been reported that 16% of UC patients are resistant against prednisolone therapy[33] and that its redundant use can cause serious side effects such as osteoporosis, hyperglycemia, hypertension, mood disorders, and increased susceptibility to infections.[34] High potency to predict the efficacy of oral prednisolone in UC patients would be a significant advantage of serum periostin as a biomarker.

Moreover, it has been demonstrated that serum periostin is effective for predicting and monitoring responses to therapeutic agents for type 2 inflammation—dupilumab, omalizumab, mepolizumab, tralokinumab, and lebrikizumab—in treatments for atopic dermatitis, asthma, and chronic rhinosinusitis.[11,21,35] Thus far, while no drug targeting type 2-dominant inflammation is available to treat UC patients, serum periostin may become a companion diagnostic for such drugs when they become available in the future.

We have previously demonstrated that periostin, a matricellular protein, acts as a downstream molecule of IL-4/IL-13 by binding to $\alpha_v\beta_3$ integrin linking type 2 inflammation and the NF-κB pathway, leading to acceleration and chronicity of skin inflammation in atopic dermatitis.[12,13] Moreover, several reports indicate some significance of periostin in the pathogenesis of UC model mice[14–16]; however, the level of significance remains unestablished. It is known that treating mice with DSS causes colitis similar to that in UC patients showing type 2-dominant inflammation,[24,25] suggesting that DSS-treated mice constitute a mouse model of type 2-dominant UC. Given that serum periostin is a signature biomarker of type 2-dominant UC, we investigated its role in the pathogenesis of type 2-dominant UC by creating a genetic deficiency of periostin in DSS-treated mice. Consequently, we found that genetic deficiency of periostin improved inflammation, especially eosinophil recruitment, in DSS-inducing model mice with colitis (Figure 6).

It has been reported that activated eosinophils accumulated in the colons of colitic mice play a critical role in exacerbating intestinal inflammation through the production of eosinophil peroxidase and inflammatory cytokines because removal of eosinophils by anti–IL-5 Abs or Siglec-F–depleting agents improved intestinal inflammation.[36] In contrast, IL-5–deficient mice showed reduced tissue eosinophilia in the DSS-inducing colitis model compared to WT controls, but no difference was found in disease severity.[37] Thus, it is still controversial whether inhibition of type 2 inflammation is effective in mice with chronic DSS colitis, as is also the case with UC patients. It has been reported that targeting IL-13 is ineffective in clinical trials of neutralizing anti–IL-13 Abs (anrukinzumab and tralokinumab),[38,39] whereas there is a report showing that administration of benralizumab, an anti–IL-5 receptor Ab, for refractory bronchial asthma improved complicated UC.[40] The failure of clinical trials may be due to using a one-size-fits-all solution for heterogeneous UC patients. Inhibitors of type 2 inflammation may be effective for type 2-dominant UC. In fact, tralokinumab, an anti–IL-13 Ab, showed a higher rate of clinical remission, a secondary endpoint, than placebo, although there was no difference in clinical response, the primary endpoint.[38] It is hoped that a clinical trial of a type 2 inhibitor for UC based on endotyping will be conducted.

The present study has several limitations. First, we used the surgical margin areas from patients with early-stage sigmoid colon cancer as normal controls because surgical specimens from healthy individuals were not available. We cannot completely exclude the possibility that adjacent cancer cells affect noncancer areas. Second, this is a single-center study with a small sample size. In particular, the number of patients using each molecularly targeted drugs ($n = 36$) and the number of patients newly introduced to prednisolone ($n = 14$) were small. We need a larger-scale study to validate it in the future. Third, we did not evaluate the usefulness of stratification of type 2-dominant UC by periostin in other agents such as biologics and JAK inhibitors. Future large-scale prospective studies are needed to clarify the usefulness of serum periostin for predicting the therapeutic efficacy of various medications including validation of steroid treatment.

In conclusion, periostin is a useful biomarker to stratify type 2-dominant UC patients and can be a promising therapeutic target for type 2-dominant UC.

Supplementary Data

Supplementary data is available at *Inflammatory Bowel Diseases* online.

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Author Contributions

H.T., S.N., Y.N., Y.H., K.Y., Y.S., M.E., and K.I. were involved in the conception and design of the study; H.T. and A.K. performed the statistical analyses. All authors contributed to data interpretation. All authors contributed to the development of the manuscript and all authors approved the final version. All authors agree to be accountable for all aspects of the work.

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Conflict of Interest

M.E. received lecture fees from AbbVie GK, Mitsubishi Tanabe Pharma Corporation, EA Pharma Co., Ltd., Janssen Pharmaceutical K.K., Takeda Pharmaceutical Co., Ltd, Pfizer Japan Inc., and Gilead Sciences, Inc., K.I. received research grants from Maruho Co., Ltd, and Torii Pharmaceutical Co., Ltd, Scholarship grants from Shino-Test Co., Ltd, outside the submitted work. All other authors have no competing interests to declare.

Data Availability

The data underlying this article are available and will be shared on reasonable request to the corresponding author.

Ethical Considerations

The study was conducted according to the principles of the Declaration of Helsinki, approved by the ethics committee at Saga University Hospital (2022-01-R-01). Written informed consent was obtained from all study patients. All animal experiments were performed following the Guidelines for Care and Use of Experimental Animals as required by the Japanese Association for Laboratory Animals Science (1987) and approved by the Saga University Animal Care and Use Committee (A2022-013-0).

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