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The utility of serum anti-tumour necrosis factor levels and biomarkers in predicting endoscopic activity in inflammatory bowel disease

Abstract

Introduction: Although higher anti-tumour necrosis factor (anti-TNF) levels are associated with higher rates of clinical remission, much less is known regarding the associations between biomarkers and endoscopic findings in patients with inflammatory bowel disease (IBD).

Materials & methods: A retrospective analysis of patients with available anti-TNF levels attending an IBD clinic at a tertiary centre from 1st January 2014 – 31st May 2020 was completed. Demographics, disease activity scores, endoscopic findings and biomarker data were collected. The area-under-curve (AUC) on the receiver operating characteristic curve was plotted to measure and compare the performance of variables in predicting endoscopic remission. This was supplemented by linear discriminant analysis.

Results: One hundred and eighty patients were included. Faecal calprotectin was better at distinguishing between quiescent and active endoscopic disease than anti-TNF level .AUC 0.78 (95% CI 0.68 – 0.89) versus 0.62 (95% CI 0.48 – 0.75). Faecal calprotectin had a higher sensitivity (77% vs 50%) and specificity (71% vs 64%) compared to anti TNF levels in predicting endoscopic activity. We found that a faecal calprotectin threshold of 200ug/g and similarly an anti TNF level of 6.21ug/ml optimally predicts endoscopic disease. Using linear discriminant analysis, faecal calprotectin was weighted against C-reactive protein (CRP), albumin, platelet count, anti-TNF, and has shown to be better at predicting mild to moderate disease activity (Log FC=0.74).

Conclusion: Faecal calprotectin as opposed to anti-TNF level is more likely to predict endoscopic disease activity. We plan to do a prospective study to confirm these findings and to develop a set of clinical cut-offs to improve disease management.

Keywords: biomarkers, faecal calprotectin, anti-tumour necrosis factor, endoscopic activity, inflammatory bowel disease

Volume 13 Issue 2 - 2022

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Received: March 12, 2020 | **Published:** March 25, 2022

Abbreviations: IBD, inflammatory bowel disease; anti-TNF, anti-tumour necrosis factor; AUC, area-under-curve; CRP, C-reactive protein; IFX, infliximab; ADA, adalimumab; UC, ulcerative colitis; CDA1, crohn's disease activity index; SD, standard deviation; ROC, receiver operating characteristic

Introduction

Anti- tumour necrosis factor (TNF) therapies such as infliximab (IFX) and adalimumab (ADA) have been widely used for the induction and maintenance of remission of inflammatory bowel disease (IBD) as well as for rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis[1] and have improved clinical outcomes in immune-mediated inflammatory conditions, although not universally effective in all patients.[2]

Therapeutic drug monitoring to guide anti-TNF dosing has emerged as a strategy to minimise primary nonresponse or secondary loss of response. It involves measuring drug levels and anti-drug antibodies to optimise treatment and to prevent further flares or failure of response.[3] The therapeutic range of serum anti-TNF may not be reached due to insufficient dosing of the drug[4] and other parameters that influence pharmacokinetics include disease activity, male sex, presence of anti-drug antibodies, lower albumin levels and body mass index.[5] Some patients may not respond as the mechanism driving mucosal inflammation in IBD may not be related to TNF.

Several studies have examined the correlation between serum anti-TNF levels and clinical outcomes, with a literature review across seven observational studies, a systematic review, meta-analysis and a post-hoc analysis of a clinical trial describing a correlation between serum anti-TNF levels and clinical remission, with an optimal therapeutic cut-off point suggested to distinguish patients with clinical remission from those with active IBD.[6-10] Serologic biomarkers such as CRP and platelet count have also been explored in relation to IBD,[11] with some studies examining the associations between biomarkers, trough levels and endoscopic remission.[12,13] Grinman et al. confirmed the presence of significant associations between anti-TNF trough levels and non-invasive biomarkers including albumin and CRP, but with no correlations to disease outcome.[12] Another study by Robin et al. outlined a model using trough level and faecal calprotectin for predicting loss of response.[14] The objective of this study was to assess anti-TNF level in relation to other biomarkers in predicting endoscopic remission in IBD.

Materials and methods

Study design and population

This is a retrospective study of 180 patients with available serum anti-TNF levels who attended an outpatient IBD clinic at a tertiary hospital from 1st January 2014 to 31st May 2020. The diagnosis of Crohn's disease (CD) or ulcerative colitis (UC) was routinely

confirmed by clinical, endoscopic, and histological parameters. All patients on anti-TNF therapy were on standard dosing regimens. The inclusion criteria were patients with a confirmed diagnosis of either CD or UC on stable maintenance therapy with an anti-TNF agent, who had serum anti-TNF levels taken within the six month overlap period from their biochemical markers including faecal calprotectin levels and colonoscopy. The study was approved by the local ethics committee and performed in accordance to the Helsinki Declaration.

Demographic data and clinical disease activity

Demographic data, clinical characteristics, date of diagnosis and disease duration were retrieved from clinical databases and electronic records. Clinical disease activity evaluation was determined by disease activity scores including Crohn's Disease Activity Index CDAI and Mayo score).

Anti-TNF levels and biomarkers

We used CRP, platelet, albumin and faecal calprotectin levels which were captured close to the time of serum anti-TNF levels. A total of 272 visits were recorded, and some patients may have had two or more laboratory results.

The anti-TNF levels were measured using an enzyme-linked immunosorbent assay. Patients on IFX and ADA were considered to have therapeutic levels if the trough level was above 3ug/ml and 5ug/ml respectively. All samples with an undetectable IFX or ADA level underwent testing for anti-drug antibodies. An elevated faecal calprotectin was defined as more than 200µg/g. The reference range for normal CRP levels was <5mg/L, platelet count was 150-400 x 10^9/L and for albumin was 35-50 g/L.

Assessment of endoscopic activity

For severity of UC, the Mayo score on a scale of 0 (least severe) to 3 (most severe) was used. For severity of CD, the presence of erythema, loss of vascular markings with or without scattered aphthae signified mild inflammation, while the presence of diffuse aphthous ulcerations or larger shallow ulcerations signified moderate inflammation. Severe inflammation was seen as deep ulcerations with or without narrowing. Endoscopic remission was defined as the lack of visible mucosal inflammation such as erosions, ulcers, granularity, or friability during endoscopy. The endoscopist reported and graded the degree of mucosal inflammation as 0 (uninflamed), 1 (mild), 2 (moderate) or 3 (severe).

Statistical analysis

Statistical analyses were performed using R software version 4.1.1. Continuous data were summarised as means with standard deviation (SD), and medians with minimum and maximum values. Pearson correlation coefficients estimated the linear association between anti-TNF serum levels and biomarkers. Associations between therapeutic levels of anti-TNF and remission were evaluated using Fisher's exact test. The Area-Under-Curve (AUC) on the receiver operating characteristic curve was plotted to measure and compare the performance of variables in predicting endoscopic remission. Linear discriminant analysis was performed to determine which biomarkers were better at predicting endoscopic remission. All tests were two-tailed, and P values less than 0.05 were assessed as statistically significant.

Results

Clinical characteristics

One hundred and eighty patients were included in this study. The median age of subjects was 36 (13, 88) years and 51% were female. All patients either had CD (n=143; 80%) or UC (n=33; 18%), with 4 patients (2%) being indeterminate. The majority received IFX (n=145; 81%) as compared to ADA (n=35; 19%). The means of albumin and platelet count were found to be within normal limits. The mean CRP was found to be borderline raised (10 mg/L) while the mean of faecal calprotectin was elevated (865µg/g). The mean CDAI score was 98.8 (≤ 150; in remission). The mean partial Mayo score was 1.27 (< 2; in remission). The clinical characteristics are shown on Table 1.

Table 1 Clinical characteristics

	Total patients=180
Age(years)	
Mean(SD)	38(15)
Median [Range]	36 [13, 88]
Gender	
Female	92(51%)
Male	88(49%)
Biologic agent	
IFX	145(81%)
ADA	35(19%)
IBD	
CD	143(80%)
UC	33(18%)
Indeterminate	4(2%)
Albumin, g/L(n=173)	
Mean(SD)	40.3(3.8)
Median [Range]	40 [28, 49]
CRP, mg/L(n=175)	
Mean(SD)	10(18)
Median [Range]	3 [0, 166]
Platelet count, x10^9/L(n=176)	
Mean(SD)	275(82)
Median [Range]	265 [142,658]
Faecal calprotectin, µg/g(n=85)	
Mean(SD)	865(1314)
Median [Range]	220 [5,5600]

Assessment of anti-TNF level in relation to other biomarkers

Using logarithmic scales, estimated correlation coefficients between anti-TNF serum levels and biomarkers confirmed the following: CRP (r=-0.359; p <0.001), platelet count (r=-0.244; p <0.001) and faecal calprotectin (r=-0.238; p <0.05) all correlated negatively while albumin (r=0.283; p <0.001) correlated positively with anti-TNF levels (Figure 1).

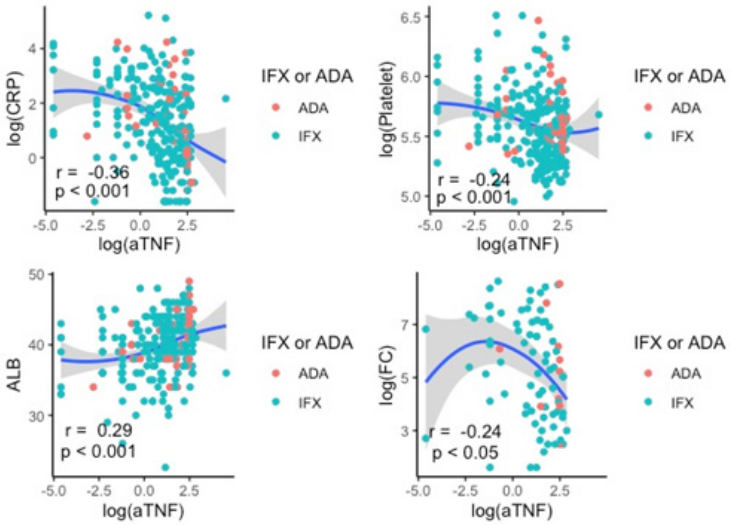

Figure 1 Logarithmic scales of biomarkers (CRP, platelet count, faecal calprotectin and albumin) against anti-TNF levels.

The total number of both available faecal calprotectin and anti-TNF levels was 83, with majority of patient visits having elevated faecal calprotectin (n=43/83; 52%). Patients with sub therapeutic anti-TNF levels had mostly raised faecal calprotectin (n=24/34; 71%) of more than 200 µg/g compared with patients with therapeutic anti-TNF levels (19/49; 49%). Overall, patients with therapeutic anti-TNF levels were less likely to have raised faecal calprotectin (p=0.007) (Table 2).

Table 2 Faecal calprotectin versus anti-TNF levels

Characteristic	Sub-therapeutic levels, N=34[1]	Therapeutic levels, N=49[1]	Overall, N=83[1]	p-value[2]
Faecal Calprotectin				**0.007**
Elevated	24(71%)	19(39%)	43(52%)	
Normal	10(29%)	30(61%)	40(48%)	

[1]n(%)

[2]Fisher's exact test

Sensitivity and specificity of anti-TNF level and faecal calprotectin in predicting endoscopic findings

There were a total of 235 available anti-TNF levels in 180 patients. In half of these cases, quiescent endoscopic disease activity was noted (n=117; 50%). Of these, 75 (64%) had therapeutic anti-TNF levels, that is, a specificity of 64%. In addition, 59/118 (50%) where disease activity was reported also reported sub therapeutic anti-TNF levels, or a sensitivity of 50%. There was a strong association between therapeutic anti-TNF levels and disease activity (p=0.003) (Table 3).

Table 3 Faecal calprotectin and anti-TNF levels versus endoscopic findings

Characteristic	quiescent, N=117[1]	mild, N=73[1]	Moderate/severe, N=45[1]	p-value[2]
Faecal calprotectin				**<0.001**
Elevated	10(29%)	19(79%)	11(73%)	
Normal	25(71%)	5(21%)	4(27%)	
Anti-TNF				**0.003**
Sub-therapeutic levels	42(36%)	30(41%)	29(66%)	
Therapeutic levels	75(64%)	43(59%)	15(34%)	

[1]n(%)

[2]Fisher's exact test

Elevated faecal calprotectin was a very specific indicator of disease activity with approximately just over 70% (n =25/117; 71%) with quiescent disease had normal faecal calprotectin. Similarly elevated faecal calprotectin was highly sensitive for disease activity. Approximately 77% (30/39) reported elevated faecal calprotectin. There was a strong association between elevated faecal calprotectin and disease activity (p < 0.001) (Table 3).

Citation: Tan J, Neeman T, Subramaniam K. The utility of serum anti-tumour necrosis factor levels and biomarkers in predicting endoscopic activity in inflammatory bowel disease. *Gastroenterol Hepatol Open Access.* 2022;13(2):53–58. DOI: 10.15406/ghoa.2022.13.00494

Diagnostic Receiver Operating Characteristic (ROC) curves mirrored these findings.[15] The AUC for anti-TNF level distinguishing between quiescent and active endoscopic disease was 0.62 (95% CI 0.48 – 0.75) indicating that anti-TNF level was at best a weak predictor of activity. In contrast, the AUC for faecal calprotectin distinguishing between quiescent and active endoscopic disease was 0.78 (95% CI 0.68 – 0.89) shown in Figure 2 and Table 4. Our study found that a faecal calprotectin threshold of 200ug/g optimally predicts endoscopic activity. Similarly, a threshold of 6.21ug/ml for anti TNF level optimally predicts endoscopic disease. However, CRP, albumin and platelet count were not predictive of endoscopic activity as can be seen in the corresponding AUC and confidence intervals.

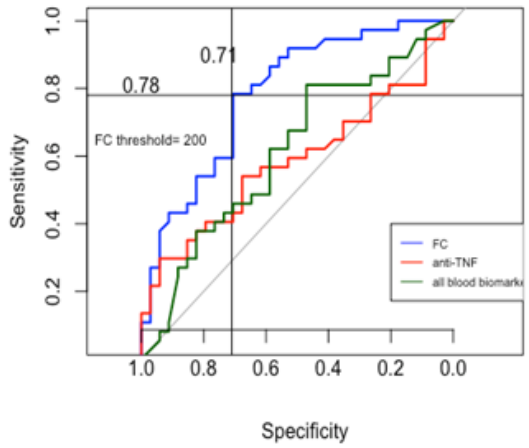

Figure 2 Area Under ROC Curves.

Table 4 Areas under ROC Curves between predictors

Predictor	AUC	Lower95	Upper95
FC	0.78	0.68	0.89
Anti-TNF	0.62	0.48	0.75
CRP	0.49	0.36	0.63
ALB	0.57	0.43	0.7
Platelets	0.53	0.39	0.66
All blood markers	0.59	0.45	0.72

Combining biomarker information to improve endoscopic disease activity prediction

In seventy-one cases, complete biomarker data for albumin, platelet count, CRP, and faecal calprotectin as well as anti TNF levels were available. We used these 71 observations in a linear discriminant analysis to explore how biomarker information (scaled) could be combined to improve endoscopic disease activity prediction (Figure 3). The linear discriminant analysis is a weighted average of the blood biomarkers to optimally discriminate between quiescent, mild, and moderate endoscopic disease. The positive scale in first linear discriminant analysis (LDA1) reflects biomarkers that predict mild, moderate and severe endoscopic disease activity. The positive scale in the second linear discriminant analysis (LDA2) reflects biomarkers that predict quiescent endoscopic disease activity. Elevated faecal calprotectin and low albumin were the strongest predictors of endoscopic disease activity (shown in Table 5). However, overall linear discriminant analysis was no better at predicting disease activity than faecal calprotectin on its own AUC 0.75 (0.63 – 0.86).

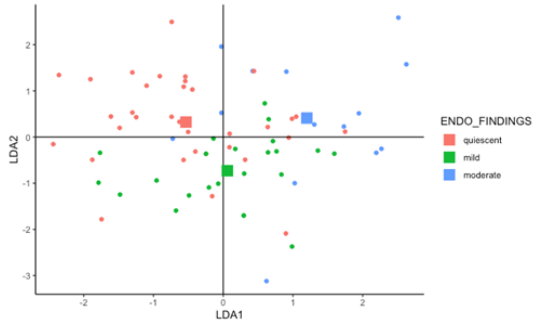

Figure 3 Linear discriminant analysis of endoscopic findings.

Table 5 Weightings of biomarkers on linear discriminant scores

Biomarker(scaled)	LDA1	LDA2
Log FC	0.75	-0.90
Log CRP	-0.23	0.29
Log anti-TNF	-0.32	-0.12
ALB	-0.62	-0.81
Log Platelets	-0.04	0.007

Discussion

As therapeutic targets have evolved from clinical remission to mucosal healing, it is crucial to have non-invasive biomarkers of mucosal inflammation for treat-to-target strategies in IBD. The Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) II guidelines by the International Organisation for the study of Inflammatory Bowel Disease examined different treatment targets including patient-reported outcome, endoscopic, histological and biomarker remission. Biomarker remission was suggested as an intermediate treatment target for both Crohn's Disease and Ulcerative colitis.[16] Non-invasive markers such as CRP and faecal calprotectin that can predict the level of disease activity and prognosis can be advantageous due to minimisation of risks and costs associated with invasive procedures. Few studies have compared the utility of routine serum anti-TNF level monitoring to faecal calprotectin and other biomarkers in patients with either CD or UC.[14]

Our study assessed various non-invasive markers (CRP, albumin, platelet count, faecal calprotectin, and anti-TNF levels) to determine a reliable method to predict endoscopic outcomes, and we found that the area under ROC curve for anti-TNF level was 0.62 (95% CI 0.48 – 0.75), lower than for faecal calprotectin at 0.78 (95% CI 0.68 – 0.89). This meant that faecal calprotectin as opposed to anti-TNF level was more likely to predict endoscopic activity in addition to distinguishing between quiescent and active endoscopic disease. Our study confirms that a faecal calprotectin threshold of 200ug/g optimally predicts endoscopic activity. Our linear discriminant analysis results also revealed that high positive faecal calprotectin was the best predictor of mild and moderate endoscopic disease activity, with high albumin being a reliable indicator of endoscopic remission. High anti-TNF level was not as good an indicator of endoscopic remission as compared to high albumin or low FC (-0.32 versus -0.62 versus 0.74). This findings were consistent with Voiosu et al. in which faecal calprotectin was a better predictor of mucosal healing than anti-TNF levels in a small population of 53 patients with UC.[17] Other studies comparing faecal calprotectin with biochemical markers such as CRP were also compatible with our study,[18-20] in which they also showed

that CRP and platelet count correlated negatively while albumin correlated positively with anti-TNF levels.

Multiple studies have compared faecal calprotectin with biochemical markers in predicting endoscopic activity.[21–25] Faecal calprotectin is a biomarker of neutrophil activation that has been reported widely to be elevated in patients with IBD.[25] It has been described as a potential non-invasive point of care testing due to its low cost. Numerous studies have concluded that faecal calprotectin is a promising and reliable non-invasive biomarker for the monitoring of endoscopic and clinical disease activity,[18-20, 26,27] with one Korean study by Lee et al. showing that faecal calprotectin could better correlate with the ulcerative colitis endoscopic disease severity than the Mayo subscore.[20] A retrospective study by Kostas et al. showed that faecal calprotectin was a biomarker of short term clinical outcome and mucosal healing.[28] This was also consistent with other retrospective studies by Zittan et al.[30] and Boon et al.[29] concluding that low faecal calprotectin was correlated with histological remission and mucosal healing in IBD.[29,30] Although there is increasing interest and evidence in its use, faecal calprotectin until recently, has not routinely been monitored in clinical practice. Therefore, our sample size for faecal calprotectin has been much lower than that for anti-TNF levels. Despite this, this study has given some insight into the value of monitoring faecal calprotectin in relation to routine anti-TNF monitoring to guide future management decisions for patients with IBD.

Similarly other studies have examined anti-TNF levels with clinical and endoscopic outcomes. A cross sectional study of 71 patients by Mazor et al.[31] showed that high ADA levels were associated with clinical remission and a cut-off point of 5.85µg/ml was suggested for its prediction.[31] Another similar prospective study on 23 patients by Bodini et al.[31] revealed that ADA levels at 48weeks and a median follow up of 102weeks were higher in patients under clinical remission than those who had moderate to severe clinical disease activity.[32] A post hoc analysis of clinical trial by Bodini et al.[32] also found higher ADA levels in patients with no recurrence during a follow up period of 2years post-surgery as compared to those who did have recurrence.[33] Similar studies also apply to IFX, in which high trough concentrations were associated with sustained histologic remission in IBD.[34] IFX trough levels were also associated with higher rates of clinical remission and quality of life.[35]

Measurement of both drug levels and biomarkers such as faecal calprotectin could improve the efficacy of anti-TNF therapy in active IBD.[36] Although the value of therapeutic drug monitoring lies in dose optimisation in view of reported correlations between anti-TNF trough concentrations and clinical outcomes in other studies, there is still contention as to whether a benefit can be demonstrated.[37] A few studies have proved that measurement of serum trough anti-TNF levels, concentration and its antibodies may help to optimise therapy for those who develop relapse or side effects.[38,39] However, there was one multicentre study showing the predictive ability for mucosal healing to be suboptimal despite an association between anti-TNF levels and mucosal healing.[40] Perhaps as pointed out by Torres et al, we should be focusing on dose intensification based on symptoms and other parameters, and not alone on trough anti-TNF concentrations.[41] Biomarkers also should be used with caution in individual clinical contexts and interpreted with consideration of performance characteristics of assays used, specified test cut-offs, and pre-test probability of disease.[42,43]

We acknowledge the limitations of this study which include being a single-centre study with a small sample size. The study population is heterogeneous including both CD and UC patients on two anti-TNF agents. As this is a retrospective study, the validity of outcome measures such as endoscopic activity assessment is open to question. It is well known that, outside randomised controlled trial or prospective trials, endoscopic disease activity assessment is hampered by unstandardized reporting and inter observer variability. It is important to note that the therapeutic level of anti TNF therapy may be different depending on the condition e.g perianal CD, acute severe ulcerative colitis, etc. Similarly the faecal calprotectin that correlate with clinical and endoscopic remission of UC or CD may be higher (up to 250µg/g) than those that discriminate between healthy controls and patients with IBD (50µg/g). Regardless further similar studies should be done to corroborate our findings so that faecal calprotectin can be routinely monitored to benefit patients with active disease who are on maintenance anti-TNF therapy.

Conclusion

In this study, we found that faecal calprotectin as opposed to anti-TNF level is more likely to predict endoscopic activity. Further prospective studies with larger sample sizes are needed to confirm our findings.

Author contributions

Joel Tan is the lead author, Teresa Neeman is the consultant statistician and Kavitha Subramaniam oversaw the planning, progress and outcome of this study.

Conflict of interest statement

The authors have no conflicts of interest to declare.

Funding sources

This research study was not funded by sponsors in its preparation, data and manuscript.

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