Original Article

Noninvasive biomarkers as surrogate predictors of clinical and endoscopic remission after infliximab induction in patients with refractory ulcerative colitis

Elham A. Hassan[1], Haidi K. Ramadan[1], Ali A. Ismael[2,3], Khaled F. Mohamed[3], Madiha M. El-Attar[1], Ihab Alhelali[4]

[1]Department of Tropical Medicine and Gastroenterology, Faculty of Medicine, Assiut University, Assiut, [2]Department of Internal Medicine, Faculty of Medicine, Zagazig University, Sharqia, Egypt, [3]Gastroenterology Unit, Farwaniya Hospital, [4]Department of Clinical Biochemistry, Ministry of Health, Kuwait

Abstract

Background/Aims: Treatment of refractory ulcerative colitis (UC) is a clinical challenge, and after biological therapy, monitoring clinical and endoscopic responses is fundamental. We aimed to investigate and compare the predictive power of different noninvasive parameters for clinical remission and mucosal healing after infliximab induction therapy in refractory UC patients.

Patients and Methods: Serum and fecal biomarkers, including hemoglobin, white blood cells, erythrocyte sedimentation rate, C-reactive protein (CRP), and fecal calprotectin (FC), and colonoscopy were assessed in 44 patients with refractory UC before and after (week 12) infliximab induction. Clinical and endoscopic responses were measured by clinical Mayo score and endoscopic Mayo subscore, respectively.

Results: After infliximab induction, 54.5% and 65.9% had clinical remission and mucosal healing, respectively. Post-induction CRP and FC were significantly lower in clinical responders versus nonresponders ($P = 0.01$ and 0.001, respectively) and in patients with mucosal healing than without ($P < 0.001$). Among all the parameters tested, FC had the best predictive value of clinical remission [Area under the curve (AUC = 0.826)] and mucosal healing (AUC = 0.949). Post-induction FC had 87.5% sensitivity and 89% specificity (cut-off <100 μg/g) for predicting clinical remission and 89.7% sensitivity and 93.3% specificity (cut-off <58 μg/g) for predicting mucosal healing.

Conclusions: Post-infliximab induction FC can be used as a surrogate marker for predicting clinical remission and mucosal healing in refractory UC patients.

Keywords: Fecal calprotectin, infliximab, mucosal healing, refractory ulcerative colitis

Address for correspondence: Dr. Elham A. Hassan, Tropical Medicine and Gastroenterology, Al-Rajhi liver Hospital, Assiut University Hospital, Assiut, Egypt.
E-mail: mam_elham75@yahoo.com

INTRODUCTION

Refractory ulcerative colitis (UC) is moderate-to-severe UC that is either refractory to or intolerant of conventional therapy and immunomodulators and its treatment is a clinical challenge that may end at colectomy.[1] Many patients present with inadequate or no response to steroid treatment (steroid-resistant or refractory colitis) or clinical relapse upon withdrawal of steroids (steroid-dependent

Access this article online	
Quick Response Code:	Website: www.saudijgastro.com
	DOI: 10.4103/sjg.SJG_599_16

How to cite this article: Hassan EA, Ramadan HK, Ismael AA, Mohamed KF, El-Attar MM, Alhelali I. Noninvasive biomarkers as surrogate predictors of clinical and endoscopic remission after infliximab induction in patients with refractory ulcerative colitis. Saudi J Gastroenterol 2017;23:238-45.

colitis) or present with active disease or relapse despite thiopurines at an appropriate dose for at least 3 months (immunomodulator-refractory colitis) and their medical treatment may prove ineffective or toxic, leading to surgical intervention.[2,3]

Early recognition of the severity of colitis, intensive treatment, and monitoring have contributed to improve the outcome.[4] The main endpoint of treatment in UC is the induction and maintenance of disease remission.[5]

In last years, infliximab (IFX), a chimeric monoclonal antibody directed against tumor necrosis factor-alpha (TNFα), has become an appropriate therapeutic approach for refractory UC patients.[6] These biological agents have proved to be effective in inducing and maintaining remission and substantially improving the clinical course.[5] Mucosal healing (MH) is considered an additional highly significant therapeutic target to achieve long-term remission and to change the natural course of UC.[7]

Colonoscopy is the gold standard in the diagnosis of mucosal healing. However, this approach has some limitations as endoscopy is invasive, costly, time-consuming, uncomfortable procedure for patients and carries the risk of perforation particularly in patients with severe disease.[8,9] To overcome these limitations, the possibility of noninvasive parameters for measuring the response to biological therapy is appealing because it could decrease the necessity of endoscopic evaluation along with reducing healthcare costs.

Numerous laboratory markers for predicting clinical and endoscopic remission, the main goals of therapy with anti-TNF-α agents in inflammatory bowel disease (IBD), have been discussed in previous studies with varying rate of success, i.e., hemoglobin (Hb), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and fecal calprotectin (FC).[8,9] Recent studies explained the role of FC as a reliable surrogate marker to estimate IBD activity and MH.[10-12]

A reliable and noninvasive marker for predicting clinical outcome and MH could provide clinicians with crucial information after the introduction of anti-TNFα treatment in these patients.[5] However, previous studies have shown conflicting results for prediction of the outcome of anti-TNF-α treatment in IBD.[13-15] The aim of our study was to investigate and compare the predictive power of different noninvasive biomarkers for clinical remission and MH to IFX induction therapy in patients with refractory UC and to identify the best parameter cut-off point to predict responses to treatment.

PATIENTS AND METHODS

Study design

This prospective study was carried out in the Gastroenterology Unit of Farwaniya (FAR) Hospital in Kuwait between January 2016 and December 2016. The study was approved by the ethical committee for medical research in the ministry of health, Kuwait and was conducted in accordance with the previsions of the Declaration of Helsinki. An informed written consent was obtained from each eligible patient who participated in the study.

Study population

Forty-four adult patients were consecutively recruited with an established diagnosis of UC based on clinical, endoscopic, and histological criteria with moderate-to-severe active disease based on Mayo clinical score[16] (≥6 points) and Mayo endoscopic subscore[17] (>2), and who were inadequately or not responding to any conventional therapy.

These patients were eligible to receive IFX induction therapy [(5 mg/Kg) intravenously at weeks 0, 2, and 6]. Patients with contraindications to anti-TNFα therapy, previously exposed to IFX, or any other anti-TNF agent, indeterminate colitis and other causes of colitis such as CMV colitis or patients with hemorrhoids were excluded.

Methods

At study entry (week 0 before IFX induction therapy), a thorough clinical history was obtained. Demographics, duration of the disease since diagnosis, and past and current medications for UC were reviewed. The severity and extent of the colitis at week 0 and at week 12 of IFX therapy were elicited. Patients underwent a complete physical examination. Blood and fecal samples were collected for laboratory investigations including complete blood count, ESR and CRP, and FC. Colonoscopy was performed at the Gastroenterology Unit, FAR hospital, Kuwait, using a CF-FH260AZL/I colonovideoscope (Olympus Inc., Tokyo Japan) by two experienced endoscopists in IBD who were blinded to medications taken by the patients, and the Mayo endoscopic subscore was calculated by consensus agreement of the two endoscopists for each patient. When disagreement between endoscopists occurred, the endoscopic subscore recorded by the senior endoscopist was approved.

Scheduled study visits were carried out and at week 12 after IFX induction therapy, clinical history, physical examination,

serum and fecal biomarker assessment, and colonoscopy were reevaluated to measure outcomes (clinical and endoscopic remission).

Disease activity and outcome measures

Clinical disease activity was assessed using Mayo clinical score where patients with a score of ≤2 were considered to be in clinical remission. Endoscopic activity was assessed by Mayo endoscopic subscore, and those with score of ≤1 were defined as having endoscopic remission (MH).

Measurement of fecal calprotectin

Fecal samples obtained prior to colonoscopy (at week 0 and 12 of IFX induction) were stored at −20°C. Fecal calprotectin levels were determined using an ELISA kit for calprotectin (Buhlmann Co., Switzerland) according to the manufacturer's instructions. The upper limit of normal FC in FAR hospital lab is <50 μg/g.

Statistical analysis

All statistical analyses were conducted using SPSS for windows version 16 (SPSS Inc., Chicago, IL. USA). Continuous data were expressed as means ± standard deviation (SD) or median (IQR) and compared using Student's and paired *t*-tests or Mann–Whitney U and Wilcoxon tests and correlated using Pearson's or Spearman's coefficient for normally or abnormally distributed data, respectively. Categorical variables were expressed as percentage and compared using Chi-square (χ^2) test. The area under receiver operating characteristic (AUC) curves were plotted to measure and compare the performance of different parameters in predicting the disease improvement and to select the best cut-off value with the highest accuracy [by calculating the sensitivity, specificity, positive (PPV), and negative predictive values (NPV), positive and negative likelihood ratios (+LR, −RL)]. Multiple regression analysis was used to determine risk factors for clinical and endoscopic remission. Statistical significance was considered when *P* value was <0.05.

RESULTS

Forty-four UC patients were consecutively and prospectively included in the study. Their mean age was 32.6 ± 1.6 years and 59.1% were men. All patients were previously treated with 5-aminosalicylates (5-ASA) and steroids, and 28 patients (64%) were steroid-dependent and 16 patients (36%) were steroid-resistant. Thiopurines were used in 25 patients (57%) and one patient (who failed to respond to thiopurines) received cyclosporine before IFX and did not respond. Disease extension was left-sided colitis (*n* = 27, 61.4%) and extensive colitis (*n* = 17, 38.6%). All patients were treated with IFX.

Table 1: Characteristics of the study patients at baseline (week 0) and after infliximab induction (follow up at week 12)

Variables	Total patients (*n*=44)
Age (mean±SD)	32.6±10.6
Sex (%)	
Males	26 (59.1%)
Females	18 (40.9%)
Median disease duration prior to IFX (range), months	56 (6-95)
Patient medication at baseline (%)	
5-Aminosalicylates	44 (100%)
Steroid therapy	
Steroid-dependent	28 (64%)
Steroid-resistant	16 (36%)
Immunosuppressant therapy (%)	
Thiopurines	25 (57%)
Cyclosporine	1 (2.3%)
Disease Extension (%)	
Left sided colitis	27 (61.4%)
Extended colitis	17 (38.6%)
Mean Hb at baseline (g/dL)	11.6±1.8
Mean Hb at follow-up (g/dL)	11.6±1.7
Mean WBCs at baseline (×109/L)	8.7±3.4
Mean WBCs at follow-up (×109/L)	8.1±2.5
Mean ESR at baseline (mm/h)	35.2±19.4
Mean ESR at follow-up (mm/h)	33±16.5
Median (mean) CRP at baseline (mg/L, IQR)	36 (35.5) (13-52.5)
Median (Mean) CRP at follow-up (mg/L, IQR)	32.5 (37.8) (12-49)
Median (mean) FC at baseline (μg/g, IQR)	393 (410) (185-625)
Median (mean) FC at follow-up (μg/g, IQR)	32 (149) (18-199)
Mean Mayo score at baseline	9.3±1.5
Mean Mayo score at follow-up	4.4±1.2
Clinical remission	24 (54.5%)
Mean endoscopic subscore at baseline	2.4±0.5
Mean Endoscopic subscore at follow-up	0.97±0.3
Mucosal healing	29 (65.9%)

SD: Standard deviation; IFX: Infliximab; Hb: Hemoglobin; WBCs: White blood cells; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; FC: Fecal calprotectin

The characteristics of the patients are shown in [Table 1]. Of the 44 patients, 24 (54.5%) had clinical remission, whereas 20 (45.5%) did not. On follow-up colonoscopy, MH was achieved in 29/44 (65.9%) patients [Table 1].

Assessment of laboratory parameters and their relation to clinical and endoscopic remission

Figure 1 shows that, in the cohort of patients with clinical remission at week 12 (follow-up), the median FC value reduced from 507 μg/g to 23 μg/g after IFX induction therapy ($P < 0.001$). In contrast, in patients who did not achieve clinical remission, the difference in FC values that reduced from 312 μg/g to 204 μg/g was not significant. At baseline, the FC values did not differ between patients who achieved clinical remission and those who did not (507 μg/g vs. 312 μg/g, $P = 0.08$), whereas after IFX induction, the latter group had significantly higher median FC values compared to the former group (204 μg/g vs. 23 μg/g, $P = 0.001$). In addition, the median CRP levels significantly decreased after anti-TNF α induction in

the group of patients with clinical remission at week 12 (37 mg/L vs. 16 mg/L, $P = 0.04$) and when compared with patients without clinical remission (42 mg/L vs. 16 mg/L, $P = 0.006$). No significant differences between patients regarding other parameters [Figure 1].

We found that CRP and FC values at follow-up colonoscopy (week 12 after IFX induction) were significantly lower in patients with MH compared to patients without MH (18 mg/L vs. 46 mg/L, $P = 0.02$, and 23 μg/g vs. 386 μg/g, $P < 0.001$, respectively), whereas CRP and FC values at baseline colonoscopy were not significantly different (40 mg/L vs. 38 mg/L, $P = 0.347$, 358 μg/g vs. 347 μg/g, $P = 0.197$, respectively). In patients with MH, CRP, and FC values at follow-up colonoscopy were significantly lower compared to CRP and FC values at baseline colonoscopy (38 mg/L vs. 18 mg/L, $P = 0.023$, and 347 μg/g vs. 23 μg/g, $P < 0.001$, respectively), whereas there was no significant difference of CRP and FC values from baseline to follow-up colonoscopy in patients without MH (40 mg/L vs. 46 mg/L, $P = 0.51$ and 358 μg/g vs. 386 μg/g, $P = 0.487$). There were no significant differences

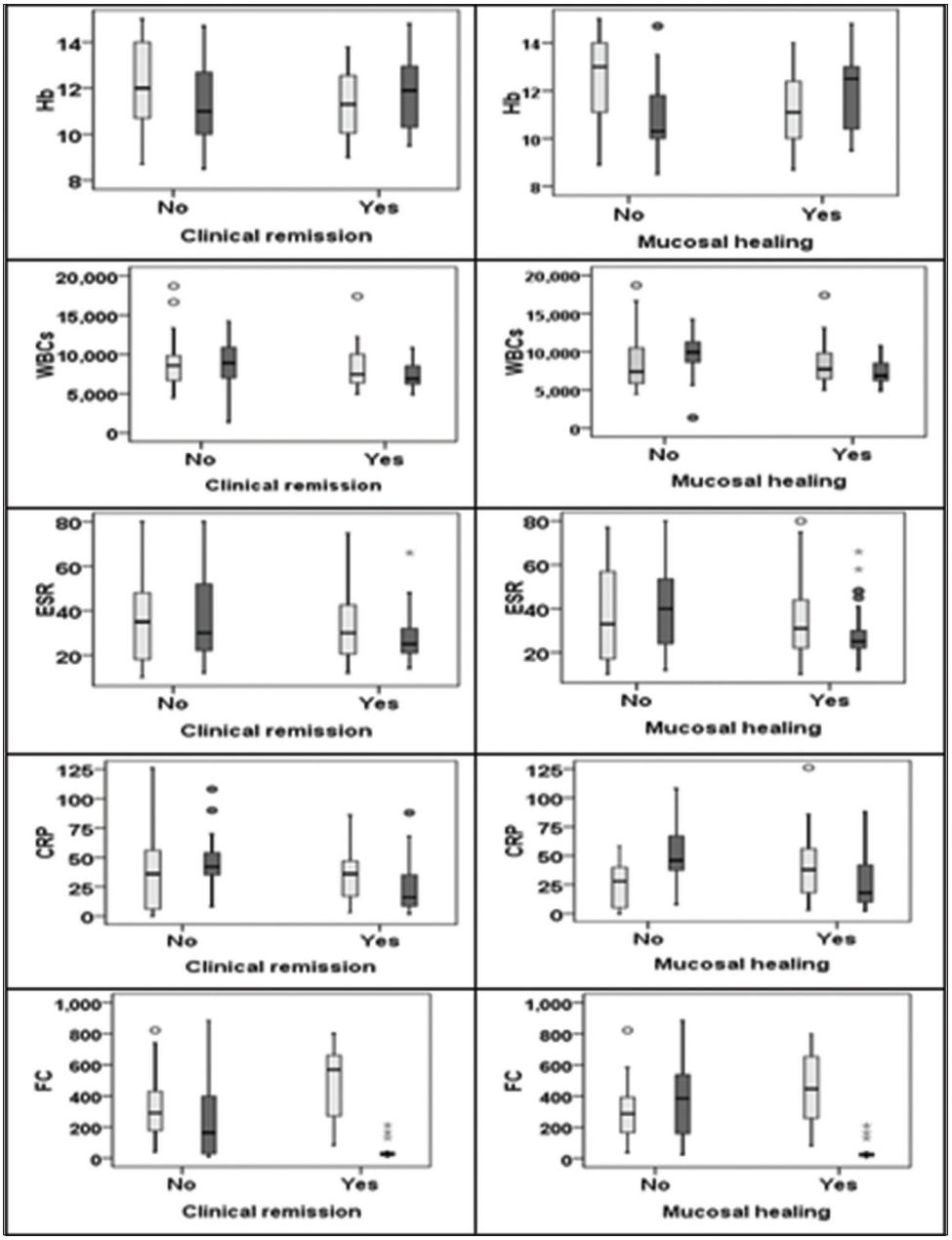

Figure 1: Laboratory parameters at baseline (Week 0) and after infliximab induction (follow-up at week 12). ☐ Variable at baseline (Week 0); ■ Variable at follow-up (Week 12). Hb: Hemoglobin; WBC: White blood cells; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; FC: Fecal calprotectin

between patients with and without MH regarding other parameters [Figure 1].

Comparison of predictive accuracy and determination of the best cut-off value of laboratory parameters for clinical and endoscopic remission

Based on the ROC curves, Hb, WBCs, ESR, CRP, and FC had good prognostic accuracy for the prediction of clinical remission after IFX induction [Figure 2a], where FC yielded the highest AUC (0.826) and 95% confidence interval (CI) (0.682–0.923, $P < 0.001$), with 87.5% sensitivity, 89% specificity, 86.9% PPV, 89.5% NPV, and 8 +LR at cut-off of <100 μg/g [Table 2].

In addition, by using the ROC curve, the ability of the post-induction FC values to predict mucosal healing at follow up colonoscopy (week 12 of IFX induction therapy) [Figure 2b] revealed the highest AUC (0.949) and 95%CI (0.838–0.992) with 89.7% sensitivity, 93.3% specificity, 96.3% PPV, 82.4% NPV and 13.4 + LR with a cut-off of <58 μg/g ($P < 0.001$) [Table 2].

Risk factor analysis for clinical and endoscopic remission

Multiple regression analysis was performed for patients with clinical remission and MH, and the variables included were age, gender, disease duration and extension, patients' medication, and laboratory data after IFX induction therapy. The analysis showed that the only variable retained in the models was the FC level, and it was the only variable that may predict clinical remission and mucosal healing ($P = 0.041$ and 0.02, respectively) [Table 3].

DISCUSSION

UC is a lifelong disease with generally unpredictable clinical course that requires colonoscopy for diagnosis and disease monitoring. Identifying markers that allow noninvasive diagnosis and disease monitoring would be of significant advantage, especially in refractory UC. Several studies have investigated the use of noninvasive markers to predict or monitor the response to medical treatment.[8-12]

This study evaluated only short-term (90 days) response to IFX in patients with refractory UC because almost always long-term remission is established in this period of time, and when a patient does not respond after three drug infusions, this predicts that he will not respond to further administration of the drug.[18,19] In the present study, clinical remission and MH were achieved in 54.5% and 65.9% after IFX induction course, respectively.

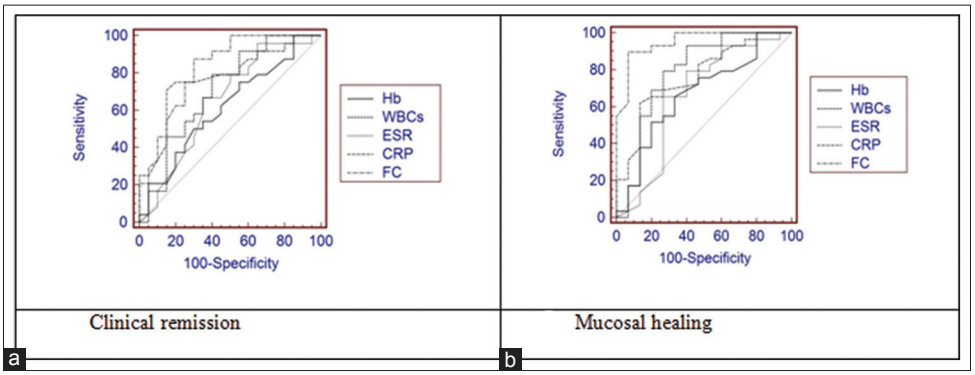

Figure 2: Area under the receiver operating characteristic curve (AUC) of laboratory parameters to predict clinical (a) and endoscopic (b) remission. FC had the highest AUC in predicting clinical remission (AUC = 0.826) and mucosal healing (AUC = 0.949). Hb: Hemoglobin; WBC: White blood cells; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; FC: Fecal calprotectin

Table 2: Diagnostic accuracy of laboratory parameters to predict clinical and endoscopic remission with the best predictive cut-offs

	AUC	SE	SP	PPV	NPV	+LR	-LR	Accuracy
Clinical remission								
Hb >10.3	0.617 (0.458-0.759)	75%	45%	62%	60%	1.36	0.56	64.8%
WBCs <8.6	0.708 (0.552-0.835)	79.2%	60%	70.3%	70.7%	1.98	0.35	72.7%
ESR <27	0.651 (0.493-0.788)	66.7%	65%	69.5%	62%	1.9	0.51	66.1%
CRP <28	0.774 (0.623-0.886)	70.8%	85%	85%	70.8%	4.72	0.34	75.6%
FC <100	0.826 (0.682-0.923)	87.5%	89%	86.9%	89.5%	8	0.14	88.3%
Endoscopic remission (mucosal remission)								
Hb >11	0.668 (0.510-0.802)	65.5%	66.7%	79.2%	50%	1.97	0.52	65.9%
WBCs <8.7	0.789 (0.639-0.897)	75.5%	73.3%	85.2%	64.7%	2.8	0.33	74.7%
ESR <27	0.661 (0.503-0.797)	65.5%	73.3%	82.6%	52.4%	2.45	0.47	68.2%
CRP <28	0.766 (0.614-0.880)	70.8%	85%	90.1%	60.1%	4.72	0.34	75.6%
FC <58	0.949 (0.838-0.992)	89.7%	93.3%	96.3%	82.4%	13.4	0.11	90.9%

Hb: Hemoglobin; WBCs: White blood cells; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; FC: Fecal calprotectin

Table 3: Multiple regression analysis of risk factors affected clinical and endoscopic remission in the studied sample

	Clinical remission		Mucosal healing	
	Odds ratio (95%CI)	*P*	Odds ratio (95%CI)	*P*
Age	3.51 (2.88-6.21)	0.498	2.08 (0.96-3.62)	0.553
Sex	1.27 (0.02-11.58)	0.803	2.6 (1.04-7.09)	0.629
Disease duration	1.08 (0.05-1.09)	0.665	1.11 (0.68-1.72)	0.503
Steroid therapy	2.28 (0.64-8.19)	0.440	1.1 (0.06-3.25)	0.720
Immunosuppressant therapy	2.64 (0.285-4.13)	0.185	2.96 (1.01-4.93)	0.186
Disease Extension	3.7 (0.99-11.4)	0.335	3.98 (0.16-11.12)	0.327
Hb	0.97 (0.36-2.54)	0.372	0.61 (0.36-1.04)	0.071
WBCs	0.85 (0.67-1.02)	0.252	0.5 (0.4-1.02)	0.625
ESR	1.02 (0.98-1.50)	0.08	0.94 (0.88-1.01)	0.121
CPR	0.95 (0.88-1.04)	0.191	0.92 (0.90-1.01)	0.085
FC	0.97 (0.94-1)	0.041	1.02 (0.99-1.16)	0.02

Hb: Hemoglobin; WBCs: White blood cells; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; FC: Fecal calprotectin

Our study assessed different noninvasive markers (Hb, WBCs, ESR, CRP, and FC) to elaborate reliable methods to predict outcome, and we demonstrated that among these markers, FC levels measured after IFX induction course can be used as a surrogate marker to estimate the efficacy of IFX as well as to predict the clinical response and MH. Our findings were compatible with that of D'Haens *et al.*[12] who found a significant correlation between FC values and endoscopic disease scores (Mayo subscores) for predicting endoscopic remission. Zittan *et al.*[20] also demonstrated that the FC test highly correlated with clinical symptoms in UC but less so in colonic CD. However, Tursi *et al.*[21] observed that FC correlated with clinical and endoscopic severity of IBD.

The present work showed that CRP and FC levels were significantly lower in patients with clinical remission than those without; however, only FC was significantly lower in patients with endoscopic remission than those without. This finding can be explained by the fact that FC appears to be a better indicator of mucosal inflammation than serum biomarkers, including CRP.[10,11,22]

On monitoring the response to IFX, FC had the highest AUC for prediction of clinical and endoscopic remission compared to serum markers. Regarding clinical remission, FC had 87.5% sensitivity, 89% specificity, and 86.9% PPV at cut-off <100 μg/g. Our results are consistent with the previously published data in this field. Molander *et al.*[14] reported that FC level is a useful marker for predicting clinical outcome after anti-TNF-α treatment. On the other hand, Tursi *et al.*[23] showed that FC test had lower diagnostic accuracy (66.7% sensitivity, 56.1% specificity, and 18.2% PPV) in predicting clinical remission in UC patients under treatment with anti-TNFα; moreover, they concluded that rapid FC seemed better in predicting persistence of endoscopic lesions than clinical remission.

In addition, many authors have observed that patients in clinical remission exhibited low FC levels, whereas patients with high levels of this biomarker were at an increased risk of relapsing, suggesting that the FC level might be used not only as a marker of clinical remission but also to predict relapse.[24,25]

Most physicians still use a clinical activity score in their everyday practice for patient monitoring, favoring it over endoscopy and biomarkers in the management of their patients.[26] However, the concept of attaining MH as the primary endpoint of treatment in IBD has been established that may be associated with lower relapse rates, hospitalization rates, and reduced need for surgery.[27]

For prediction of MH in our study, FC had 89.7% sensitivity, 93.3% specificity, and 96.3% PPV with cut-off <58 μg/g. In addition, about two-thirds of UC patients showed normalization of FC levels at week 12 of follow-up, and all of them achieved MH according to endoscopic Mayo subscore; hence, no discrepancies between MH and FC level normalization were observed in our study. Our results are consistent with De Vos *et al.*[13] who showed that FC value <50 mg/kg was a very good predictor of mucosal healing at week 10 of IFX induction. Szczepański *et al.*[28] stated that FC is a good biomarker of MH with values below 54 μg/g enabling to select 77% patients with full MH.

Unlike our results, several studies experienced different FC cut-off values for predicting MH in UC; Lin *et al.*[29] and Theede *et al.*[30] demonstrated that a calprotectin level below 190 μg/g was associated with mucosal healing in UC (UCEIS and Mayo Endoscopic Score). These findings confirmed that FC can reflect endoscopic disease activity with different endoscopic scores.

Calprotectin is a marker of neutrophil migration to the intestinal lumen,[31] and its high level is associated with increased inflammation in the intestine.[32,33] Further, FC is a better predictor of mucosal inflammation than other acute

phase reactants such as ESR and CRP, which is consistent with data from other studies.[31,34] Therefore, monitoring FC may decrease the need for invasive procedures to distinguish patients with quiescent disease who develop functional symptoms from those with active inflammation and who may benefit from escalation in therapy.[20] FC is a reliable, simple, inexpensive, and safe test that correlates closely with endoscopic findings in UC.[35,36] It provides more objective results than fecal microscopy and bypasses the difficulty of identifying stool leukocytes.[37]

Our limitations were being a single-center and small sample sized study, as well as lack of histological assessment, and hence, lack of estimation of biomarker cut-off values for prediction of histological remission.

To our knowledge, it is one of the first studies in Gulf countries to address IFX therapy in refractory colitis. Therefore, these findings need to be confirmed by more prospective studies with larger populations and to determine whether these biomarkers will be of benefit for the more severely affected patients who are unable to undergo endoscopic screening. In addition, further studies are needed to assess long-term outcome after the induction therapy of IFX.

CONCLUSION

Post-IFX induction FC can be used as a surrogate marker for predicting clinical remission and MH in patients with refractory UC.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Garud S, Peppercorn MA. Ulcerative colitis: Current treatment strategies and future prospects. Therap Adv Gastroenterol 2009;2:99-108.
2. Chey WY. Infliximab for Patients With Refractory Ulcerative Colitis. Inflamm Bowel Dis 2001;7: S30-3.
3. Stange EF, Travis SP, Vermeire S, Reinisch W, Geboes K, Barakauskiene A, *et al.* European evidence-based Consensus on the diagnosis and management of ulcerative colitis: Definitions and diagnosis. J Crohns Colitis 2008;2:1-23.
4. Rizzello F, Gionchetti P, Venturi A, Campieri M. Review article: Medical treatment of severe ulcerative colitis. Aliment Pharmacol Ther 2003;17:7-10.
5. Guidi L, Marzo M, Andrisani G, Felice C, Pugliese D, Mocci G, *et al.* Faecal calprotectin assay after induction with anti-Tumour Necrosis Factor α agents in inflammatory bowel disease: Prediction of clinical response and mucosal healing at one year. Dig Liver Dis 2014;46:974-9.
6. Chey WY, Hussain A, Ryan C, Potter GD, Shah A. Infliximab for refractory ulcerative colitis. Am J Gastroenterol 2001;96:2373-81.
7. Rutgeerts P, Vermeire S, Van Assche G. Mucosal healing in inflammatory bowel disease: Impossible ideal or therapeutic target. Gut 2007;56:453-5.
8. Schoepfer AM, Beglinger C, Straumann A, Trummler M, Renzulli P, Seibold F. Ulcerative colitis: Correlation of the Rachmilewitz endoscopic activity index with fecal calprotectin, clinical activity, C-reactive protein, and blood leukocytes. Inflamm Bowel Dis 2009;15:1851-8.
9. Angriman I, Scarpa M, D'Inca R, Basso D, Ruffolo C, Polese L, *et al.* Enzymes in feces: Useful markers of chronic inflammatory bowel disease. Clin Chim Acta2007;381:63-8.
10. Schoepfer AM, Beglinger C, Straumann A, Trummler M, Vavricka SR, Bruegger LE, *et al.* Faecal calprotectin correlates more closely with the Simple Endoscopic Score for Crohn's disease (SES-CD) than CRP, blood leukocytes, and the CDAI. Am J Gastroenterol2010;105:162-9.
11. Schoepfer AM, Beglinger C, Straumann A. Faecal calprotectin more accurately reflects endoscopic activity of ulcerative colitis than the Lichtiger Index, C-reactive protein, platelets, hemoglobin, and blood leukocytes. Inflamm Bowel Dis2013;19:332-41.
12. D'Haens G, Ferrante M, Vermeire S, Baert F, Noman M, Moortgat L, *et al.* Faecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. Inflamm Bowel Dis 2012;18:2218-24.
13. De Vos M, Dewit O, D'Haens G, Baert F, Fontaine F, Vermeire S, *et al.* Fast and sharp decrease in calprotectin predicts remission by infliximab in anti-TNF naïve patients with ulcerative colitis. J Crohn's Colitis 2012;6:557-62.
14. Molander P, af Björkesten CG, Mustonen H, Haapamäki J, Vauhkonen M, Kolho KL, *et al.* Faecal calprotectin concentration predicts outcome in inflammatory bowel disease after induction therapy with TNFα blocking agents. Inflamm Bowel Dis 2012;18:2011-7.
15. Laharie D, Mesli S, El Hajbi F, Chabrun E, Chanteloup E, Capdepont M, *et al.* Prediction of Crohn's disease relapse with faecal calprotectin in infliximab responders: A prospective study. Aliment Pharmacol Ther 2011;34:462-9.
16. Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. N Engl J Med 1987;317:1625-9.
17. Samaan MA, Mosli MH, Sandborn WJ, Feagan BG, D'Haens GR, Dubcenco E, *et al.* A systematic review of the measurement of endoscopic healing in ulcerative colitis clinical trials: Recommendations and implications for future research. Inflamm Bowel Dis 2014;20:1465-71.
18. Florholmen J, Øverland G, Olsen T, Rismo R, Cui G, Christiansen I, *et al.* Short-and Long-Term Clinical Outcomes of Infliximab in Fulminant Ulcerative Colitis. Ulcers 2011;2011:156407.
19. Nieminen U, Turunen U, Arkkila P, Sipponen T, Färkkilä M. Infliximab for active ulcerative colitis: A prospective study on patients' short-term response and long-term outcome. Poster presentations (P594). Clinical Therapy & Observation; 2016.
20. Zittan E, Kelly OB, Kirsch R, Milgrom R, Burns J, Nguyen GC, *et al.* Low Fecal Calprotectin Correlates with Histological Remission and Mucosal Healing in Ulcerative Colitis and Colonic Crohn's Disease. Inflamm Bowel Dis 2016;22:623-30.
21. Tursi A, Elisei W, Giorgetti G, Picchio M, Brandimarte G. Rapid fecal calprotectin correlates with clinical and endoscopic severity of inflammatory bowel diseases. Scand J Gastroenterol 2013;48:1359-60.
22. Yoon JY, Park SJ, Hong SP, Kim TI, Kim WH, Cheon JH. Correlations of C-reactive protein levels and erythrocyte sedimentation rates with endoscopic activity indices in patients with ulcerative colitis. Dig Dis Sci 2014;59:829-37.
23. Tursi A, Elisei W, Picchio M, Giorgetti G, Brandimarte G. Accuracy of Rapid Fecal Calprotectin Test in Monitoring Inflammatory Bowel Diseases Under Treatment with TNFα Antagonists. Dig Dis Sci 2015;60:1406-13.
24. Tibble JA, Sigthorsson G, Bridger S, Fagerhol MK, Bjarnason I. Surrogate markers of intestinal inflammation are predictive of relapse in patients with inflammatory bowel disease. Gastroenterology 2000;119:15-22.
25. Gisbert JP, Bermejo F, Pérez-Calle JL, Taxonera C, Vera I,

McNicholl AG, *et al*. Faecal calprotectin and lactoferrin for the prediction of inflammatory bowel disease relapse. Inflamm Bowel Dis 2009;15:1190-8.
26. Schoepfer AM, Vavricka S, Zahnd-Straumann N, Straumann A, Beglinger C. Monitoring inflammatory bowel disease activity: Clinical activity is judged to be more relevant than endoscopic severity or biomarkers. J Crohns Colitis2012;6:412-8.
27. Colombel JF, Rutgeerts P, Reinisch W, Esser D, Wang Y, Lang Y, *et al*. Early mucosal healing with infliximab is associated with improved long-term clinical outcomes in ulcerative colitis. Gastroenterology 2011;141:1194-201.
28. Szczepański M, Dądalski M, Szymańska E, Meglicka M, Woynarowski M, Kierkuś J. Fecal calprotectin is a good biomarker of mucosal healing in monitoring of children with IBD. Postępy Nauk Medycznych 2014;27:150-3.
29. Lin W, Wong J, Tung C, Lin CP, Chou JW, Wang HY, *et al*. Fecal calprotectin correlated with endoscopic remission for Asian inflammatory bowel disease patients. World J Gastroenterol 2015;21:13566-73.
30. Theede K, Holck S, Ibsen P, Ladelund S, Nordgaard-Lassen I, Nielsen AM. Level of Fecal Calprotectin Correlates With Endoscopic and Histologic Inflammation and Identifies Patients With Mucosal Healing in Ulcerative Colitis. Clin Gastroenterol Hepatol 2015;13:1929-36.
31. Costa F, Mumolo MG, Ceccarelli L, Bellini M, Romano MR, Sterpi C, *et al*. Calprotectin is a stronger predictive marker of relapse in ulcerative colitis than in Crohn's disease. Gut 2005;54:364-8.
32. Langhorst J, Elsenbruch S, Koelzer J, Rueffer A, Michalsen A, Dobos GJ. Noninvasive markers in the assessment of intestinal inflammation in inflammatory bowel diseases: Performance of fecal lactoferrin, calprotectin, and PMN-elastase, CRP, and clinical indices. Am J Gastroenterol 2008;103:162-9.
33. Canani RB, Terrin G, Rapacciuolo L, Miele E, Siani MC, Puzone C, *et al*. Faecal calprotectin as reliable non-invasive marker to assess the severity of mucosal inflammation in children with inflammatory bowel disease. Dig Liver Dis 2008;4:547-53.
34. Johne B, Fagerhol MK, Lyberg T, Prydz H, Brandtzaeg P, Naess-Andresen CF, *et al*. Functional and clinical aspects of the myelomonocyte protein calprotectin. Mol Pathol 1997;50:113-23.
35. van Rheenen PF, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: Diagnostic meta- analysis. BMJ2010;341:c3369.
36. Yang Z, Clark N, Park KT. Effectiveness and cost-effectiveness of measuring fecal calprotectin in diagnosis of inflammatory bowel disease in adults and children. Clin Gastroenterol Hepatol 2014;12:253-62.
37. Limburg PJ, Ahlquist DA, Sandborn WJ, Mahoney DW, Devens ME, Harrington JJ, *et al*. Fecal calprotectin levels predict colorectal inflammation among patients with chronic diarrhea referred for colonoscopy. Am J Gastroenterol2000;95:2831-7.