Advantages of Fecal Lactoferrin Measurement during Granulocyte and Monocyte Adsorptive Apheresis Therapy in Ulcerative Colitis

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The authors report no conflict of interest regarding this manuscript.

ABSTRACT:

Background: Fecal lactoferrin has been introduced as a useful tool for the diagnosis and monitoring of IBD. The aim of this study was to assess if fecal lactoferrin can be employed to predict or estimate the effect of granulocyte and monocyte adsorptive apheresis (GMA) in UC.

Methods: This was a prospective study involving 21 patients with UC. Patients with moderately-to-severely active UC who were scheduled to undergo GMA were recruited. Changes in fecal lactoferrin concentration were compared between the GMA-responder and -nonresponder groups.

Results: In the GMA-responder group, fecal lactoferrin significantly increased 1 week after the introduction of GMA and then significantly decreased after GMA sessions. Fecal lactoferrin concentrations were significantly higher in the GMA-responder group than in the GMA-nonresponder group at 1 and 2 weeks after the introduction of GMA. Multivariate logistic regression analysis revealed that fecal lactoferrin concentration 1 week after the introduction of GMA was the most contributing factor for the effectiveness of GMA in patients with UC.

Conclusions: In the GMA-responder group, fecal lactoferrin concentration significantly increased 1 week after the introduction of GMA. Fecal lactoferrin may be beneficial for predicting clinical

response of GMA in patients with UC at an early stage of GMA treatment.

Keywords: Ulcerative colitis, fecal lactoferrin, granulocyte and monocyte adsorptive apheresis, predictive factor

1. INTRODUCTION AND BACKGROUND:

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) characterized by periods of remission and recurrent relapses. Although factors that initiate and perpetuate UC are not well understood at present, it is considered that dysregulated immune activation causes tissue injury and gut inflammation. In active phase, excessively activated leukocytes are major sources of inflammatory cytokines [1-8]. Elevated peripheral blood granulocytes and monocytes/macrophages are shown to mediate exacerbation and perpetuation of IBD [5, 7, 9].

Although conventional medications including 5-aminosalicylic acid, prednisolone, and immunomodulators have been used for many years in the treatment of UC, their use has often been associated with intolerance, treatment failure, and drug-related adverse side effects [10-13]. Granulocyte and monocyte adsorptive apheresis (GMA) is a nonpharmacologic therapy for IBD that depletes activated leukocytes. Adacolumn® consists of a column filled with cellulose acetate beads of 2 mm in diameter. The column adsorbs granulocytes and monocytes/macrophages (FcγR and complement receptors bearing leukocytes). Therefore, the column selectively adsorbs most of the granulocytes, monocytes/macrophages, and a significant fraction of platelets from the blood. Therefore, lymphocytes are spared and subsequently increase after GMA treatment [8, 14]. GMA is

also associated with marked increase in serum level of the anti-inflammatory cytokine, IL-10 [15]. Several studies have reported significant efficacy in patients with UC following a course of GMA [8,14,16-21]. It has been reported that the overall response (remission or significantly improvement) of GMA treatment was from 58.5% to 77.7% [14, 18, 20]. Meta-analysis has also revealed that GMA induced a clinical remission in a higher proportion of patients with UC compared with conventional medical therapy from seven randomized controlled trials [19]. One of the advantages of GMA treatment is the lower frequency of side effects compared to steroid treatment [14, 18]. In addition, reduction in prednisolone dose is expected after GMA treatment. Therefore, GMA treatment is suitable for patients with active UC refractory to conventional drug therapy.

Because the treatment with GMA is very expensive and the duration of GMA treatment sessions is long, useful predictive factors are needed to identify responders to the treatment. Previous studies have shown that patients with a short duration of UC, higher baseline granulocyte fraction, younger age, and steroid naïveté appeared to respond well to GMA [18, 22-27]. Although these factors are useful for deciding whether to introduce GMA treatment or not, there are no indicators that detect responders at an early phase after starting GMA treatment.

Several biomarkers to diagnose IBD or monitor disease activity have been studied as

candidate surrogate markers in recent reports [28-42]. These biomarkers have the potential to avoid invasive procedures to estimate disease activity if they closely correlate with disease activity in the colonic mucosa. Among the available fecal biomarkers, lactoferrin and calprotectin have been introduced as useful clinical tools for the diagnosis and monitoring of IBD [28-42]. Lactoferrin is an iron binding glycoprotein with a molecular weight of approximately 80,000. It is secreted by most mucosal membranes and is a major component of secondary granules of polymorphonuclear granulocytes, a component of the inflammatory response. The potential role of lactoferrin as a marker for intestinal inflammation in patients with chronic intestinal disease has been investigated. A correlation between fecal lactoferrin concentrations and clinical, endoscopic, and histological parameters of IBD has been confirmed. Fecal lactoferrin determination may be useful in predicting impending clinical relapse in patients with IBD [29, 34-37, 39, 41, 42]. These observations led to a hypothesis that fecal lactoferrin may be suitable for the prediction and effect measurement of GMA. It remains unclear whether fecal lactoferrin could be useful in evaluating the effect of GMA in patients with UC. The aim of this study was to assess if fecal lactoferrin can be employed to predict or estimate the effect of GMA in patients with UC.

2. METHODS:

2.1. Study design

This was a prospective study conducted at the Nagasaki University Hospital and five associated medical institutions in Nagasaki, Japan. Patients were eligible if they had been treated with GMA between November 2011 and January 2014 at these institutions.

2.2. Patients

At entry, patients were classified as having moderately-to-severely active UC according to the diagnostic criteria for UC set by the Japan Ministry of Health Expert Committee on Inflammatory Bowel Disease which is decided by the frequency of defecation, melena, and systemic symptoms (fever, tachycardia, anemia and erythrocyte sedimentation rate). Moreover, patients with steroid-refractory UC who had previously undergone corticosteroid therapy, steroid-dependent UC that required continuous prednisolone treatment to prevent flare-ups, intolerance to steroid treatment, and disinclination for steroid treatment were recruited in this study and were scheduled to undergo GMA. Endoscopic examination was performed and disease activity index (DAI) was assessed at entry for all patients. Any concomitant medication that had been started before the introduction of GMA was allowed during the study period. In cases of patients who were

already on steroid treatment, corticosteroid dose was tapered when DAI decreased to remission level (≤2).

2.3. GMA procedure

GMA treatment was performed with the Adacolumn® (JIMRO, Takasaki, Japan), as previously described [8, 14]. Each patient received 1 or 2 GMA session(s)/week, up to 11 sessions.

2.4. Stool analysis/fecal tests

Fecal lactoferrin was measured four times: before the introduction of GMA, 1 and 2 weeks after the introduction of GMA, and after GMA sessions. The collected feces were stored at room temperature and immediately analyzed at the Kyoto Medical Science Laboratory (Kyoto, Japan). Fecal lactoferrin was measured by a sandwich enzyme-linked immunosorbent assay, which has been previously described in detail [33].

2.5. Outcome measurement

The efficacy of GMA was clinically and endoscopically evaluated with DAI before and after GMA therapy. We defined the GMA-responder group as follows: remission; a total of DAI score ≤2 points and effective; a decrease in DAI score by ≥ 3 points from baseline. The GMA-nonresponder group was defined when the remission and effective group were excluded. Changes in fecal lactoferrin

concentrations, white blood cell (WBC) count, and C-reactive protein (CRP) during GMA sessions were assessed for each group. Fecal lactoferrin concentrations were also compared between the GMA-responder and -nonresponder groups. In addition, correlation between fecal lactoferrin and endoscopic score or serological markers was assessed.

2.6. Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki and in compliance with the Good Clinical Practices. In addition, the study was approved by the Ethics Committee of the Nagasaki University Hospital (Office for Human Research Protection Number: IORG 0007678). All patients provided written informed consent before any study-related procedures were performed.

2.7. Statistical analyses

The JMP Pro version 10.0.2 software (The SAS Institute, Cary, NC, USA) was used for all analyses. When the distribution of values was appropriate, numerical data are presented as mean ± standard deviation values. Because fecal lactoferrin concentrations did not adapt to normal distribution, data of fecal lactoferrin concentrations are presented as median ± standard error values. Wilcoxon signed-ranks tests were employed for comparison of changes in fecal lactoferrin concentrations during GMA sessions. Wilcoxon rank-sum tests were used for comparison of fecal lactoferrin

concentrations between the GMA-responder and -nonresponder groups. Correlation between fecal lactoferrin and endoscopic score or serological markers was assessed using the Spearman's correlation coefficient. To determine predictive factors that are closely related to the response of GMA, we plotted receiver operating characteristic (ROC) curves for fecal lactoferrin concentrations and other clinicopathological factors. Subsequently, area under the curve (AUC) was calculated, and the point with the largest AUC was defined as the point having the greatest association with the effectiveness of GMA. The best cut-off values of the predictive factors had a minimum distance from the upper left corner to the point on the ROC curve and were distinguishable between the GMA-responder and -nonresponder groups. According to the cut-off values, univariate analysis and multiple logistic regression analysis were applied to determine contributing factors for the response of GMA. *P* values of <0.05 were considered statistically significant.

3. RESULTS:

3.1. Patients characteristics

Of the 23 recruited patients, two did not meet the inclusion criteria. The remaining 21 eligible patients were selected for this study. As shown in Table 1, UC was moderate in 18 and

severe in three patients. Ten patients had total colitis and 11 had left-sided colitis. Eleven of 21 patients were already on steroid treatment (prednisolone; 5–70 mg/day). Of these 11 patients, six had steroid-refractory UC who had previously undergone corticosteroid therapy and five had steroid-dependent UC that required continuous prednisolone treatment to prevent flare-ups. Twenty of 21 patients were on aminosalicylate for at least 8 weeks prior to study enrollment. Four of 21 patients were on tacrolimus for at least 1 week prior to enrollment. Four of 21 patients were on azathioprine for at least 2 weeks prior to enrollment. Another three patients started receiving azathioprine during the observation period.

3.2. Response rate of GMA and Changes of WBC count and CRP

Overall, the response rate of GMA was 61.9% (13 of 21 patients). The patients were categorized into GMA-responder and -nonresponder groups. Thirteen of 21 patients were in the GMA-responder group; the remaining eight patients were in the GMA-nonresponder group.

Median WBC counts were significantly decreased from 6800 ± 1380 to 6000 ± 570 μl after GMA sessions in the GMA-responder group (P =0.01). In the GMA-nonresponder group, median WBC counts were decreased from 6350 ± 840 to 5650 ± 880 μl after GMA sessions, but this change was not significant (P = 0.50). Median CRP was significantly decreased at 1 week and 2

weeks after introduction of GMA and after GMA sessions in comparison with the baseline in the GMA-responder group (0.54 ± 1.46 to 0.28 ± 1.22 mg/dl, $P = 0.03$; 0.54 ± 1.46 to 0.24 ± 0.53 mg/dl, $P = 0.02$; 0.54 ± 1.46 to 0.07 ± 0.55 mg/dl, $P = 0.01$, respectively). No significant change was observed in the GMA-nonresponder group.

3.3. Changes of fecal lactoferrin and comparison of fecal lactoferrin during GMA

As shown in figure 1, median fecal lactoferrin in the GMA-responder group significantly increased from 1335.7 ± 380.6 to 2676.1 ± 676.1 ng/ml at 1 week after the introduction of GMA ($P = 0.03$). After GMA sessions, median fecal lactoferrin decreased and there was significant difference between values before and after treatments (1335.7 ± 380.6, 149.5 ± 287.1 ng/ml, respectively; $P = 0.03$). As with the GMA-nonrespoder group, median fecal lactoferrin also increased from 506.1 ± 198.2 to 998.8 ± 189.6 ng/ml at 1 week after the introduction of GMA. However, this change was not significant ($P = 0.06$). After GMA sessions, median fecal lactoferrin decreased and there was no significant difference between values before and after treatments (506.1 ± 198.2, 608.0 ± 1081.5 ng/ml, respectively; $P = 0.3$).

Figure 2 shows the comparison of fecal lactoferrin concentrations between the GMA-responder and -nonresponder groups. Median fecal lactoferrin concentrations before the

introduction of GMA were higher in the GMA-responder group than that in the GMA-nonresponder group (1335.7 ± 380.6, 506.1 ± 198.2 ng/ml, respectively), although this difference was not significant ($P = 0.1$). Moreover, at 1 week after the introduction of GMA, median fecal lactoferrin concentrations were significantly higher in the GMA-responder group than that in the GMA-nonresponder group (2676.1 ± 676.1, 998.8 ± 189.6 ng/ml, respectively; $P = 0.04$). Significant difference was also observed at 2 weeks after the introduction of GMA between the GMA-responder and -nonresponder group (1531.5 ± 455.2, 556.8 ± 184.3 ng/ml, respectively; $P = 0.04$). With regard to lactoferrin value after GMA, there were no statistical differences between the GMA-responder and -nonresponder groups (149.5 ± 287.1, 608.0 ± 1081.5 ng/ml, respectively; $P = 0.2$).

3.4. Correlation between fecal lactoferrin and endoscopic score or serological marker

Endoscopic score was calculated from a part of the DAI score. Only endoscopic score had a weak positive correlation with fecal lactoferrin (Figure 3). CRP or WBC count did not have correlation with fecal lactoferrin (data not shown).

3.5. ROC and cut-off value

On the base of the ROC analysis using fecal lactoferrin concentrations for all 21 patients, the optimal cut-off value for fecal lactoferrin concentration could be determined. Figure 4 shows the

ROC curve for fecal lactoferrin concentrations (before the introduction of GMA, 1 week after the introduction of GMA, and 2 weeks after the introduction of GMA) in the GMA-responder vs. -nonresponder groups. The optimal cut-off value estimated for these factors that allows the distinction of GMA-responder group compared with those of the GMA-nonresponder group was 1335.7, 1767.0, and 1471.7 ng/ml, respectively. As shown in Table 2, the AUC of the ROC values, cut-off values, sensitivity, and specificity were estimated; the sensitivity values were 53.8%, 76.9%, and 66.6%, respectively. All specificities were 87.5%. Other clinicopathological factors were also estimated by the same method using the optimal cut-off values.

3.6. Univariate analysis and multivariate logistic regression analysis

According to the above cut-off values, univariate analysis was performed to identify contributing factors for the response to GMA. As shown in Table 3, age ≤44 years, ≥1335.7 ng/ml of fecal lactoferrin concentration before the introduction of GMA, ≥1767.0 ng/ml of fecal lactoferrin concentration at 1 week after the introduction of GMA, ≥1471.7 ng/ml of fecal lactoferrin concentration at 2 weeks after the introduction of GMA, and ≤0.325 mg/dl of CRP at 2 weeks after the introduction of GMA were indicative of effective GMA. Other factors did not show significant correlation with effective GMA. Multivariate logistic regression analysis showed that ≥1767.0 ng/ml

of fecal lactoferrin concentration at 1 week after the introduction of GMA was the most significant independent predictive factor for response to GMA ($P = 0.005$). Age ≤44 years was also a significant independent predictive factor for response to GMA ($P = 0.04$).

4. DISCUSSION:

This study demonstrated that fecal lactoferrin is an inexpensive and noninvasive surrogate marker to monitor disease activity and predict the response to GMA in patients with UC. In particular, elevated fecal lactoferrin at 1 week after the introduction of GMA was considered an indication of clinical effectiveness. Fecal lactoferrin dramatically fluctuated in the GMA-responder group during treatment, whereas no significant change was observed in the GMA-nonresponder group. In addition, fecal lactoferrin significantly declined after GMA sessions in the GMA-responder group. Previous lactoferrin studies have shown its usefulness for diagnostically distinguishing between organic and functional intestinal diseases, diagnosing IBD, and correlation between fecal lactoferrin and IBD activity estimated with clinical, endoscopic, and histological parameters [28, 29, 31-42]. To the best of our knowledge, this study is the first to present data that indicate the correlation of fecal lactoferrin with response to GMA. Lactoferrin has antibacterial activity and is

resistant to proteolysis in the feces. Stability of fecal lactoferrin is unaffected by multiple freeze/thaw cycles and may remain stable in the stool for as long as 5 days. It has been shown that fecal lactoferrin concentrations were 90% of initial concentrations after storage at room temperature for 48 h [28-31]. This characteristic allows the collection of feces from each patient at home and storing at room temperature. Another advantage of fecal lactoferrin assessment is that it may detect slight mucosal inflammatory activity that may be detected at a concentration insufficient to cause an increase in ESR and CRP. Furthermore, lactoferrin concentrations in the stool appear to be unaffected by a variety of nonintestinal conditions [37].

In the current study, the correlation between fecal lactoferrin and endoscopic score showed weak positive correlation. Moreover, fecal lactoferrin did not have significant correlation with CRP or WBC count. Nevertheless, several studies have reported positive correlations between fecal lactoferrin and endoscopic and histological scores and serological markers [16, 17, 23, 30, 37, 43, 44]. This could be explained by the deviation of disease severity; most patients with UC had moderate disease, whereas a few had severe disease and none had mild UC.

GMA therapy has been reported as an effective therapy with minimal side effects for patients with UC, particularly in refractory cases to conventional drug therapy [8, 14, 16-21]. In

contrast, a study in the United States reported no significant difference between the sham group and the GMA group [45]. These conflicting results may be because of a short period of treatment with weekly GMA and a high rate of withdrawal. A recent study showed that an intensive course of GMA such as twice-weekly apheresis appears to produce a more and rapid efficacy compared with weekly GMA without increasing the incidence of side effects [46]. In the current study, most of the patients received 2 sessions/week of GMA, up to 11 sessions, which may have contributed to significant improvement.

Fecal lactoferrin at 1 week after the introduction of GMA increased in both of the GMA-responder and –nonresponder groups, although the change in the GMA-nonresponder group was not significant. There are no reports that measured fecal lactoferrin at an early phase after starting GMA treatment or other treatment such as 5-ASA or steroids in the patients with ulcerative colitis. It is unknown whether these changes are specific for GMA. As for Crohn's disease, Buderus et al. [47] demonstrated that a mean level of fecal lactoferrin decreased at 7-10 days following initial infliximab infusion and correlated with a mean decrease of Pediatric Crohn's Disease Activity Index. At present, the underlying mechanism of this phenomenon is not completely understood. GMA carriers adsorb

approximately 65% of granulocytes, 55% of monocytes, and 2% of lymphocytes from the blood into the column [8]. It has been reported that extracorporeal circulation induces temporal serum lactoferrin increase because of degranulation of granulocytes [48, 49]. Because GMA carriers absorb approximately 65% of granulocytes, it is possible that remaining granulocytes that returned to the blood system subsequently localized to intestinal mucosa. Therefore, in cases in which fecal lactoferrin increased 1 week after the introduction of GMA, relocalization of mucosal granulocytes from blood vessels may have intensively occurred. In these cases, granulocyte turnover in the inflamed mucosa may have been promoted, leading to a better response to GMA. Fecal calprotectin is also a sensitive and specific surrogate marker for evaluating intestinal inflammation in IBD. Hanai et al. [50] demonstrated that fecal calprotectin decreased significantly after 10 GMA sessions, although they did not examine it at an early phase after starting GMA treatment. In the present study, we did not measure fecal calprotectin. Fecal calprotectin is also primarily derived from granulocytes and is shown to correlate with fecal lactoferrin value [51]. It is possible that fecal calprotectin may also increase 1 week after GMA introduction similarly with lactoferrin. Further studies assessing levels of fecal calprotectin at an early phase after starting GMA treatment are thus warranted.

Multivariate logistic regression analysis revealed that cases with high fecal lactoferrin

concentrations at 1 week after the introduction of GMA were responders in our study. Previous studies have shown that patients with a short duration of UC appeared to respond well to GMA [24-26]. Hibi, et al. reported that higher baseline granulocyte fraction was an independent predictor of clinical response in GMA [18]. Low cumulative prednisolone doses, receiving GMA immediately after a clinical relapse, low WBC count at the first GMA session, and younger age were also reported as responders [26, 27]. Our study also showed younger patients were responders. Several studies showed that steroid naiveté was a good predictor of response to GMA, whereas Hanai, et al. reported that steroid could enhance the efficacy of GMA [22, 23, 25, 52]. In our study, GMA was more effective in the group receiving steroids than in the group not receiving them. This result maybe explained by the presence of few steroid-naïve patients in our study, and steroid treatment enhanced the efficacy of GMA. Among the clinicopathological factors, fecal lactoferrin may be most objective predictor of response to GMA. Subsequently, fecal lactoferrin concentrations at 1 week after the introduction of GMA would be helpful in the assessment of the effectiveness of the GMA treatment. Because the treatment with GMA is very expensive and the duration of GMA treatment sessions is long, fecal lactoferrin measurements have cost-saving benefits and may help avoid unnecessary GMA treatments.

In conclusion, this study indicated that patients with UC showing high fecal lactoferrin concentrations at 1 week after the introduction of GMA would be the responders of GMA treatment. Further prospective studies with large sample sizes are certainly warranted to confirm the clinical significance of these findings.

CONFLICT OF INTEREST STATEMENT:

The authors have no conflict of interest in relation with the publication of this manuscript.

ACKNOWLEDGEMENTS:

We would like to express our gratitude to Dr. M. Yamakawa, Dr. D. Norimura, Dr. R. Uehara, and Dr. S. Mine for providing the clinical data. We also thank the Kyoto Medical Science Laboratory (Kyoto, Japan) for measuring fecal lactoferrin concentrations and Enago (Tokyo, Japan) for the English language review.

FIGURE LEGENDS:

FIGURE 1. Changes of fecal lactoferrin concentrations during granulocyte and monocyte adsorptive apheresis (GMA) sessions

Median fecal lactoferrin concentrations significantly increased at 1 week after the introduction of GMA and significantly decreased after GMA sessions in the GMA-responder group ($P < 0.05$). No significant change was observed in the GMA-nonresponder group.

FIGURE 2. Fecal lactoferrin concentrations in the granulocyte and monocyte adsorptive apheresis (GMA)-responder and -nonresponder groups

At 1 and 2 weeks after the introduction of GMA, fecal lactoferrin concentrations were significantly higher in the GMA-responder group than that in the GMA-nonresponder group. Before the introduction of GMA and after GMA sessions, there was no statistical difference between the GMA-responder and -nonresponder groups.

FIGURE 3. Correlation between fecal lactoferrin and endoscopic score

Fecal lactoferrin had a weak positive correlation with endoscopic score but not with C-reactive

protein (CRP) or white blood cell (WBC) count.

FIGURE 4. Receiver operating characteristic (ROC) curve for fecal lactoferrin concentrations in the granulocyte and monocyte adsorptive apheresis (GMA)-responder group vs. -nonresponder group

ROC curve for fecal lactoferrin concentrations (before the introduction of GMA, 1 week after the introduction of GMA, 2 week after the introduction of GMA) in the GMA-responder group vs. the GMA-nonresponder group was shown. On the basis of ROC analysis, using the fecal lactoferrin concentration for all 21 subjects, the optimal cut-off value for fecal lactoferrin concentration was determined.

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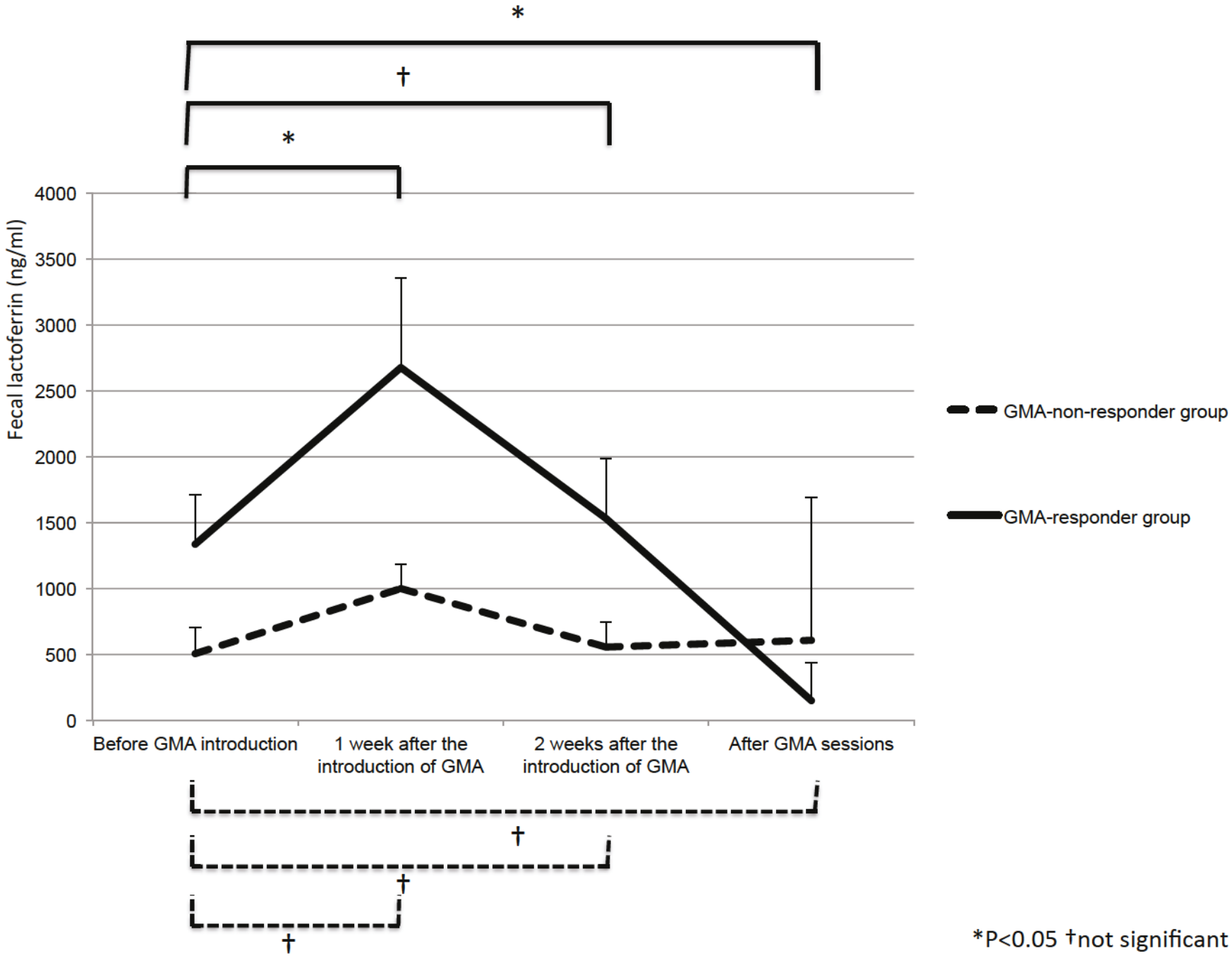
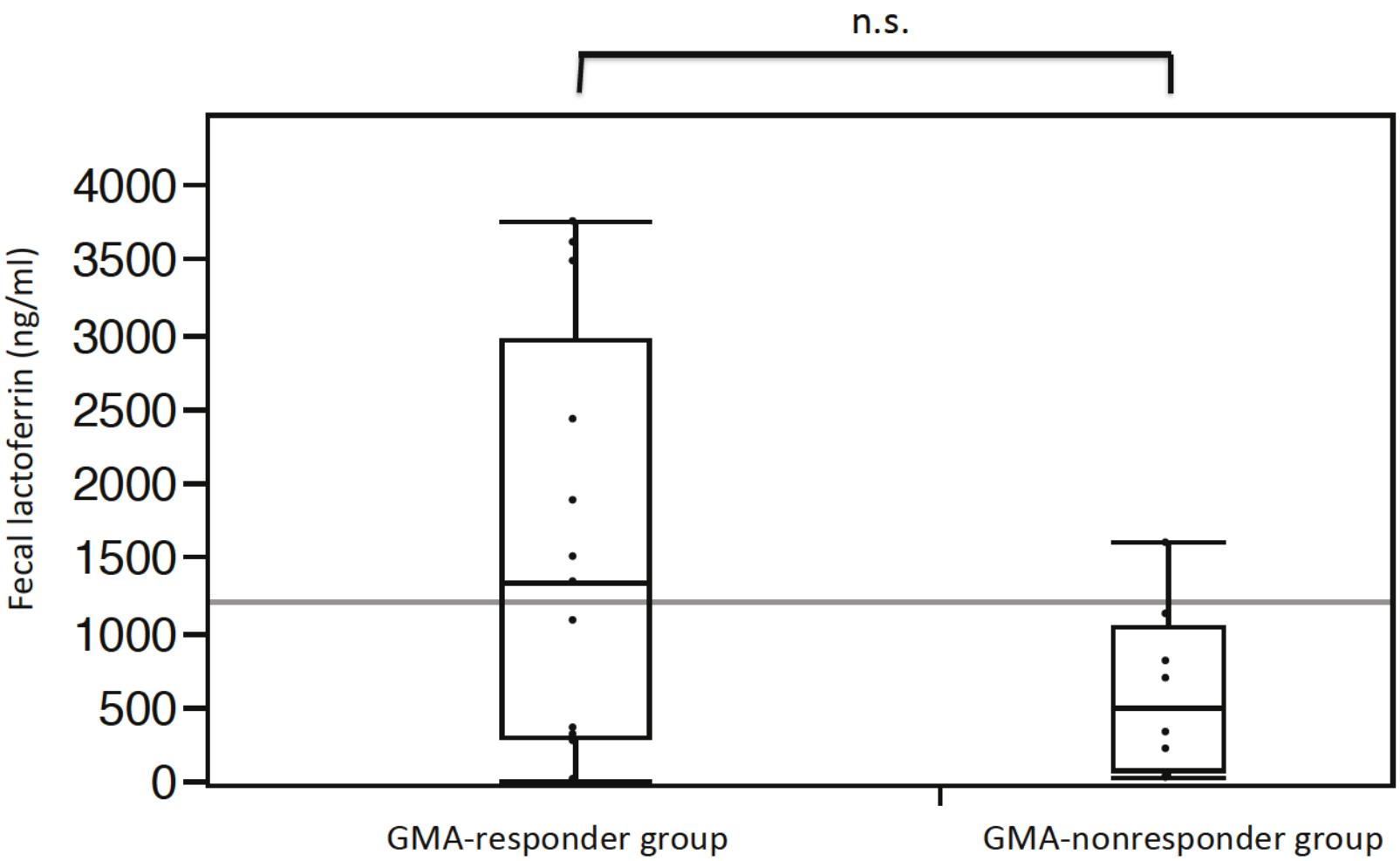
Figure 1

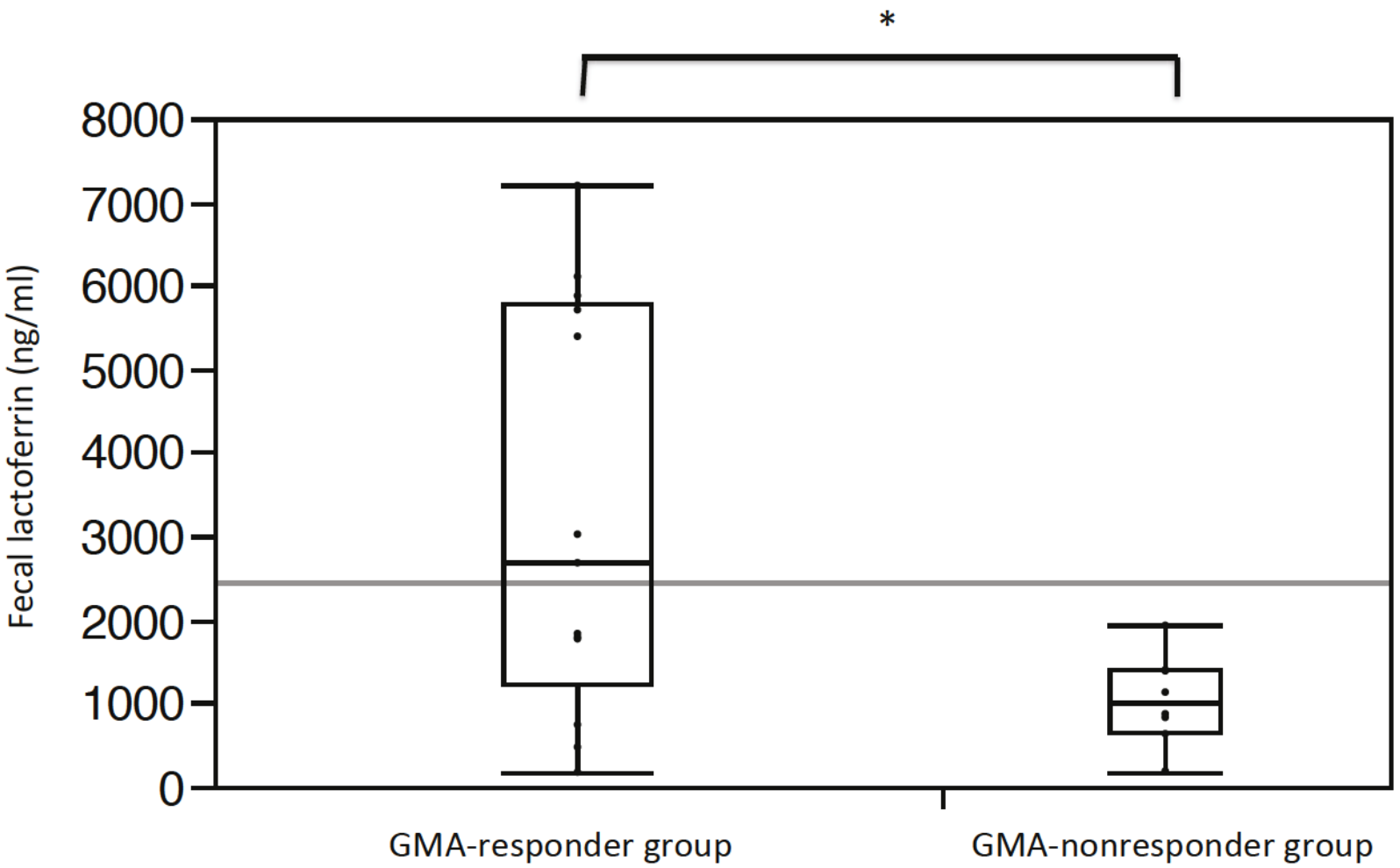


*P<0.05 †not significant

Figure 2

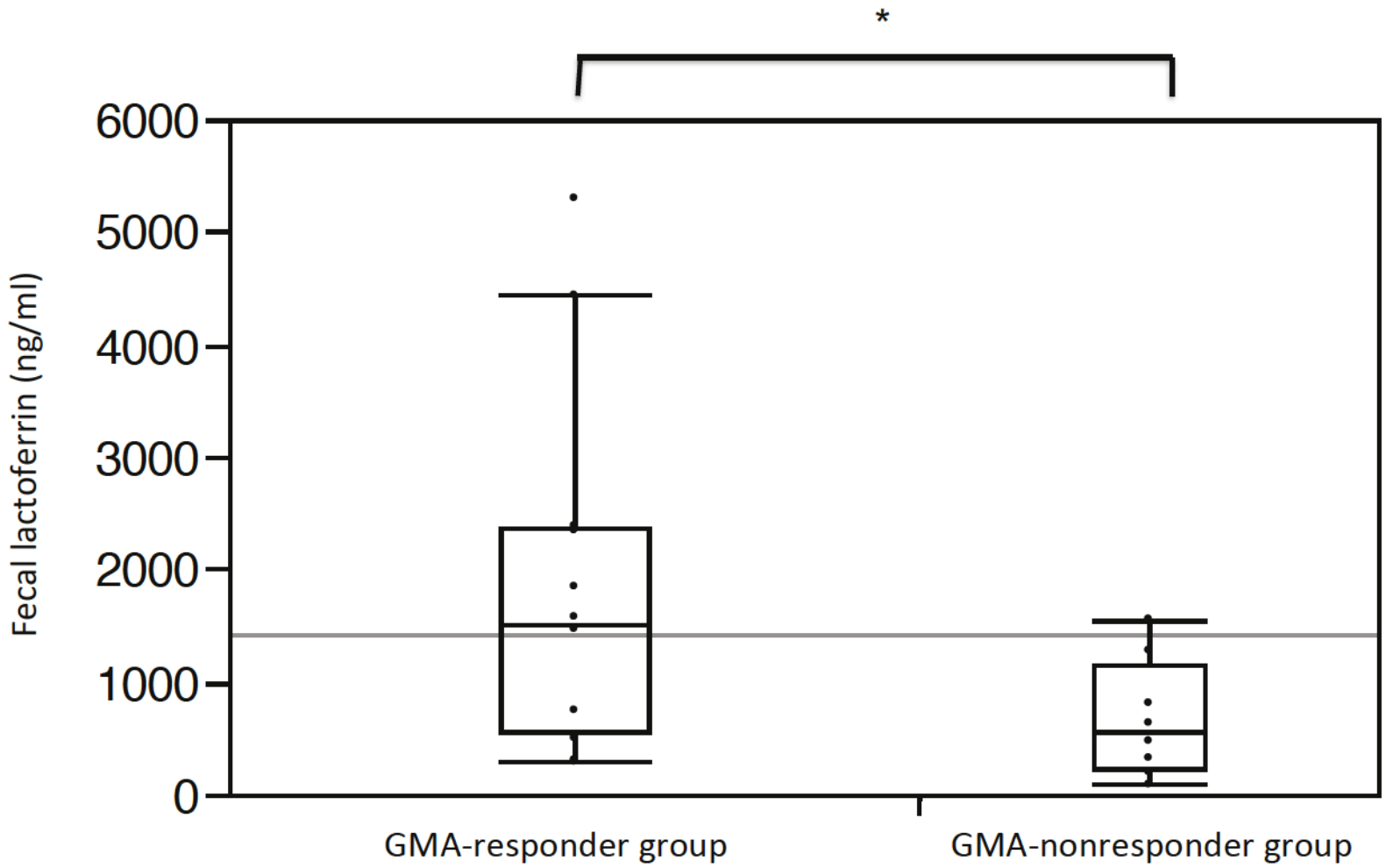

(a) Before GMA introduction
n.s.
Fecal lactoferrin (ng/ml)
4000
3500
3000
2500
2000
1500
1000
500
0
GMA-responder group
GMA-nonresponder group

(b) 1 week after the introduction of GMA



*P<0.05

(c) 2 weeks after the introduction of GMA



*P<0.05

(d) After GMA sessions

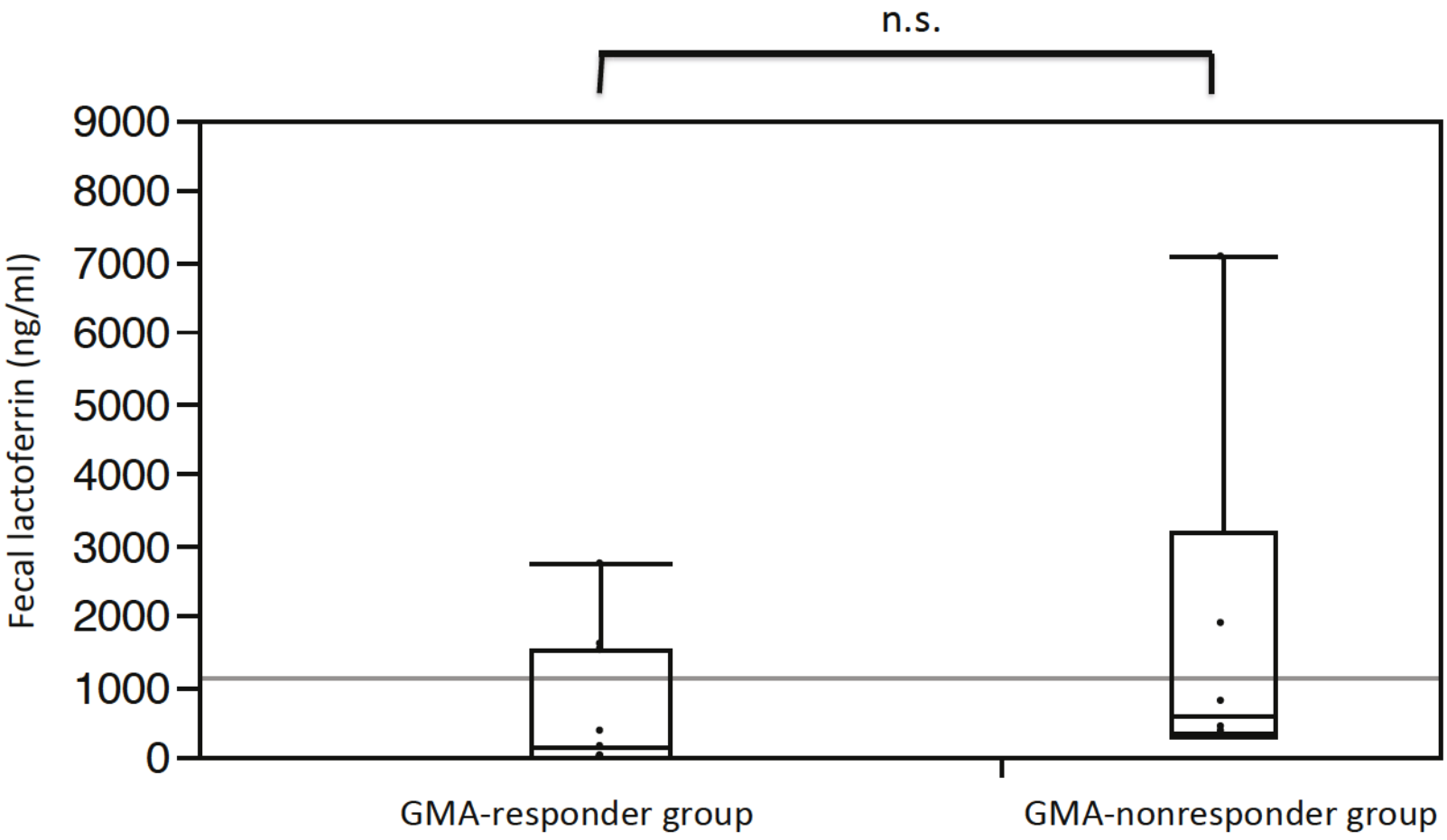
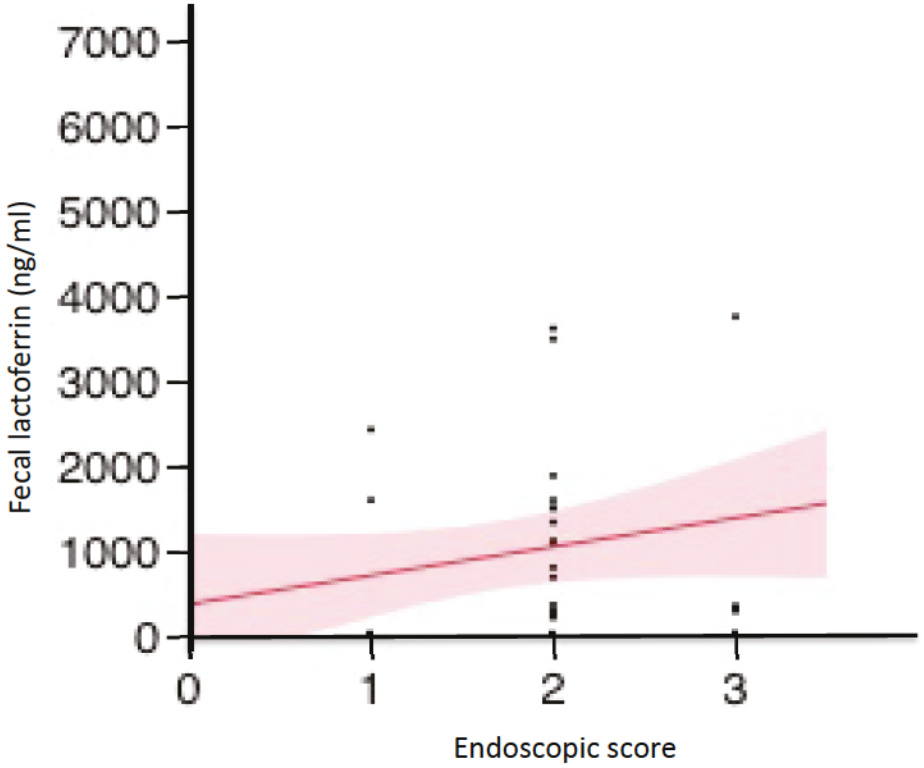


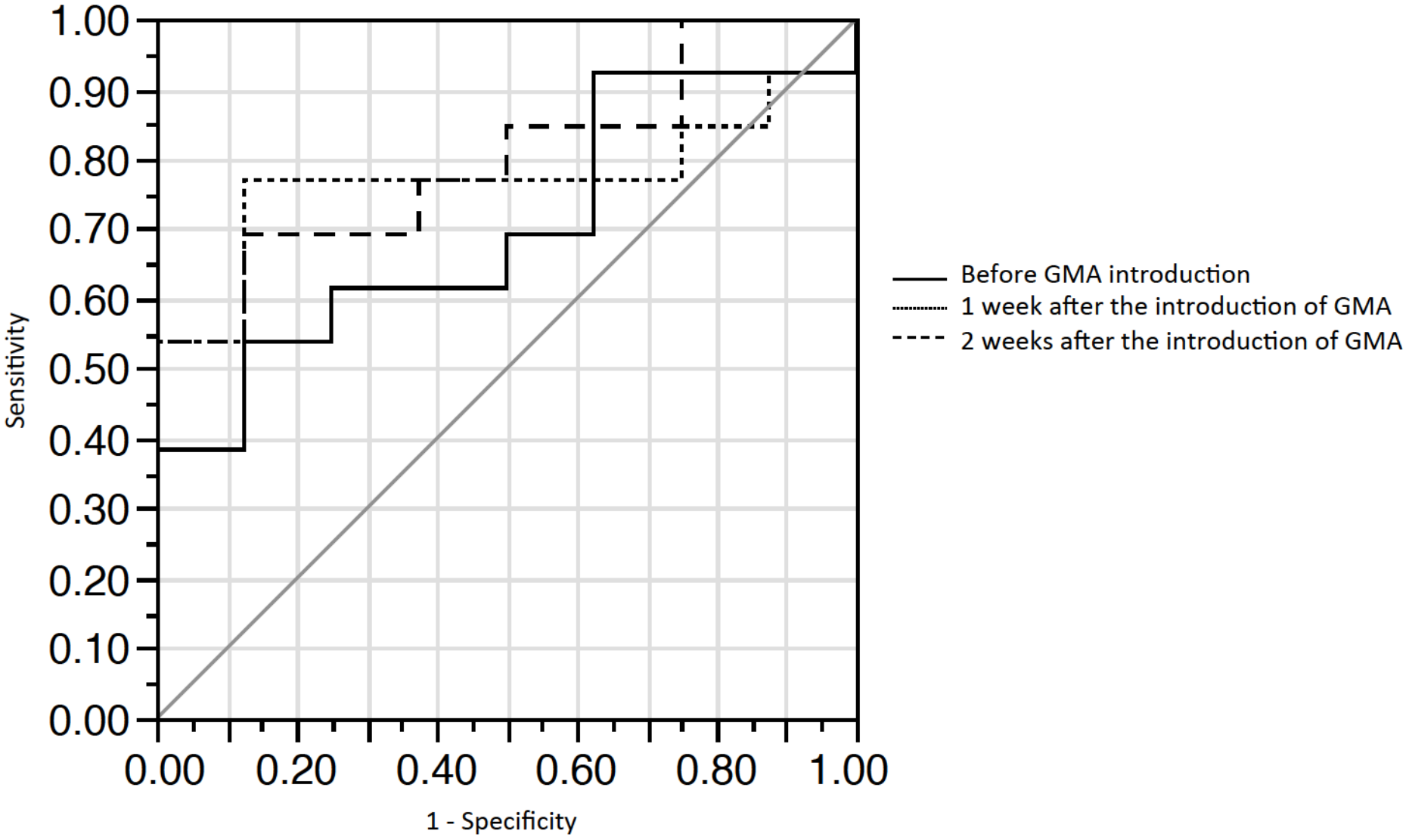
Figure 3



Spearman's rank correlation coefficient = 0.3760

P<0.05

Figure 4


1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Sensitivity
0.00
0.20
0.40
0.60
0.80
1.00
1 - Specificity
Before GMA introduction
1 week after the introduction of GMA
2 weeks after the introduction of GMA

TABLES:

TABLE 1. Baseline Patient Characteristics	
Gender (male/female)	11/10
Age in years, mean (SD)	44.0 (15.5)
Duration of UC in years, median (SE)	3.75 (2.0)
Location of disease	
Total colitis	10
Left-sided colitis	11
Severity	
Moderate	18
Severe	3
Surgery intended (Yes/No)	0/21
Clinical course	
Relapse-remitting type	14
Chronic continuous type	5
One attack only	2
Steroid use (Yes/No)	11/10
Immunosuppressant	
Azathioprine	7
Tacrolimus	4
Aminosalicylate use (Yes/No)	20/1
Number of GMA sessions, median (SE)	10 (0.4)
DAI score before GMA introduction, median (SE)	9.0 (0.2)

SD, standard deviation; SE, standard error; GMA, granulocyte and monocyte adsorptive apheresis; DAI, Disease activity index.

TABLE 2. Cut-off value of fecal lactoferrin with sensitivity, specificity, AUC, and CI in the determination of effectiveness to GMA

Measurements	Cut-off value	Sensitivity (%)	Specificity (%)	AUC (95%CI)
Fecal lactoferrin concentrations (Before GMA introduction)	≥1335.7 ng/ml	53.8	87.5	0.70 (0.43-0.87)
Fecal lactoferrin concentrations (1 week after GMA introduction)	≥1767.0 ng/ml	76.9	87.5	0.76 (0.49-0.91)
Fecal lactoferrin concentrations (2 week after GMA introduction)	≥1471.7 ng/ml	66.6	87.5	0.78 (0.51-0.92)

AUC, area under the curve; CI, confidence interval; GMA, granulocyte and monocyte adsorptive apheresis.

TABLE 3. Significant predictors of effectiveness in GMA by univariate analyasis and multivariate logistic regression analysis

Univariate analysis				
	Cut-off value	Odds ratio	95% CI	*P*-value
Gender	male	2.6	0.44-18.3	n.s.
Age in years	≤ 44	11.2	1.39-244.7	0.02
Duration of UC in years	≤ 3.75	1.4	0.23-9.48	n.s.
Location of disease	Total colitis	4.8	0.75-42.8	n.s.
	Left-sided colitis	Reference	-	-
Severity	Severe	Reference	-	-
	Moderate	1.2	0.10-30.4	n.s.
Clinical course	Relapse-remitting type			
	Chronic continuous type	-	-	n.s.
	One attack only			
Steroid use	Yes	3.5	0.55-30.6	n.s.
Immunosuppressant	Azathioprine	3	0.28-70.8	n.s.
	Tacrolimus	4	0.42-91.3	n.s.
	None	Reference	-	-
Number of GMA sessions	≥10	1.83	0.18-18.7	n.s.
Lactoferrin (before GMA introduction)	≥ 1335.7 ng/ml	8.1	1.02-176.6	0.047
Lactoferrin (1 week after GMA introduction)	≥ 1767.0 ng/ml	23.3	2.7-543.2	0.002
Lactoferrin (2 week after GMA introduction)	≥ 1471.7 ng/ml	14	1.67-314.2	0.012
WBC (before GMA introduction)	≤ 6800/μl	1.1	0.19-7.07	n.s.
WBC (1 week after GMA introduction)	≤ 7900/μl	1.0	0.08-11.3	n.s.
WBC (2 week after GMA introduction)	≤ 7650/μl	1.5	0.09-4.10	n.s.

CRP (before GMA introduction)	≤ 0.72mg/dL	1.1	0.19-7.07	n.s.
CRP (1 week after GMA introduction)	≤ 0.51mg/dL	4.4	0.40-111.0	n.s.
CRP (2 week after GMA introduction)	≤ 0.325 mg/dL	13.5	1.59-304.2	0.014
DAI score before GMA introduction	≥ 9	2.0	0.28-14.7	n.s.
Multivariate logistic regression analysis				
	Cut-off value	Odds ratio	95% CI	*P*-value
Age in years	≤ 44	15.2	1.09-628.7	0.041
Lactoferrin (1 week after GMA introduction)	≥ 1767.0 ng/ml	29.8	2.5-1153.3	0.005

GMA, granulocyte and monocyte adsorptive apheresis; CI, confidence interval; n.s., not significant