One-Year Clinical Outcomes in an IBD Cohort Who Have Previously Had Anti-TNFa Trough and Antibody Levels Assessed

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Background: Loss of response (LOR) is a big concern for anti-TNFa therapies in inflammatory bowel disease. Immunomonitoring may be useful to optimize response rates and overcome secondary LOR.

Methods: This was an observational retrospective cohort study of a group of patients with inflammatory bowel disease on infliximab (IFX) and adalimumab (ADA) who had anti-TNFa trough and antibody levels measured, during maintenance phase of treatment. Anti-TNFa trough and antibody levels were measured using standard enzyme-linked immunosorbent assay techniques. Baseline patient characteristics were determined and patients were reviewed 1 year later. Clinical assessment took place with partial Mayo scores for ulcerative colitis and Harvey–Bradshaw index for Crohn's disease. C-reactive protein (CRP) and albumin were also measured. Poor outcomes were defined as the following: need for rescue steroids, dose intensification, surgery, or treatment discontinuation.

Results: Seventy-four patients were included in the study, 37 (50%) were female, mean age 41 years, 61 (82%) had Crohn's disease, and 42 (57%) ulcerative colitis. Forty-two (57%) patients received IFX and 32 (43%) ADA. Mean IFX trough was 3.6 μg/mL and mean ADA troughs were 3.78 μg/mL. Twenty-seven percent of patients (n = 20) overall had a poor outcome, with a similar proportion in each group 24% (n = 10) IFX and 31% (n = 10) ADA (*P* value 0.24). Of the cohort, 14.2% (6/42) treated with IFX had subtherapeutic trough levels, 6.2% (2/32) of ADA patients had a trough level <1 μg/mL (*P* value = 0.273) There was no difference in mean trough according to outcome (4.9 μg/mL poor versus 5.4 μg/mL good, *P* value 0.14). Low IFX trough levels did correlate with high CRP, low albumin and response rates, mean CRP 6.66 μg/mL (n = 3), mean albumin 37 g/L for patients with low trough levels and poor response versus CRP 2.0 μg/mL (n = 24), mean albumin 43 g/L for patients with high trough levels and good response (*P* = 0.009, 95% confidence interval, −0.78 to −0.12).

Conclusions: LOR is still a big concern with anti-TNFa therapies. Stand-alone anti-TNFa trough and antibody levels are not useful at predicting LOR/disease progression at 1 year, but low trough levels do correlate well with elevated CRP, hypoalbuminaemia, and poor response rates.

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Key Words: inflammatory bowel disease, Crohn's disease, ulcerative colitis, immunomonitoring, loss of response, anti-TNFa trough and antibody

Tissue necrosis factor alpha (anti-TNFa) therapies have revolutionized the management of inflammatory bowel disease (IBD). Their earlier introduction, and use in combination with immunomodulators, has resulted in a significant improvement in response and remission rates in both ulcerative colitis (UC) and Crohn's disease (CD).[1,2] In addition, they help to induce long-lasting mucosal healing and deep remission, and may alter the natural history of the disease, and reduce the risk of future complications. However, overtime, response to anti-TNFa therapy can be lost, resulting in clinical relapse and disease progression. Eighty percent of patients treated with infliximab (IFX) in CD respond initially, but overtime, 30% of patients will lose response, requiring dose or interval adjustments.[3] Loss of response (LOR) is associated with flares of disease, increased hospitalization rates, need for surgical interventions, and decline in quality of life.

Response may be lost for a multitude of reasons. Immunogenicity may account for some of this LOR. Immunogenicity is the formation of antibodies against anti-TNFa therapies. This can be associated with reduced drug trough levels and a loss of clinical efficacy.[4] Immunogenicity is associated with increased drug clearance, which directly leads to reduced trough levels. This can ultimately lead to LOR, infusion reactions and the need for dose intensification, or the need to switch to an alternative agent. A 2-compartment pharmacokinetic model for IFX has shown that the clearance increases 2.7-fold in patients positive for antibodies to IFX as compared with patients without antibodies to IFX.[5] In addition, a recent meta-analysis by Moore et al looked specifically at optimal targets for IFX.[6] They found 12 studies that reported

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IFX levels in a manner suitable for determining effect estimates. During maintenance therapy, patients in clinical remission had significantly higher mean trough IFX levels than those of patients not in remission—3.1 μg/mL versus 0.9 μg/mL. Patients with an IFX level >2 μg/mL were more likely to be in clinical remission (risk ratio 2.9, 95% confidence interval [CI], 1.8–4.7, $P < 0.001$) or achieve endoscopic remission (risk ratio 3, 95% CI, 1.4–6.5, $P = 0.004$) than patients with levels <2 μg/mL.

Similarly for adalimumab (ADA), antibody formation is associated with a reduction in ADA trough levels and an increased risk of future inflammation and subsequent LOR.[7] This and other studies suggest that immunomonitoring has an important role to play in evaluating LOR and developing strategies to overcome this difficult problem.

Calculation of anti-TNFa drug and antibody levels may identify patients who are losing response and may benefit from drug intensification or alternative therapies. Steenholdt et al[8] established a cutoff of <0.5 μg/mL as being associated with LOR. There is expanding evidence from the literature illustrating the association between trough levels and response rates.[9–11]

There is in addition an association between low trough levels, elevated CRP, and LOR.[12] Post hoc analysis of the ACCENT 1 trial also confirmed these important predictors of LOR. Patients with durable sustained response to maintenance IFX 5 mg/kg had higher postinduction trough levels than that of patients without durable sustained response. Serum IFX trough levels ≥3.5 μg/mL and ≥60% CRP decrease were significantly associated with durable sustained response.[13] Thus, immunomonitoring alongside biochemical markers of disease activity have a role to play in assessing LOR.

The aim of our study was to examine 1-year clinical outcomes in a cohort of patients with IBD on maintenance anti-TNFa therapy at our center, who had once off anti-TNFa antibody and trough levels measured.

MATERIALS AND METHODS

The cohort consisted of a group of patients with UC and CD treated with either IFX or ADA at our centre in 2014. The cohort had responded initially to standard induction therapy and was on maintenance therapy. Anti-TNFa trough levels were measured during the maintenance phase of patients' anti-TNFa therapy, in a random fashion. That is, there was no distinction made between those who were responding and those who were experiencing a secondary LOR. This cohort was followed retrospectively at 1 year to assess clinical outcomes. Poor outcomes were defined as follows: need for steroids, dose intensification, treatment discontinuation, hospitalization, or surgery. Clinical assessment took place in the form of partial Mayo scores for UC and Harvey–Bradshaw scores for CD. Biochemical parameters of disease activity were measured, including CRP (normal <5 μg/mL) and serum albumin (normal >35 g/L) levels. Low trough levels for IFX were defined as <1 μg/mL and high trough levels >3 μg/mL IFX[4] and low trough levels for ADA were defined as <1 μg/mL and high trough levels were defined as >5 μg/mL.[7]

Cutoffs for IFX and ADA antibodies were defined as 2.5 and 0.45 μg/mL, respectively. Trough and antibody status were correlated with outcomes; a P value of < 0.05 was considered significant.

Anti-TNFa trough and antibodies were measured as follows. Drug levels were assayed using a protocol adopted from Ungar et al.[14] Briefly, enzyme-linked immunosorbent assay plates (Thermo Scientific NUNC, Basingstoke, United Kingdom) were coated with 500 ng/mL recombinant human TNFα (PeproTech, London, United Kingdom) overnight at room temperature. After blocking and washing steps, 100 μL of serum (diluted 1:100) was added to each well of the enzyme-linked immunosorbent assay plates for 90 minutes. After washing, horseradish peroxidase–conjugated goat anti-human IgG Fc fragment antibody (MP Biomedicals, Illkirch Cedex, France) was added at a concentration of 0.62 μg/mL for 60 minutes and subsequently reacted with tetramethylbenzidine substrate (Thermo Scientific). After addition of the stop solution (2N H_2SO_4), absorbance was read at 450 nm on an EL-800 plate reader (BioTek, Bad Friedrichshall, Germany). Drug concentrations in serum samples were determined using a standard curve generated from absorbance readings of IFX or ADA added at concentrations from 0 to 400 ng/mL. The drug concentration cutoff level was calculated using the average concentration obtained from unexposed controls plus 3 SDs.[15]

Antidrug antibody levels were assayed using a protocol adopted from Ungar et al.[14] Enzyme-linked immunosorbent assay plates (Thermo Scientific NUNC) were coated overnight with 500 ng/mL TNFα (PeproTech), as outlined above. After blocking and washing, 100 μL of drug (0.1 mg/mL IFX or ADA) was added to the plates for 90 minutes, followed by 100 μL of diluted serum (1:10 dilution) for 90 minutes. After washing, goat anti-human λ chain horseradish peroxidase–conjugated antibody (AbD Serotec, Oxford, United Kingdom) was added at a dilution of 2.5×10^4 for 60 minutes, subsequently reacted with tetramethylbenzidine substrate and the reaction stopped using 2N H_2SO_4. Absorbance at 450 nm was determined on an EL-800 plate reader. Antidrug antibody concentrations were determined by calibration to a standard curve generated using horseradish peroxidase–labeled goat anti-human IgG F(ab')2 fragment antibody (MP Biomedicals) at concentrations from 0 to 600 ng/mL. The antidrug antibody concentration cutoff was calculated using the average concentration obtained from unexposed controls plus 3 SDs.[15]

ETHICAL CONSIDERATIONS

The study was approved by the local ethics committee, and informed consent was obtained from patients for enrollment in the study.

RESULTS

Baseline patient characteristics for our cohort are shown in Table 1. Total number of patients in our cohort were 74, 37 (50%)

TABLE 1. Patient Baseline Characteristics

	IFX	ADA	*P*	Total
Sex				
Male	22 (52%)	15 (47%)	0.32	37 (50%)
Female	20 (48%)	17 (53%)		37 (50%)
Age, mean	38 yr	44 yr		41 yr
Disease phenotype				
CD	34 (81%)	27 (84%)	0.27	61 (82%)
UC	8 (19%)	5 (16%)	0.35	13 (18%)
Anti-TNFα				
IFX				42 (57%)
ADA				32 (43%)
Dose (IFX)				
5 mg/kg	38 (90%)			
10 mg/kg	4 (10%)			
Dose (ADA)				
40 mg fortnightly		24 (75%)		
40 mg weekly		8 (25%)		
Immunomodulators (azathioprine)				
Yes	22 (52%)	1 (3%)	<0.001	23 (31%)
No	20 (48%)	31 (97%)		51 (69%)
CRP, mean	3.33	6.5	0.05	4.03
Albumin, mean	43.34	42.33		42.77
Clinical assessment				
HBI, mean	3.85	2.88		
Partial Mayo, mean	0.75	0.40		

were female, mean age 41 years. In terms of phenotype overall, 61 (82%) had CD. Forty-two (57%) patients received IFX and 32 (43%) ADA. A statistically significant larger number of patients treated with IFX were on combination therapy with azathioprine. Twenty-two (52%) of patients treated with IFX were on combination therapy versus only 1 (3%) for ADA (*P* value <0.001, 95% CI, 0.31–0.68) Patients treated with ADA had a slightly increased CRP at baseline compared with IFX 6.5 versus 3.33 (*P* value = 0.05). Baseline mean Harvey–Bradshaw indexes for IFX and ADA were 3.85 and 2.88, respectively. Mean partial Mayo scores for IFX and ADA were 0.75 and 0.40, respectively. Mean CRP for ADA-treated group was slightly higher compared with IFX (6.5 versus 3.3, *P* value = 0.05). Mean serum albumin rates were similar for the 2 groups (43.3, for IFX, 42.3 for ADA). In terms of drug dosing, 90% (n = 38) of IFX were treated with 5 mg/kg and 10% (n = 4) were on 10 mg/kg. Likewise, for ADA, 75% (n = 24) were on 40 mg every fortnight, with 25% (n = 8) on 40 mg every week.

In terms of trough levels, overall 11% (n = 8) had a low trough level (<1 μg/mL). 14.2% (6/42) of the cohort treated with IFX had subtherapeutic trough levels and 6.2% (2/32) of patients with ADA had a trough level <1 μg/mL (*P* value = 0.273). (Fig. 1).

In addition, 35.2% (26/74) patients had positive antibodies, 14.3% (6/42) for IFX, and 62.5% (20/32) for ADA (*P* value <0.0001, 95% CI, −0.68 to −0.29) (Fig. 2). However, only 9% of ADA antibodies were strongly positive.

Overall, mean IFX trough level was 3.6 μg/mL. In total, 13/42 (30%) had clinically active disease, whereas 8/42 (18.6%) had low serum trough levels, mean 0.57 μg/mL. Antibody status and drug trough level did not correlate with CRP; however, there was a trend toward increased clinical disease activity with low drug trough level (12.5% inactive disease versus 36% with active disease), but this did not reach statistical significance

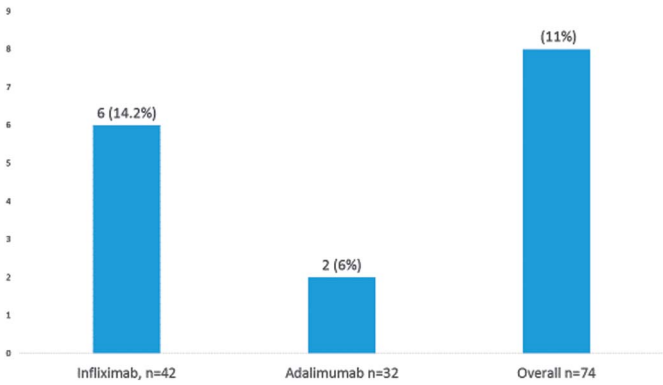

FIGURE 1. Patients on IFX/ADA with low trough levels <1 μg/mL.

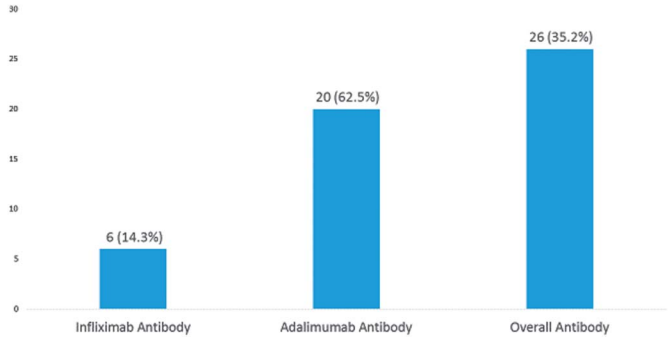

FIGURE 2. Rates of antibody formation against IFX/ADA.

($P < 0.08$). In addition, a low trough level was not associated with biochemical activity (CRP 2.8 versus 3.3). Four patients (50%) had clinically active disease in the low trough group versus 9 (26%) in the normal trough group. This difference did not reach statistical significance (odds ratio (OR) 3.3; $P = 0.08$). Mean ADA troughs were 3.78 μg/mL.

In terms of 1-year outcomes, disappointingly, 27% (n = 20) overall had a poor outcome, with a similar proportion in each group—24% (n = 10) IFX and 31% (n = 10) ADA (*P* value 0.24) (Fig. 3). Subclassifying poor outcomes, 20% (n = 2) of IFX patients required surgery, 30% (n = 3) required a dose escalation, 20% (n = 2) had to stop because of side effects/toxicity, 10% (n = 1) required rescue steroids, and 20% (n = 2) had to switch to alternative agent because of LOR. Subclassifying for ADA 30% (n = 3) of patients required surgery, 20% (n = 2) required a dose escalation, 20% (n = 2) had to stop because of side effects/toxicity, 20% (n = 2) required steroids, and 10% (n = 1) had to switch to an alternative agent. For patients with CD, 22.2% (18/81) had a poor response, versus 15.4% (2/13) for UC (*P* value = 0.278). Thus, LOR is a real concern and has a big impact on patient outcomes. Any strategies to help overcome LOR are to be welcomed.

In Crohn's, overall mean IFX trough levels were 6.38 μg/mL, versus 6.74 μg/mL for UC, and for ADA 3.94 μg/mL versus 2.92 μg/mL. There was no difference in mean trough according to outcome (4.9 μg/mL poor versus 5.4 μg/mL good, *P* value 0.14) (Fig. 4). Antibody positivity did not correlate with low trough levels (16.6% versus 83.3%).

Although 72% (n = 31) on IFX achieved a recommended trough >3 μg/mL, none on ADA reached a target of >5 μg/mL, ($P < 0.0001$, 95% CI, 0.58–0.90). A higher IFX trough was not associated with better outcomes, 3/10 poor versus 8/32 good response.

Low IFX trough levels did correlate with high CRP, low albumin and response rates, mean CRP 6.66 μg/mL (n = 3), mean albumin 37 g/L for patients with low trough levels and poor response versus CRP 2.0 μg/mL (n = 24), mean albumin 43 g/L for patients with high trough levels and good response ($P = 0.009$, 95% CI, −0.78 to −0.12) (Fig. 5).

DISCUSSION

LOR is a big concern for anti-TNFa therapies. Immunogenicity, the formation of antibodies against anti-TNFa, leads to increased drug clearance and reduced trough levels. This process leads to an increase likelihood of treatment failure, disease relapse, and disease progression, as well as increased need for surgical intervention.[16]

Immunomonitoring has been increasingly recognized as a useful tool to explore an immune basis behind LOR to anti-TNFa therapy. It can be used alongside other biochemical predictors of LOR such as CRP and faecal calprotectin.[12,17] Our study was a 1-year retrospective analysis of a cohort of patients who previously had stand-alone anti-TNFa trough and antibodies measured. We aimed to see whether these stand-alone anti-TNFa trough and antibody levels would be useful in predicting future outcomes.

Similar to other studies, a significant number of our cohort treated with anti-TNFa had a negative outcome (27% 20/74).

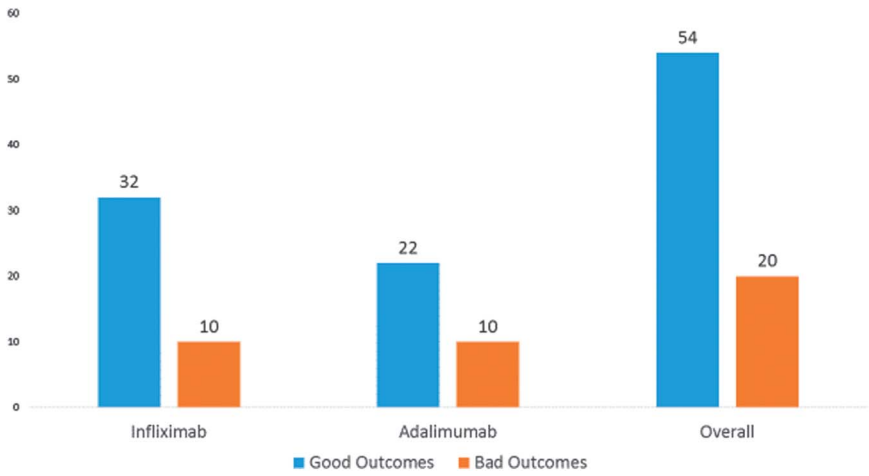

FIGURE 3. One-year clinical outcomes for patients on maintenance IFX/ADA, n = 74.

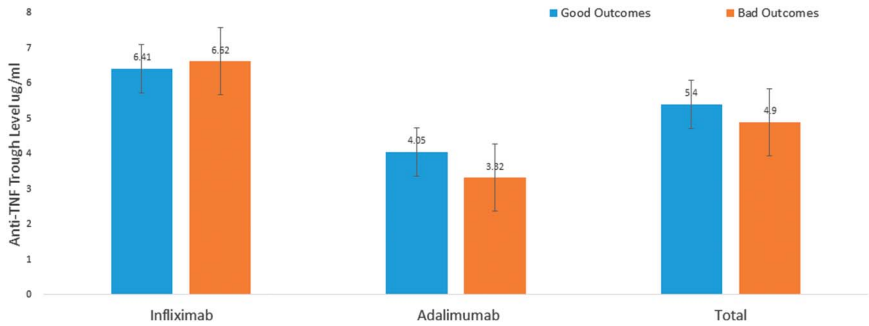

FIGURE 4. Mean trough levels for IFX and ADA based on outcomes.

LOR leads to increased hospitalizations, need for further steroid usage, and increased surgical intervention. Indeed, as mentioned above, 22% of patients treated with IFX required surgery and a similar number for ADA 27%. One can therefore see a need to use new strategies that will help overcome treatment failure, prevent LOR, or facilitate a regain in response, leading to an improved likelihood of long-lasting deep remission.

As mentioned, antibody formation is a drawback to long-term anti-TNFa use and may impact LOR. Our study has shown increased antibody formation against ADA compared with IFX for our cohort. Likely explanations include possible mode of administration, subcutaneous versus intravenous, and a larger proportion of IFX patients being on combination therapy, which is associated with reduced antibody formation. However, not all antibodies are clinically significant, and research is ongoing exploring this link and deciphering differences between clinically significant and insignificant anti-TNF antibodies.

For our cohort, stand-alone anti-TNFa trough and antibody levels did not prove useful, in predicting clinical outcomes, based on our 1-year retrospective study. As mentioned above, there was no difference in trough levels, according to response rates: 4.9 μg/mL in patients with poor response versus 5.4 μg/mL in patients with good response rates, *P* value 0.14 (Fig. 4). This data suggest that immunomonitoring is not helpful, when used in a stand-alone manner, and may be best used at more important timepoints in a patients treatment, such as the end of induction or when evaluating for secondary LOR. In addition, again there was no relationship between stand-alone anti-TNFa antibody levels and response rates. This could suggest that it is the impact of disease activity on anti-TNFa trough and antibody levels rather than trough levels predicting disease activity.

Possible explanations for our results were that trough and antibody levels were performed on a broad cohort of patients. All patients were on maintenance phase of their treatment and relatively well. It maybe that measuring anti-TNFa trough and antibody levels will prove more informative in the setting of secondary LOR. Another possibility is that cutoffs used, for high trough levels, <3 μg/mL for IFX or >5 μg/mL for ADA were too high. Finally, there is ongoing work into ascertaining the impact of clinically significant versus clinically insignificant antibodies. It's a possibility that our antibody assay was detecting a higher degree of clinically insignificant antibodies, hence our high reported rate of anti-TNFa antibody formation, particularly for ADA.

Our data are similar to other studies, confirming the association between low trough levels, LOR, and elevated CRP.[13] Thus, the combination of a patient with low anti-TNFa trough levels and elevated CRP is a strong predictor of LOR. Our study did not confirm an association between trough levels and response rates. Possible explanations include that the patient population was a heterogenous group, i.e. was not solely focused on those loosing response. In addition, there are ongoing studies

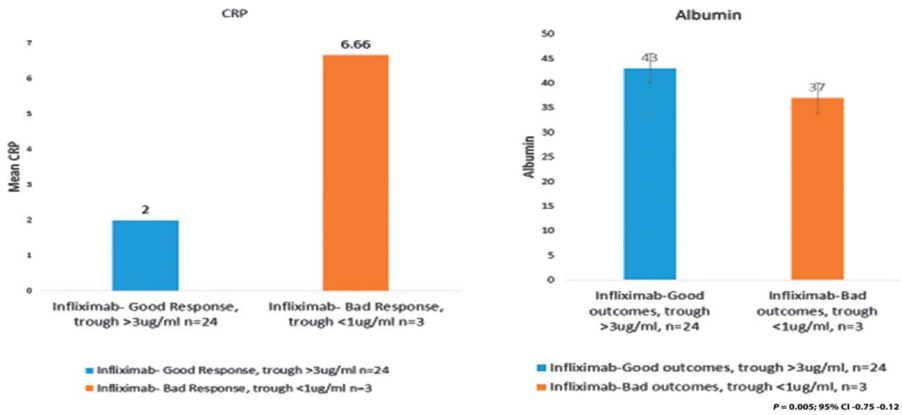

FIGURE 5. Relationship between mean CRP, albumin, and IFX outcomes.

exploring the role of clinically significant versus insignificant anti-TNFa antibodies and their role in LOR.

Our data did show that high CRP/low albumin correlated with low anti-TNFa trough levels and LOR. Therefore, in patients with a high CRP and/or a low albumin, we believe its worthwhile checking anti-TNFa trough and antibody levels to explore an immune basis behind LOR.

Going forward, it is likely that immunomonitoring will have an increasingly important role to play in fine-tuning the management of IBD. Stand-alone anti-TNFa trough and antibody levels are unlikely however to be useful in helping guide the treatment decision-making process. Careful thought needs to take place, before governing bodies and societies incorporate immunomonitoring into treatment algorithms. As mentioned, the use of immunomonitoring at the completion of induction phase of therapy, or during an episode of LOR, are likely to prove more beneficial. Another potential role for immunomonitoring is in patients at higher risk of LOR, such as those with elevated CRP or low albumin levels. Furthermore, immunomonitoring may be more helpful in predicting good response in those higher trough levels and low CRP levels after induction. Further work is required to define optimal trough levels.

Finally, overall outcomes were somewhat disappointing, despite adequate median anti-TNFa trough levels, potentially suggesting other nonimmune issues could be contributing to LOR.

CONCLUSION

LOR is still a big concern for anti-TNFa therapy. Stand-alone anti-TNFa and antibody levels are not useful predictors of LOR. The use of immunomonitoring needs to be fine-tuned, to best address this important aspect of IBD management.

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