

Clinical impact of immunomonitoring in the treatment of inflammatory bowel disease

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Abstract

Despite improvement in outcomes, loss of response (LOR) to tumor necrosis factor-alpha (TNF α) therapies is a big concern in the management of inflammatory bowel disease. LOR is associated with flares of disease, increased hospitalisation rates, need for surgical interventions, and decline in quality of life. LOR may be multifactorial, but immunogenicity makes a significant contribution. Traditionally doses of anti-TNF α have been adjusted based on clinical response, using a standard approach. Immunomonitoring involves the measurement of anti-TNF α trough and antibody levels. It takes into account the underlying pharmacokinetics of anti-TNF α therapies. Expanding on this a treat to target approach may be used, where doses are intensified, or tailored to the individual based on the measurement of anti-TNF α trough and antibody levels. This review looks at the history, evolution, and clinical impact that immunomonitoring is having in the treatment of inflammatory bowel disease. It will focus on the role of immunomonitoring in helping to achieve long lasting deep remission and mucosal healing. It will explore the different options in terms of best measuring trough and antibody levels, explore possible advantages of immunomonitoring, and discuss its role in best optimising response, at induction, during the maintenance phase of treatment, as well as a role in withdrawing or switching therapy.

Key words: Inflammatory bowel disease; Ulcerative colitis; Immunomonitoring; loss of response; Anti-TNF α trough and antibody levels; Crohn's disease; immunogenicity

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Core tip: Immunomonitoring is being increasingly used to

optimise response rates in inflammatory bowel disease. The aim of this review article is to explore the available literature, and to understand the rationale for using immunomonitoring and to see how this approach can be best incorporated into inflammatory bowel disease treatment algorithms. The focus of this review article is the role for immunomonitoring at the key points of induction, and at loss of response. It will emphasise the possible advantages of immunomonitoring. It will define optimal trough levels, plus targets required to achieve mucosal healing, and help alter the natural history of the disease.

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INTRODUCTION

Response and remission rates for inflammatory bowel disease (IBD) have improved considerably with greater utilisation of immunomodulators, like azathiopurine and earlier introduction of anti-tumor necrosis factor- α (TNF α) therapies. TNF α is a major driver of the inflammatory cascade and a key molecule for targeting in managing IBD^[1]. Anti-TNF α therapies have proven efficacy in inducing and maintaining remission in both ulcerative colitis (UC) and Crohn's disease (CD)^[2-9].

Anti-TNF α therapies have revolutionised the management of IBD, improving response rates, and helping to alter the natural history of the disease, and achieve, long term goals of mucosal healing and deep remission. However loss of response and immunogenicity is a big concern. 80% of patients treated with infliximab in CD respond initially, but overtime 30% of patients will lose response, requiring dose and interval adjustments^[7,10]. LOR is associated with flares of disease, increased hospitalisation rates, need for surgical interventions, and decline in quality of life. Historically doses have been intensified in a stepwise fashion, based on clinical response. This standard approach involves increasing the doses of anti-TNF α used, and shortening frequency of administration. However this approach does not take into account the underlying pharmacokinetics of anti-TNF α , and lacks flexibility for individual patients. Immunomonitoring offers an alternative, treat to target approach, where doses are tailored to the individual, based on individual anti-TNF α trough and antibody levels.

There is increasing evidence to suggest that utilisation of immunomonitoring, is helping to overcome LOR, improve remission rates, achieve mucosal healing and thus help alter the natural history of IBD. By targeting treatment to the individual overall response rates can be improved, and this can be further linked to both

biochemical and mucosal healing. Immunomonitoring also facilitates more cost effective use of anti-TNF α therapy, and helps reduce side-effect burden, and limit toxicity.

This review article looks at the history, evolution, and clinical impact that immunomonitoring is having in the treatment of IBD. It will focus on the role of immunomonitoring in helping to achieve long lasting deep remission and mucosal healing.

WHAT IS IMMUNOMONITORING?

Overtime the body's immune system may recognise anti-TNF α molecules, as foreign antigens, and result in antibody formation against them. This process is called immunogenicity, and can result in increased drug clearance and subsequent loss of response. Antibodies against Anti-TNF α (ADA) formation can lead to failure of anti-TNF α which may be due to a change in pharmacokinetics causing a faster clearance or by blocking the drug's activity in case of neutralizing ADA. Therefore, drug trough concentrations could be the missing link to help understand the clinical impact of ADA^[11].

Infliximab (IFX) is a chimeric mouse-human IgG1 molecule and antibodies to IFX (ATIs) are directed against the murine F(ab)2 fragment of the drug. Antibodies may form against infliximab in a large number of patients (8%-60%), and indeed may form as soon as after the first infusion^[10]. Immunogenicity, is associated with increased drug clearance, which directly leads to reduced trough levels. This can ultimately lead to loss of response, infusion reactions and the need for dose intensification, or the need to switch to an alternative agent. A two-compartment pharmacokinetic model for infliximab has shown that the clearance increases 2.7-fold in patients positive for ATIs as compared with patients without ATIs^[12].

Adalimumab is a fully human IgG monoclonal antibody against anti-TNF α , which has proven efficacy in the treatment of moderate to severe UC or CD. It was initially thought that antibody formation against adalimumab would be reduced, as it's a fully human IgG antibody, but studies have shown that antibodies against adalimumab (AAA), are a real problem^[13]. AAA formation are again associated with low trough levels, and loss of response.

Expanding on this, immunomonitoring is the use of laboratory techniques to measure anti-TNF α drug and antibody levels. Historically using a standard approach, drug doses are adjusted based on clinical need. However this approach is not patient focused, and doesn't explore the underlying pharmacokinetics of anti-TNF α therapy. A treat to target approach with the use of anti-TNF α drug and antibody levels, could help tailor treatment to the individual and may help improve response rates, overcome loss of response, and achieve mucosal healing. An example of this approach used to good effect, is from the recent TAXIT

study, whereby patients infliximab doses were proactively intensified if trough levels were $< 3 \mu\text{g/mL}$ ^[14].

Key issues at present concerning immunomonitoring include, defining optimal trough levels. Clarifying why low trough levels develop, and forming strategies to improve them. Do we need higher troughs to achieve mucosal healing? Distinguishing between clinically significant and insignificant antibody levels. Clarifying the role for combination therapy, and its impact on immunogenicity. When best to check levels, with perhaps strong consideration to targeting the period around completion of induction therapy. Improvement in laboratory enzyme-linked immunosorbent assay (ELISA) techniques, reproducibility, and comparability of different assays.

DEFINING TROUGH AND ANTIBODY LEVELS?

As mentioned immunogenicity leads to ADA formation, and sub-therapeutic anti-TNF α trough levels, which can lead to loss of response. It's important to define targets when incorporating immunomonitoring into the treatment algorithm of IBD. Low trough levels, are defined as trough levels $< 1 \mu\text{g/mL}$. The treat to target approach, involves aiming for optimal rather than minimal anti-TNF α trough levels. Therapeutic trough levels are currently being redefined, and targets will likely be drug specific, and trough levels will need to be improved, to achieve mucosal healing. This review will explore these targets in further detail. ADA may be defined as being detectable or undetectable, with specific cut-offs depending on the assay used.

Concerns have been expressed about the risk of high or supra-therapeutic trough levels. A recent study however has shown IBD patients with higher anti-TNF serum concentrations had significantly better disease-specific quality-of-life. Fatigue, arthralgia, skin lesions and other side-effects do not occur more often in these patients. This study is reassuring in that high serum concentrations of anti-TNF antibodies are not toxic^[15].

Antibody formation to TNF α is not a fixed process. A prospective study of 125 patients with IBD, treated with infliximab showed that ATI formation can fluctuate^[16]. Clinically relevant ATI were typically formed within the first 12 mo but transient ATI, which are of little clinical significance, can be formed at any time during treatment. Loss of response can be predicted based on a combination of C-reactive protein (CRP), trough levels and stable antibodies with a high degree of accuracy^[17]. Transient antibody formation is not associated with a loss of response.

Recently there has been an introduction of biosimilar anti-TNF α molecules for treating IBD. European agencies have approved Remsima (CT-P13) for use in Europe across all indications^[18]. A recent study has confirmed the cross-reactivity of Remsima and Remicade (infliximab) suggesting similar immunodominant epit-

opes and immunogenic potential of the two agents^[19]. It confirms that patients with IBD who develop high-titre antibodies and infusion reaction/loss of response to Remicade should probably not be considered for switching to Remsima. In contrast, patients who develop anti-adalimumab antibodies may be considered for a switch to either Remicade or Remsima, if clinically indicated.

IMMUNOMONITORING, TROUGH AND ANTIBODY ASSAYS

There are a number of different ways of measuring drug concentration of anti-TNF α molecules in serum. For convenience trough levels are measured. The most commonly used method is ELISA^[20-21]. The advantage of monoclonal or monospecific polyclonal anti-drug antibody is the specificity toward the anti-TNF drug, which results in lower specific binding^[22]. This reduces the risk of false positives.

With regard to detecting ADA the most commonly used assay is the double-antigen (a.k.a. bridging) ELISA in which the anti-TNF drug is both used as capture and detecting antibody^[23]. Despite developments and improvements in the assays to measure anti-TNF α and ADA levels, there is still a lack of standardization and quality control between the established tests^[24]. This can have clinical implications, as well as issues around reproducibility of results between different centres.

A number of factors can influence ADA formation, including the type of assay used, timing involved in antibody measurement as well as the study population. Taking this into account there have been attempts to standardize measurements, specifically for ADA against adalimumab^[25].

ELISA techniques whilst reproducible and accurate do not offer single patient testing. Ongoing research is looking at more rapid turnaround alternatives. Lu *et al*^[26] have developed a fast bioassay for determining IFX concentration in serum using an in-house developed fiber-optic surface plasmon resonance biosensor. The assay turn-around time, was considerably reduced compared to ELISA.

Tables 1 and 2^[21,23-32] illustrate the different options available in terms of measuring anti-TNF α trough and antibody levels. Measuring ADA have proved problematic, and there are ongoing attempts to develop drug tolerant ELISA assays.

IMPACT OF IMMUNOGENICITY ON LOSS OF RESPONSE

As mentioned above antibody formation against anti-TNF α is associated with LOR. In general low trough levels ($< 1 \mu\text{g/mL}$) and the presence of detectable antibodies are associated with worse clinical outcomes. Steenholdt *et al*^[33] established a cut off of < 0.5

Table 1 Detecting anti-tumor necrosis factor- α trough levels

Study	Technique	Key Points
Ternant <i>et al</i> ^[21]	ELISA	
Vande Casteele <i>et al</i> ^[24]	ELISA	
Lu <i>et al</i> ^[26]	FO-SPR based sandwich bioassay	Faster than ELISA, correlates well
Malíčková <i>et al</i> ^[27]	ELISA (for CT-P13 biosimilar to infliximab)	
Corstjens <i>et al</i> ^[28]	Rapid lateral flow (LF)-based assay	
Wang <i>et al</i> ^[29]	Non-radiolabeled homogeneous mobility shift assay (HMSA)	

ELISA: Enzyme-linked immunosorbent assay; FO-SPR: Fiber-optic surface plasmon resonance.

Table 2 Detecting anti-tumor necrosis factor- α antibody levels

Ref.	Technique	Key points
Van Stappen <i>et al</i> ^[30]	Solid-phase ELISA	Lacks the ability to detect ADA in the presence of drug
Gils <i>et al</i> ^[25]	Solid-phase ELISA	
Bloem <i>et al</i> ^[31]	Drug tolerant ELISA	
Imaeda <i>et al</i> ^[23]	ELISA	Measures AAAs in the presence of free ADA
Van Stappen <i>et al</i> ^[32]	ELISA	Converts drug-sensitive bridging ELISA into a drug-tolerant bridging ELISA

ELISA: Enzyme-linked immunosorbent assay; ADA: Anti-tumor necrosis factor- α ; AAA: Antibodies against adalimumab.

$\mu\text{g/mL}$, has been associated with loss of response. It's clear, that the level of anti-TNF α will impact on response rates, and there is ample evidence that higher trough levels are associated with sustained response, and likewise low or undetectable trough levels, increase the likelihood of loss of response. The following table from published papers explores the current evidence of the relationship between infliximab trough levels and loss of response (Table 3^[33-42]). The data for adalimumab is somewhat more limited (Table 4^[43,44]).

In addition, to ADA formation the inflammatory burden may impact on response to anti-TNF α . For example, Brandse *et al*^[45] have shown that in UC, patients with more severe disease lose infliximab in stool, to a greater level than clinical responders. This emphasises the concept of the leaky, inflamed gut, with increased loss of anti-TNF α in severely inflamed tissue. This links in with further research by Gibson *et al*^[46] showing increase response rates and reduced colectomy rates in patients treated with an accelerated induction course of infliximab for severe UC. Going forward, it will be useful to check trough levels, during this accelerated protocol, to further define the best treatment strategy for severe UC.

Table 3 Relationship between infliximab trough levels and response rates

Ref.	n	Mean IFX trough levels $\mu\text{g/mL}$	Mean IFX trough levels $\mu\text{g/mL}$	P value
		Lost response	Maintained response	
Ainsworth <i>et al</i> ^[34]	27	0 (0-0.1)	2.9 (0.9-4.3)	0.002
Yamada <i>et al</i> ^[35]	31 (CD)	6.3	4.7	NS
Steenholdt <i>et al</i> ^[33]	69 (CD)	N/A	2.8 (0.8-5.3)	< 0.0001
Pariente <i>et al</i> ^[36]	76 (CD)	3.3 \pm 4.1	2.3 \pm 2.2	NS
Steenholdt <i>et al</i> ^[33]	13 (UC)	N/A	3.8 (1.1-8.5)	< 0.0001
Karmiris <i>et al</i> ^[37]	136 (CD)	0.3 (0.3-3.6)	4.9 (1.7-8.2)	0.01
Marits <i>et al</i> ^[38]	79 (CD)	1.8	4.1	< 0.001
Bortlik <i>et al</i> ^[39]	84 (CD)	N/A	> 3	N/A
Adedokun <i>et al</i> ^[40]	728 (UC)	N/A	3.7	N/A
Cornillie <i>et al</i> ^[41]	147 (CD)	1.9	4.0	0.0331
Reinisch <i>et al</i> ^[42]	203 (CD)	0.8	2.14	0.006

IFX: Infliximab; UC: Ulcerative colitis; CD: Crohn's disease.

Table 4 Relationship between adalimumab trough levels and response rates

Study	n	Mean Adalimumab trough levels $\mu\text{g/mL}$	Mean Adalimumab trough levels $\mu\text{g/mL}$	P value
		Lost response	Maintained response	
Imaeda <i>et al</i> ^[43]	40 (CD)	N/A	5.9	N/A
Roblin <i>et al</i> ^[44]	40 (UC/CD)	3.2	6.2	0.12

UC: Ulcerative colitis; CD: Crohn's disease.

The ATLAS study in addition explored the relationship between serum and intestinal anti-TNF levels, with endoscopic disease activity and levels of TNF^[47]. This study of a cross-sectional group of 30 patients with UC or CD, treated with IFX or ADA showed that anti-TNF α levels were higher in mild to moderately inflamed than in non-inflamed tissue, but this increase was more than negated by the proportionally greater level of TNF in inflamed tissue. Anti-TNF concentration in tissue correlated with degree of endoscopic inflammation, except for tissue with severe inflammation in which anti-TNF levels were again lower (mean normalised anti-TNF in tissue: uninfamed = 0.93, mild = 2.17, moderate = 13.71, severe = 2.2 inflammation ($P = 0.0042$). This may explain why patients with satisfactory anti-TNF levels, have active disease, as the inflamed tissue characterised by an abundance of TNF acts as a sink for the anti-TNF. This in turn increases the risk of ADA formation. These patients might therefore benefit from drug dose intensification.

Going forward more work is required to tease out the distinction between clinically significant and insignificant ADA, which undoubtedly has a big impact on loss of response.

Finally one must also consider alternative explanations for loss of response. Overlap with functional symptoms, small bowel bacterial malabsorption, non-inflammatory strictures, could all explain alternatives

Table 5 Strategies to overcome loss of response

Dose escalate	Alternative cause for LOR?
Low trough	Adequate trough
No ADA	No ADA
Combination therapy	Alternative anti-TNF α /agent
Low trough	Low trough
ADA	ADA

ADA: Anti-TNF α ; LOR: Loss of response.

to immunogenicity, in causing loss of response.

POSSIBLE ADVANTAGES OF IMMUNOMONITORING?

Dose intensification and treatment outcomes based on anti-TNF α trough and ADA

Immunomonitoring has an increasingly important role to play in managing IBD. A prospective examination of a cohort in The Netherlands has shown absence of IFX-trough levels in a significant proportion of their population, suggesting a vital role for immunomonitoring, in identifying and managing loss of response to anti-TNF therapies^[48].

As mentioned LOR is a big concern with anti-TNF α therapy. Immunomonitoring has a role to play in helping to explore the pharmacokinetics behind LOR and to develop strategies to overcome it. For example, if patients have low trough levels, and no ADA, they may benefit from dose intensification, whereas patients, with adequate trough, and no ADA, are unlikely to benefit. Furthermore in the setting of ADA, and low trough, one strategy is the use of combination therapy, to reduce ADA and improve trough levels. However in the setting of ADA, and adequate trough levels, intensifying doses, will have no impact, and a drug switch should be considered (Table 5). There is increasing evidence that adaption of a treat to target approach, with dose intensification based on anti-TNF α trough and antibody levels, alongside appropriate treatment selection, helps improve response rates, and achieve mucosal healing.

There is now proven evidence, that dose escalation of anti-TNF based on low drug trough levels, not only leads to improved clinical response rates, but also to increased mucosal healing. The TAXIT study looked at patients on stable maintenance doses of infliximab in remission and adjusted their infliximab dose to obtain a fixed drug level between 3–7 $\mu\text{g/mL}$ ^[14]. This resulted in a higher proportion of CD patients in remission than before dose escalation (88% vs 65%, $P = 0.020$). This approach was also cost-effective, with 72 patients with trough levels > 7 $\mu\text{g/mL}$, 67 patients (93%) achieved through levels of 3–7 $\mu\text{g/mL}$ after dose reduction. This resulted in a 28% reduction in drug cost from before dose reduction ($P < 0.001$).

In addition a recent study has also shown that

a therapeutic week 2 IFX trough level is associated with higher likelihood of mucosal healing in a UC population^[49].

Treatment selection based on trough and ADA

Early trough level assessment is useful at predicting both short and long-term outcomes, as well as facilitating earlier decision making between continuing with the drug or considering alternative options. There is ample evidence from the literature, that escalating doses of anti-TNF α in patients with ADA is unlikely to improve response rates, and alternative agents should be considered^[50]. Immunomonitoring helps explore this immunogenicity, and helps identify patients losing response for immune reasons, and to develop strategies to regain response.

Economic benefit

A Danish study by Steenholdt also confirms that an individualised approach, with adjustment of infliximab doses based on drug antibody and trough levels, is more cost effective, without any obvious negative clinical effect on efficacy^[51]. Costs for intention-to-treat patients were substantially lower (34%) for those treated in accordance with the algorithm than by IFX dose intensification: € 6038 vs € 9178, $P < 0.001$. However, disease control, as judged by response rates, was similar: 58% and 53%, respectively, $P = 0.81$; difference 5% (-19% to 28%). For per-protocol patients, treatment costs were even lower (56%) in the algorithm-treated group (€ 4062 vs € 9178, $P < 0.001$) and with similar response rates [47% vs 53%, $P = 0.78$; difference -5% (-33% to 22%)].

Reduced toxicity

In addition immunomonitoring can be utilised to manage complications or drawbacks to anti-TNF α therapy. As well as impacting on loss of response, Anti-TNF α antibody formation is also associated with transfusion related reactions and anaphylaxis^[52]. For example patients with ATI (antibodies against infliximab) are at increased risk of acute transfusion reaction, and loss of response, compared to those patients without ATI^[53]. In addition a study by Baert *et al*^[20] in which an arbitrary figure for ATI was used in a population with CD, they showed that patients with an ATI greater than 8 $\mu\text{g/mL}$, had increased risk of loss of response, and 2.4 fold increased risk of infusion reaction.

COMBINATION THERAPY AND IMMUNOMONITORING

There is ample evidence that the addition of an immunomodulator like a thiopurine or methotrexate to anti-TNF α therapy is associated with improved response rates in IBD. In the SUCCESS (Efficacy and Safety of Infliximab, as Monotherapy or in Combination with Azathioprine, versus Azathioprine

Monotherapy in Moderate to Severe Ulcerative Colitis) trial in UC, steroid-free remission was achieved by 40% of patients receiving infliximab and azathioprine, compared with 22% receiving infliximab alone ($P = 0.017$)^[54]. Furthermore it has been shown that combination therapy of infliximab and azathioprine is associated with reduced infliximab antibody formation, as well as reduced systemic inflammation. Post hoc analysis of the SONIC trial data has shown that at week 30, trough levels for the combination of IFX and azathioprine were 3.5 µg/mL vs 1.6 µg/mL for the IFX group alone ($P < 0.001$). The authors also found that only 1 out of 116 (0.9%) in the combination group had drug antibodies compared to 15 out of 103 (14.6%) in the IFX group alone^[55]. Combination therapy has been shown to require less need for dose escalation, surgical intervention or the need for switching to a different class of anti-TNF or alternative agents.

In addition data from a Dutch study has confirmed the benefits of combination therapy in overcoming the problems of immunogenicity^[56]. In a study involving 217 patients (108 patients IFX; 109 patients' adalimumab). Mean trough levels in the IFX group was higher in the combination therapy group compared with the monotherapy group, 4.6 µg/mL vs 7.5 µg/mL, $P = 0.04$. In the adalimumab group, the difference was not significant. In patients with IFX monotherapy, the incidence of antibody formation was higher compared with patients with combination therapy (29.8% vs 5.7%, $P = 0.001$). The incidence of antibody formation was lower in IFX patients who immediately started with immunomodulators compared with patients who did not (33.3% vs 66.7%, $P = 0.04$). Thus combination therapy, through a synergistic effect on immunogenicity clearly results in reduced antibody formation, and leads to a greater likelihood of improved response rates.

There is also role for measuring 6-TG levels, the active metabolite of azathioprine^[57]. This offers the potential to even further optimise the combination approach. An interesting study by Yarur *et al*^[58] looked at the relationship between 6-TG, infliximab trough and antibody levels. They performed a cross-sectional study of 72 patients receiving maintenance therapy with infliximab and a thiopurine for IBD. They found that levels of 6-TG correlated with those of infliximab ($\rho = 0.53$, $P < 0.0001$). The cut-off point of 6-TG that best predicted a higher level of infliximab was 125 pmol/8 × 10⁸ red blood cells (RBCs) ($P < 0.001$). Patients with 6-TG levels less than 125 pmol/8 × 10⁸ RBCs were significantly more likely to have ATI (OR = 1.3, 95%CI: 2.3-72.5, $P < 0.01$). Historically a 6-TG level 230 pmol/8 × 10⁸ RBCs have been associated with better response rates in patients on monotherapy, a level of 6-TG of 125 pmol/8 × 10⁸ RBCs or greater may be adequate to achieve therapeutic levels of infliximab. In the long term, this may minimize the toxicity and adverse side effects, like malignancy for patients on combination therapy.

However measuring 6-TG levels is complicated, with concerns over reproducibility of the assay, as well as the potential for increased toxicity, in patients with high 6-TG levels. This may restrict the wide-spread use of 6-TG monitoring, as part of the treatment algorithm.

IMMUNOMONITORING TO FACILITATE DRUG WITHDRAWAL

The benefits of combination therapy are proven, with improved response and remission rates. There is however long terms concerns about the side-effects of combination therapy, and concerns expressed about risks of lymphoproliferative disorders in particular. Therefore discussions about withdrawal of immunomodulators in well patients, achieving remission have been debated. Concerns have been expressed about relapse of disease, with their withdrawal. In a retrospective study, among co-treated patients, levels of infliximab remained stable after immunomodulators were withdrawn after at least 6 mo of therapy (before: 3.2 µg/mL, 95%CI: 1.6-5.8 µg/mL and after: 3.7 µg/mL, 95%CI: 1.3-6.3 µg/mL, $P = 0.70$)^[59]. The most striking observation in this study was the fact that none of the 27 patients with infliximab trough levels > 5 µg/mL at the time of immunomodulator withdrawal lost response to infliximab after withdrawal of immunomodulator during the median follow-up of 29 mo. The authors propose that it is safe to stop immunomodulators in patients with IFX trough levels greater than 5 µg/mL.

OPTIMAL TROUGH

As mentioned above the use of anti-TNFα trough and antibody levels, may be helpful in identifying loss of response. Also of interest is the potential to develop strategies to improve response rates. However there is a need to define optimal trough levels, in terms of what's required not only to achieve clinical remission, but also what's necessary for achieving mucosal healing. As mentioned the TAXIT study, looking at patients who have secondary loss of response to infliximab doses can be safely intensified aiming for a trough level of between 3-7 µg/mL^[14].

Similarly Bortlik *et al*^[39] showed that an infliximab trough of greater than 3 µg/mL, at the start of a maintenance regime was associated with sustained clinical response to infliximab. A recent meta-analysis by Moore *et al*^[60] has shed further light on optimal targets for infliximab. They found twelve studies reported 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 patients not in remission; 3.1 µg/mL vs 0.9 µg/mL. Patients with an IFX level > 2 µg/mL were more likely to be in clinical remission (RR = 2.9, 95%CI: 1.8-4.7, $P < 0.001$), or achieve endoscopic

Table 6 Trough levels associated with mucosal healing for infliximab

Study	n	Mean Infliximab Trough level $\mu\text{g/mL}$	Mean Infliximab Trough level $\mu\text{g/mL}$	P value	95%CI
		Mucosal Healing	No mucosal Healing		
Paul <i>et al</i> ^[61]	52 (UC/CD)	Delta IFX > 0.5		0.0001	
Ungar <i>et al</i> ^[63]	78 (UC/CD)	4.3	1.7	0.002	
Imaeda <i>et al</i> ^[43]	45 (CD)	> 4.0			0.56-0.70
Reinisch <i>et al</i> ^[42]	123 (CD)	> 3.0			1.53-7.28
Colombel <i>et al</i> ^[64]	188 (CD)	3.51	1.72	0.0018	
Papamichael <i>et al</i> ^[62]	101(UC)	> 15 (wk 6)		0.025	
		> 2.1 (wk 14)		0.004	

IFX: Infliximab; UC: Ulcerative colitis; CD: Crohn's disease.

Table 7 Trough levels associated with mucosal healing for adalimumab

Study	n	Mean Adalimumab Trough level $\mu\text{g/mL}$	Mean Adalimumab Trough level $\mu\text{g/mL}$	P value
		Mucosal Healing	No mucosal Healing	
Ungar <i>et al</i> ^[63]	67 (UC/CD)	6.7	3.1	0.01
Roblin <i>et al</i> ^[44]	40 (UC/CD)	6.5	4.2	< 0.05
Zittan <i>et al</i> ^[68]	60 (UC/CD)	14.7	3.4	< 0.0001

UC: Ulcerative colitis; CD: Crohn's disease.

remission (RR = 3, 95%CI: 1.4-6.5, $P = 0.004$) than patients with levels < 2 $\mu\text{g/mL}$.

In addition evidence is emerging that in order to achieve the more stringent target of mucosal healing, higher trough levels are essential. Table 6^[42,43,61-64] illustrates the data for infliximab trough levels, and mucosal healing, and Table 7, the data for adalimumab. In a French study looking at response to infliximab dose intensification in patients losing response, the only factor associated with a greater likelihood of mucosal healing, was an increase in drug trough levels^[61]. A recent meta-analysis by Barnes *et al*^[65] showed that among patients with IBD, anti-TNF α trough levels above pre-specified values were associated with increased rates of mucosal healing (OR = 5.57, 95%CI: 3.80-8.15).

In a retrospective study of 145 IBD patients Ungar *et al*^[63] recently found significant association between serum levels of anti-TNF α agents and level of mucosal healing. Median serum levels of infliximab and adalimumab were significantly higher in patients with mucosal healing than patients with active disease (based on endoscopy) (for infliximab, 4.3 $\mu\text{g/mL}$ vs 1.7 $\mu\text{g/mL}$, $P = 0.0002$ and for adalimumab, 6.2 $\mu\text{g/mL}$ vs 3.1 $\mu\text{g/mL}$, $P = 0.01$). Levels of infliximab above 5 $\mu\text{g/mL}$ (area under the curve = 0.75, $P < 0.0001$) and levels of adalimumab above 7.1 $\mu\text{g/mL}$ (area under the curve = 0.7, $P = 0.004$) identified patients with mucosal healing with 85% specificity. Increasing levels of infliximab beyond 8 $\mu\text{g/mL}$ produced only minimal increases in the rate of mucosal healing, whereas the association between higher level of adalimumab and increased rate of mucosal healing reached a plateau at 12 $\mu\text{g/mL}$. They propose that serum levels of 6-10 $\mu\text{g/mL}$ for infliximab and 8-12 $\mu\text{g/mL}$ for adalimumab are required to achieve mucosal healing in 80%-90% of patients with IBD, and that this could be considered

as a "therapeutic window". Exceeding these levels produces only a negligible gain in proportion of patients with mucosal healing. Further studies are required, but this suggests, that in order to alter the natural history of IBD, and achieve mucosal healing, we need robust and sustained trough levels of anti-TNF α .

With regard to adalimumab there is less available research in the field of immunomonitoring. Post-hoc analysis of the Karmiris trial by Baert *et al*^[66] has shown that a low serum adalimumab concentration after the induction regimen increases the risk of ATA formation. A trough level of < 5 $\mu\text{g/mL}$, increased the risk of ATA formation. In addition ATA formation is associated with a future risk of inflammation and disease relapse. Further analysis of the CHARM trial data also identified a positive association between serum adalimumab concentration and remission at several time points^[67]. However the authors did not identify a threshold concentration reliably associated with remission. Roblin *et al*^[44] also showed that in a cohort of 40 patients with IBD, on maintenance therapy, trough levels of adalimumab were higher in patients with mucosal healing (6.5 $\mu\text{g/mL}$) than in patients without (4.2 $\mu\text{g/mL}$, $P < 0.005$). Zittan *et al*^[68] similarly showed that higher adalimumab trough levels are associated with mucosal healing. In a cohort of 60 patients, on maintenance adalimumab therapy, a median trough of 14.7 $\mu\text{g/mL}$ was found in those with mucosal healing vs 3.4 $\mu\text{g/mL}$ in those without, ($P = 6.25 \times 10^{-5}$). They propose a cut-off of 8.14 $\mu\text{g/mL}$, be used, as a target to achieve mucosal healing.

APPLICATION OF IMMUNOMONITORING

Immunomonitoring may be utilised at different stages of the treatment pathway, from induction, to mainte-

nance phase and finally remission.

Induction/Early immunomonitoring

As ever with the management of IBD timing is crucial, specifically at the key time points of induction, and maintenance. Low anti-TNF α trough levels, in the induction phase is linked to increase risk of antibody formation for both infliximab and adalimumab^[69,70]. Data though on optimal trough levels at induction phase is limited. However a recent Belgian study of 101 patients with UC, who completed induction therapy with infliximab has demonstrated that higher infliximab trough levels are associated with increased likelihood of short term mucosal healing^[62]. Multiple logistic regression analysis identified infliximab concentration ≥ 15 at week 6 ($P = 0.025$, OR = 4.6, 95%CI: 1.2-17.1) and ≥ 2.1 $\mu\text{g/mL}$ at week 14 ($P = 0.004$, OR = 5.6, 95%CI: 1.7-18) as independent factors associated with short term mucosal healing. Further randomised studies are required, but this suggests that a targeted approach, achieving therapeutic trough levels, particularly after completion of induction phase of therapy, will help optimise response and remission rates.

Brandse *et al.*^[71] have also shown significant differences in IFX trough levels at 6 wk in responders compared to non-responders after completion of induction course of IFX. The median serum concentrations of infliximab at week 6 were 8.1 $\mu\text{g/mL}$ in responders (interquartile range, 3.0-13.7 $\mu\text{g/mL}$) and 2.9 $\mu\text{g/mL}$ in non-responders (interquartile range, 0.01-5.8 $\mu\text{g/mL}$) ($P = 0.03$). In addition they found that early development of ATIs during induction therapy reduces the serum concentration of infliximab and is associated with nonresponse to treatment. Patients with high baseline serum levels of CRP had lower serum concentrations of infliximab. Thus it's clear, that immunogenicity is a concern from the outset of treatment, and particularly for the severely inflamed colon, accelerated induction courses, with therapeutic trough levels may be aimed for, to best optimise response.

Maintenance phase immunomonitoring

With regard to maintenance phase of treatment, as mentioned above there is evidence from the literature, backing up a targeted approach to therapy. As mentioned The TAXIT study, where patients on maintenance infliximab were randomised between a treat to target approach, aiming for trough levels between 3-7 $\mu\text{g/mL}$, and the current standard approach. During optimization stage, response rates were improved, in patients with sub-therapeutic levels, who had their IFX doses intensified. In addition patients with supra-therapeutic levels, had doses safely reduced, allowing a more cost effective use of anti-TNF α ^[14].

There is however a lack of association between once off maintenance phase immunomonitoring with outcomes. Our group have done a retrospective anal-

ysis of 82 patients on maintenance infliximab and adalimumab, and we found no association between once off, anti-TNF α trough and ADA levels, with clinical response, similar to other data^[72]. In future, event triggered immunomonitoring may be the best approach to incorporate immunomonitoring. For example, in the patient with a relapse, or who is losing response, calculation of trough and antibody levels, may help explore this deterioration and help guide therapy as previously discussed.

Stopping therapy

Immunomonitoring may also be useful in guiding when to stop therapy. Amiot *et al.*^[73] have shown that in a cohort of 80 patients with inflammatory bowel disease in clinical remission on infliximab therapy, de-escalation of infliximab therapy should be considered based on therapeutic drug monitoring rather than the current blind standard approach based on symptoms and CRP. In addition a study of patients with Crohn's disease in remission, who had their infliximab discontinued, has shown that an infliximab trough level < 6 $\mu\text{g/mL}$, is associated with long lasting clinical remission^[74]. Patients with higher trough levels at the time of IFX discontinuation were more prone to relapse suggests that these patients probably require continued anti-TNF α administration and an adequate drug concentration to maintain clinical remission and therefore are more likely to relapse once this therapy is discontinued. Further randomised controlled trials are required to confirm this, but these two studies suggests that immunomonitoring may be useful in guiding when it is safe to stop anti-TNF α therapy.

OPTIMISING THERAPY: SWITCHING AGENTS

Despite attempts to optimise response to anti-TNF α therapy, some patients will require a switch to another agent within the anti-TNF family, as well as alternative biologic agents, or immunomodulators. Patients with adequate trough levels, who loose response, are unlikely to benefit from further dose escalation. In a study of 247 IBD patients, with suspected loss of response, trough levels of adalimumab greater than 4.5 $\mu\text{g/mL}$ and infliximab greater than 3.8 $\mu\text{g/mL}$ identified patients who failed to respond to an increase in drug dosage or a switch to another anti-TNF agent with 90% specificity^[75]. In addition levels of antibodies against adalimumab > 4 $\mu\text{g/mL}$ or antibodies against infliximab > 9 $\mu\text{g/mL}$ identified patients who did not respond to increase in doses of anti-TNF, with 90% specificity.

Switching from one class on anti-TNF to an alternative agent is associated with modest response rates. It's worth noting that patients who develop antibodies to one anti-TNF agent, are more likely to develop antibodies to an alternative anti-TNF agent. For example

Frederiksen *et al*^[76] has shown that in patients who failed infliximab, antibody formation to adalimumab was increased which was associated with minimal drug level, and a clear lack of response. They propose that it is prudent to assess ADA immunogenicity in anti-IFX antibody-positive switchers to ensure optimal interventions at inadequate treatment responses and to avoid inappropriate ADA intensification regimens. In addition data from the SWITCH trial has shown that elective switching to a subcutaneous regimen is not efficacious and is associated with a high likelihood of losing response^[77].

In patients with ADA's and low anti-TNF α trough levels, consideration can be given to alternative anti-TNF α agents, like golimumab, or newer agents, like vedolizumab but data on immunomonitoring for these agents is lacking.

EXPANDING ROLE OF IMMUNOMONITORING

Immunomonitoring has the potential to drive forward the management of IBD, by tailoring treatment to the individual. However it's important that its role is incorporated smoothly into treatment algorithms. Furthermore immunomonitoring needs to be utilised at key points in the treatment process. While further prospective data and studies are required there is evidence to support its usage in current practice. After completion of induction therapy, assessment of anti-TNF α trough and antibody levels, may be performed, with strong consideration on dose intensification, if trough levels are sub-therapeutic. In addition for patients on maintenance therapy, it may be appropriate to assess anti-TNF α trough and antibody levels, when loss of response occurs. Treatment may be intensified, by way of dose escalation where necessary to target trough levels, or the addition of an IM to improve trough levels, and reduce antibody formation. In addition immunomonitoring will offer guidance when contemplating a switch to an alternative anti-TNF α agent as discussed above.

CONCLUSION

Immunomonitoring is helping us to understand the pharmacokinetics behind anti-TNF α therapies, and also how best to optimise management of IBD. Tailoring treatment to the individual in a treat to target fashion, offers the hope of improving response and remission rates, as well as achieving mucosal healing. This needs to be verified, using randomised clinical trials, comparing with the current standard approach. Going forward, we need to understand further the significance of immunogenicity, the impact of anti-TNF α antibody formation, and there is a strong need for greater availability, of more affordable and rapid turnaround ELISA or alternative techniques, to fully implement the

potential of immunomonitoring.

REFERENCES

- 1 Diveu C, McGeachy MJ, Cua DJ. Cytokines that regulate autoimmunity. *Curr Opin Immunol* 2008; **20**: 663-668 [PMID: 18834938 DOI: 10.1016/j.coi.2008.09.003]
- 2 Costa J, Magro F, Caldeira D, Alarcão J, Sousa R, Vaz-Carneiro A. Infliximab reduces hospitalizations and surgery interventions in patients with inflammatory bowel disease: a systematic review and meta-analysis. *Inflamm Bowel Dis* 2013; **19**: 2098-2110 [PMID: 23860567 DOI: 10.1097/MIB.0b013e31829936c2]
- 3 Hanauer SB, Sandborn WJ, Rutgeerts P, Fedorak RN, Lukas M, MacIntosh D, Panaccione R, Wolf D, Pollack P. Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn's disease: the CLASSIC-I trial. *Gastroenterology* 2006; **130**: 323-333; quiz 591 [PMID: 16472588 DOI: 10.1053/j.gastro.2005.11.030]
- 4 Reinisch W, Sandborn WJ, Hommes DW, D'Haens G, Hanauer S, Schreiber S, Panaccione R, Fedorak RN, Tighe MB, Huang B, Kampman W, Lazar A, Thakkar R. Adalimumab for induction of clinical remission in moderately to severely active ulcerative colitis: results of a randomised controlled trial. *Gut* 2011; **60**: 780-787 [PMID: 21209123 DOI: 10.1136/gut.2010.221127]
- 5 Rutgeerts P, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johans J, Travers S, Rachmilewitz D, Hanauer SB, Lichtenstein GR, de Villiers WJ, Present D, Sands BE, Colombel JF. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2005; **353**: 2462-2476 [PMID: 16339095 DOI: 10.1056/NEJMoa050516]
- 6 Sandborn WJ, van Assche G, Reinisch W, Colombel JF, D'Haens G, Wolf DC, Kron M, Tighe MB, Lazar A, Thakkar RB. Adalimumab induces and maintains clinical remission in patients with moderate-to-severe ulcerative colitis. *Gastroenterology* 2012; **142**: 257-265.e1-3 [PMID: 22062358 DOI: 10.1053/j.gastro.2011.10.032]
- 7 Hanauer SB, Feagan BG, Lichtenstein GR, Mayer LF, Schreiber S, Colombel JF, Rachmilewitz D, Wolf DC, Olson A, Bao W, Rutgeerts P. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. *Lancet* 2002; **359**: 1541-1549 [PMID: 12047962 DOI: 10.1016/S0140-6736(02)08512-4]
- 8 Lichtenstein GR, Yan S, Bala M, Blank M, Sands BE. Infliximab maintenance treatment reduces hospitalizations, surgeries, and procedures in fistulizing Crohn's disease. *Gastroenterology* 2005; **128**: 862-869 [PMID: 15825070 DOI: 10.1053/j.gastro.2005.01.048]
- 9 Feagan BG, Panaccione R, Sandborn WJ, D'Haens GR, Schreiber S, Rutgeerts PJ, Loftus EV, Lomax KG, Yu AP, Wu EQ, Chao J, Mulani P. Effects of adalimumab therapy on incidence of hospitalization and surgery in Crohn's disease: results from the CHARM study. *Gastroenterology* 2008; **135**: 1493-1499 [PMID: 18848553 DOI: 10.1053/j.gastro.2008.07.069]
- 10 Lee LY, Sanderson JD, Irving PM. Anti-infliximab antibodies in inflammatory bowel disease: prevalence, infusion reactions, immunosuppression and response, a meta-analysis. *Eur J Gastroenterol Hepatol* 2012; **24**: 1078-1085 [PMID: 22647738 DOI: 10.1097/MEG.0b013e328355558c]
- 11 Vande Casteele N, Gils A. Pharmacokinetics of anti-TNF monoclonal antibodies in inflammatory bowel disease: Adding value to current practice. *J Clin Pharmacol* 2015; **55** Suppl 3: S39-S50 [PMID: 25707962 DOI: 10.1002/jcph.374]
- 12 Ternant D, Aubourg A, Magdelaine-Beuzelin C, Degenne D, Watier H, Picon L, Paintaud G. Infliximab pharmacokinetics in inflammatory bowel disease patients. *Ther Drug Monit* 2008; **30**: 523-529 [PMID: 18641542 DOI: 10.1097/ftd.0b013e318180e300]
- 13 Schellekens H. Factors influencing the immunogenicity of therapeutic proteins. *Nephrol Dial Transplant* 2005; **20** Suppl 6: vi3-vi9 [PMID: 15958824 DOI: 10.1093/ndt/gfh1092]
- 14 Stanton PK, Mody I, Heinemann U. A role for N-methyl-D-aspartate receptors in norepinephrine-induced long-lasting potentiation in the dentate gyrus. *Exp Brain Res* 1989; **77**: 517-530

- [PMID: 2572445 DOI: 10.1053/j.gastro.2015.02.0315]
- 15 **Brandse JF**, Vos LM, Jansen J, Schakel T, Ponsioen CI, van den Brink GR, D'Haens GR, Löwenberg M. Serum Concentration of Anti-TNF Antibodies, Adverse Effects and Quality of Life in Patients with Inflammatory Bowel Disease in Remission on Maintenance Treatment. *J Crohns Colitis* 2015; **9**: 973-981 [PMID: 26116557 DOI: 10.1093/ecco-jcc/jjv116]
 - 16 **Ungar B**, Chowers Y, Yavzori M, Picard O, Fudim E, Har-Noy O, Kopylov U, Eliakim R, Ben-Horin S. The temporal evolution of antidrug antibodies in patients with inflammatory bowel disease treated with infliximab. *Gut* 2014; **63**: 1258-1264 [PMID: 24041539 DOI: 10.1136/gutjnl-2013-305259]
 - 17 **Roblin X**, Marotte H, Leclerc M, Del Tedesco E, Phelip JM, Peyrin-Biroulet L, Paul S. Combination of C-reactive protein, infliximab trough levels, and stable but not transient antibodies to infliximab are associated with loss of response to infliximab in inflammatory bowel disease. *J Crohns Colitis* 2015; **9**: 525-531 [PMID: 25895875 DOI: 10.1093/ecco-jcc/jjv061]
 - 18 **European Medicines Agency: Inflectra. European public assessment report.** Available from: URL: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/002778/human_med_001677.jsp&mid=WC0b01ac0
 - 19 **Ben-Horin S**, Yavzori M, Benhar I, Fudim E, Picard O, Ungar B, Lee S, Kim S, Eliakim R, Chowers Y. Cross-immunogenicity: antibodies to infliximab in Remicade-treated patients with IBD similarly recognise the biosimilar Remsima. *Gut* 2016; **65**: 1132-1138 [PMID: 25897019 DOI: 10.1136/gutjnl-2015-309290]
 - 20 **Baert F**, Noman M, Vermeire S, Van Assche G, D'Haens G, Carbonez A, Rutgeerts P. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. *N Engl J Med* 2003; **348**: 601-608 [PMID: 12584368 DOI: 10.1056/NEJMoa020888]
 - 21 **Ternant D**, Mulleman D, Degenne D, Willot S, Guillaumin JM, Watier H, Goupille P, Paintaud G. An enzyme-linked immunosorbent assay for therapeutic drug monitoring of infliximab. *Ther Drug Monit* 2006; **28**: 169-174 [PMID: 16628126 DOI: 10.1097/01.fdt.0000189901.08684.4b]
 - 22 **Buurman DJ**, Vande Castele N, Sturkenboom MG, Kleibeuker JH, Vermeire S, van der Kleij D, Rispens T, Gils A, Dijkstra G. Letter: detection of infliximab levels and anti-infliximab antibodies--comparison of three different assays; authors' reply. *Aliment Pharmacol Ther* 2013; **37**: 282 [PMID: 23252783 DOI: 10.1111/apt.12128]
 - 23 **Imaeda H**, Takahashi K, Fujimoto T, Bamba S, Tsujikawa T, Sasaki M, Fujiyama Y, Andoh A. Clinical utility of newly developed immunoassays for serum concentrations of adalimumab and anti-adalimumab antibodies in patients with Crohn's disease. *J Gastroenterol* 2014; **49**: 100-109 [PMID: 23575576 DOI: 10.1007/s00535-013-0803-4]
 - 24 **Vande Castele N**, Buurman DJ, Sturkenboom MG, Kleibeuker JH, Vermeire S, Rispens T, van der Kleij D, Gils A, Dijkstra G. Detection of infliximab levels and anti-infliximab antibodies: a comparison of three different assays. *Aliment Pharmacol Ther* 2012; **36**: 765-771 [PMID: 22928581]
 - 25 **Gils A**, Vande Castele N, Poppe R, Van de Wouwer M, Compennolle G, Peeters M, Brouwers E, Vermeire S, Geukens N, Declercq PJ. Development of a universal anti-adalimumab antibody standard for interlaboratory harmonization. *Ther Drug Monit* 2014; **36**: 669-673 [PMID: 24906181 DOI: 10.1097/FTD.0000000000000074]
 - 26 **Lu J**, Van Stappen T, Spasic D, Delpont F, Vermeire S, Gils A, Lammertyn J. Fiber optic-SPR platform for fast and sensitive infliximab detection in serum of inflammatory bowel disease patients. *Biosens Bioelectron* 2016; **79**: 173-179 [PMID: 26706938 DOI: 10.1016/j.bios.2015.11.087]
 - 27 **Malíčková K**, Ďuricová D, Bortlík M, Hind'oš M, Machková N, Hrubá V, Lukáš M, Zima T, Lukáš M. Serum trough infliximab levels: A comparison of three different immunoassays for the monitoring of CT-P13 (infliximab) treatment in patients with inflammatory bowel disease. *Biologicals* 2016; **44**: 33-36 [PMID: 26603635 DOI: 10.1016/j.biologicals.2015.09.005]
 - 28 **Corstjens PL**, Fidler HH, Wiesmeijer KC, de Dood CJ, Rispens T, Wolbink GJ, Hommes DW, Tanke HJ. A rapid assay for on-site monitoring of infliximab trough levels: a feasibility study. *Anal Bioanal Chem* 2013; **405**: 7367-7375 [PMID: 23836086 DOI: 10.1007/s00216-013-7154-0]
 - 29 **Wang SL**, Ohrmund L, Hauenstein S, Salbato J, Reddy R, Monk P, Lockton S, Ling N, Singh S. Development and validation of a homogeneous mobility shift assay for the measurement of infliximab and antibodies-to-infliximab levels in patient serum. *J Immunol Methods* 2012; **382**: 177-188 [PMID: 22691619 DOI: 10.1016/j.jim.2012.06.002]
 - 30 **Van Stappen T**, Billiet T, Vande Castele N, Compennolle G, Brouwers E, Vermeire S, Gils A. An Optimized Anti-infliximab Bridging Enzyme-linked Immunosorbent Assay for Harmonization of Anti-infliximab Antibody Titers in Patients with Inflammatory Bowel Diseases. *Inflamm Bowel Dis* 2015; **21**: 2172-2177 [PMID: 26284296 DOI: 10.1097/MIB.0000000000000434]
 - 31 **Bloem K**, van Leeuwen A, Verbeek G, Nurmohamed MT, Wolbink GJ, van der Kleij D, Rispens T. Systematic comparison of drug-tolerant assays for anti-drug antibodies in a cohort of adalimumab-treated rheumatoid arthritis patients. *J Immunol Methods* 2015; **418**: 29-38 [PMID: 25637408 DOI: 10.1016/j.jim.2015.01.007]
 - 32 **Van Stappen T**, Brouwers E, Vermeire S, Gils A. Validation of a sample pretreatment protocol to convert a drug-sensitive into a drug-tolerant anti-infliximab antibody immunoassay. *Drug Test Anal* 2016; Epub ahead of print [PMID: 26990872 DOI: 10.1002/dta.1968]
 - 33 **Steenholdt C**, Bendtzen K, Brynskov J, Thomsen OØ, Ainsworth MA. Cut-off levels and diagnostic accuracy of infliximab trough levels and anti-infliximab antibodies in Crohn's disease. *Scand J Gastroenterol* 2011; **46**: 310-318 [PMID: 21087119 DOI: 10.3109/00365521.2010.536254]
 - 34 **Ainsworth MA**, Bendtzen K, Brynskov J. Tumor necrosis factor- α binding capacity and anti-infliximab antibodies measured by fluid-phase radioimmunoassays as predictors of clinical efficacy of infliximab in Crohn's disease. *Am J Gastroenterol* 2008; **103**: 944-948 [PMID: 18028512 DOI: 10.1111/j.1572-0241.2007.01638.x]
 - 35 **Yamada A**, Sono K, Hosoe N, Takada N, Suzuki Y. Monitoring functional serum antitumor necrosis factor antibody level in Crohn's disease patients who maintained and those who lost response to anti-TNF. *Inflamm Bowel Dis* 2010; **16**: 1898-1904 [PMID: 20310016 DOI: 10.1002/ibd.21259]
 - 36 **Pariente B**, Pineton de Chambrun G, Krzysiek R, Desroches M, Louis G, De Cassan C, Baudry C, Gornet JM, Desreumaux P, Emilie D, Colombel JF, Allez M. Trough levels and antibodies to infliximab may not predict response to intensification of infliximab therapy in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2012; **18**: 1199-1206 [PMID: 22127789]
 - 37 **Karmiris K**, Paintaud G, Noman M, Magdelaine-Beuzelin C, Ferrante M, Degenne D, Claes K, Coopman T, Van Schuerbeek N, Van Assche G, Vermeire S, Rutgeerts P. Influence of trough serum levels and immunogenicity on long-term outcome of adalimumab therapy in Crohn's disease. *Gastroenterology* 2009; **137**: 1628-1640
 - 38 **Marits P**, Landucci L, Sundin U, Davidsdottir L, Nilsson J, Befrits R, Wikström AC, Eberhardsson M. Trough s-infliximab and antibodies towards infliximab in a cohort of 79 IBD patients with maintenance infliximab treatment. *J Crohns Colitis* 2014; **8**: 881-889 [PMID: 24486178 DOI: 10.1016/j.crohns.2014.01.009]
 - 39 **Bortlík M**, Ďuricová D, Malíčková K, Machková N, Bouzkova E, Hrdlicka L, Komarek A, Lukas M. Infliximab trough levels may predict sustained response to infliximab in patients with Crohn's disease. *J Crohns Colitis* 2013; **7**: 736-743 [PMID: 23200919 DOI: 10.1016/j.crohns.2012.10.019]
 - 40 **Adedokun OJ**, Sandborn WJ, Feagan BG, Rutgeerts P, Xu Z, Marano CW, Johanns J, Zhou H, Davis HM, Cornillie F, Reinisch W. Association between serum concentration of infliximab and efficacy in adult patients with ulcerative colitis. *Gastroenterology* 2014; **147**: 1296-1307.e5 [PMID: 25173754 DOI: 10.1053/j.gastro.2014.08.035]

- 41 **Cornillie F**, Hanauer SB, Diamond RH, Wang J, Tang KL, Xu Z, Rutgeerts P, Vermeire S. Postinduction serum infliximab trough level and decrease of C-reactive protein level are associated with durable sustained response to infliximab: a retrospective analysis of the ACCENT I trial. *Gut* 2014; **63**: 1721-1727 [PMID: 24474383 DOI: 10.1136/gutjnl-2012-304094]
- 42 **Reinisch W**, Colombel JF, Sandborn WJ, Mantzaris GJ, Kornbluth A, Adedokun OJ, Miller M, Tang KL, Rutgeerts P, Cornillie F. Factors associated with short- and long-term outcomes of therapy for Crohn's disease. *Clin Gastroenterol Hepatol* 2015; **13**: 539-547.e2 [PMID: 25245629 DOI: 10.1016/j.cgh.2014.09.031]
- 43 **Imaeda H**, Bamba S, Takahashi K, Fujimoto T, Ban H, Tsujikawa T, Sasaki M, Fujiyama Y, Andoh A. Relationship between serum infliximab trough levels and endoscopic activities in patients with Crohn's disease under scheduled maintenance treatment. *J Gastroenterol* 2014; **49**: 674-682 [PMID: 23666424 DOI: 10.1007/s00535-013-0829-7]
- 44 **Roblin X**, Marotte H, Rinaudo M, Del Tedesco E, Moreau A, Phelip JM, Genin C, Peyrin-Biroulet L, Paul S. Association between pharmacokinetics of adalimumab and mucosal healing in patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol* 2014; **12**: 80-84.e2 [PMID: 23891927 DOI: 10.1016/j.cgh.2013.07.010]
- 45 **Brandse JF**, van den Brink GR, Wildenberg ME, van der Kleij D, Rispens T, Jansen JM, Mathôt RA, Ponsioen CY, Löwenberg M, D'Haens GR. Loss of Infliximab Into Feces Is Associated With Lack of Response to Therapy in Patients With Severe Ulcerative Colitis. *Gastroenterology* 2015; **149**: 350-355.e2 [PMID: 25917786 DOI: 10.1053/j.gastro.2015.04.016]
- 46 **Gibson DJ**, Heetun ZS, Redmond CE, Nanda KS, Keegan D, Byrne K, Mulcahy HE, Cullen G, Doherty GA. An accelerated infliximab induction regimen reduces the need for early colectomy in patients with acute severe ulcerative colitis. *Clin Gastroenterol Hepatol* 2015; **13**: 330-335.e1 [PMID: 25086187 DOI: 10.1016/j.cgh.2014.07.041]
- 47 **Yarur AJ**, Jain A, Sussman DA, Barkin JS, Quintero MA, Princen F, Kirkland R, Deshpande AR, Singh S, Abreu MT. The association of tissue anti-TNF drug levels with serological and endoscopic disease activity in inflammatory bowel disease: the ATLAS study. *Gut* 2016; **65**: 249-255 [PMID: 25670812 DOI: 10.1136/gutjnl-2014-308099]
- 48 **Warman A**, Straathof JW, Derijks LJ. Therapeutic drug monitoring of infliximab in inflammatory bowel disease patients in a teaching hospital setting: results of a prospective cohort study. *Eur J Gastroenterol Hepatol* 2015; **27**: 242-248 [PMID: 25569569 DOI: 10.1097/MEG.0000000000000279]
- 49 **Kobayashi T**, Suzuki Y, Motoya S, Hirai F, Ogata H, Ito H, Sato N, Ozaki K, Watanabe M, Hibi T. First trough level of infliximab at week 2 predicts future outcomes of induction therapy in ulcerative colitis-results from a multicenter prospective randomized controlled trial and its post hoc analysis. *J Gastroenterol* 2016; **51**: 241-251 [PMID: 26162647 DOI: 10.1007/s00535-015-1102-z]
- 50 **Affif W**, Loftus EV, Faubion WA, Kane SV, Bruining DH, Hanson KA, Sandborn WJ. Clinical utility of measuring infliximab and human anti-chimeric antibody concentrations in patients with inflammatory bowel disease. *Am J Gastroenterol* 2010; **105**: 1133-1139 [PMID: 20145610 DOI: 10.1038/ajg.2010.9]
- 51 **Steenholdt C**, Brynskov J, Thomsen OØ, Munck LK, Fallingborg J, Christensen LA, Pedersen G, Kjeldsen J, Jacobsen BA, Oxholm AS, Kjellberg J, Bendtzen K, Ainsworth MA. Individualised therapy is more cost-effective than dose intensification in patients with Crohn's disease who lose response to anti-TNF treatment: a randomised, controlled trial. *Gut* 2014; **63**: 919-927 [PMID: 23878167 DOI: 10.1136/gutjnl-2013-305279]
- 52 **Ben-Horin S**, Yavzori M, Katz L, Kopylov U, Picard O, Fudim E, Coscas D, Bar-Meir S, Goldstein I, Chowers Y. The immunogenic part of infliximab is the F(ab')₂, but measuring antibodies to the intact infliximab molecule is more clinically useful. *Gut* 2011; **60**: 41-48 [PMID: 20519742]
- 53 **O'Meara S**, Nanda KS, Moss AC. Antibodies to infliximab and risk of infusion reactions in patients with inflammatory bowel disease: a systematic review and meta-analysis. *Inflamm Bowel Dis* 2014; **20**: 1-6 [PMID: 24280879 DOI: 10.1097/01.MIB.0000436951.80898.6d]
- 54 **Panaccione R**, Ghosh S, Middleton S, Márquez JR, Scott BB, Flint L, van Hoogstraten HJ, Chen AC, Zheng H, Danese S, Rutgeerts P. Combination therapy with infliximab and azathioprine is superior to monotherapy with either agent in ulcerative colitis. *Gastroenterology* 2014; **146**: 392-400.e3 [PMID: 24512909 DOI: 10.1053/j.gastro.2013.10.052]
- 55 **Colombel JF**, Sandborn WJ, Reinisch W, Mantzaris GJ, Kornbluth A, Rachmilewitz D, Lichtiger S, D'Haens G, Diamond RH, Broussard DL, Tang KL, van der Woude CJ, Rutgeerts P. Infliximab, azathioprine, or combination therapy for Crohn's disease. *N Engl J Med* 2010; **362**: 1383-1395 [PMID: 20393175 DOI: 10.1056/NEJMoa0904492]
- 56 **van Schaik T**, Maljaars JP, Roopram RK, Verwey MH, Ipenburg N, Hardwick JC, Veenendaal RA, van der Meulen-de Jong AE. Influence of combination therapy with immune modulators on anti-TNF trough levels and antibodies in patients with IBD. *Inflamm Bowel Dis* 2014; **20**: 2292-2298 [PMID: 25230167 DOI: 10.1097/MIB.0000000000000208]
- 57 **Dubinsky MC**, Lamothe S, Yang HY, Targan SR, Sinnett D, Théorêt Y, Seidman EG. Pharmacogenomics and metabolite measurement for 6-mercaptopurine therapy in inflammatory bowel disease. *Gastroenterology* 2000; **118**: 705-713 [PMID: 10734022 DOI: 10.1016/S0016-5085(00)70140-5]
- 58 **Yarur AJ**, Kubiliun MJ, Czul F, Sussman DA, Quintero MA, Jain A, Drake KA, Hauenstein SI, Lockton S, Deshpande AR, Barkin JS, Singh S, Abreu MT. Concentrations of 6-thioguanine nucleotide correlate with trough levels of infliximab in patients with inflammatory bowel disease on combination therapy. *Clin Gastroenterol Hepatol* 2015; **13**: 1118-1124.e3 [PMID: 25562796 DOI: 10.1016/j.cgh.2014.12.026]
- 59 **Drobne D**, Bossuyt P, Breynaert C, Cattaert T, Vande Casteele N, Compennolle G, Jürgens M, Ferrante M, Ballet V, Wollants WJ, Cleynen I, Van Steen K, Gils A, Rutgeerts P, Vermeire S, Van Assche G. Withdrawal of immunomodulators after co-treatment does not reduce trough level of infliximab in patients with Crohn's disease. *Clin Gastroenterol Hepatol* 2015; **13**: 514-521.e4 [PMID: 25066841 DOI: 10.1016/j.cgh.2014.07.027]
- 60 **Moore C**, Corbett G, Moss AC. Systematic Review and Meta-Analysis: Serum Infliximab Levels During Maintenance Therapy and Outcomes in Inflammatory Bowel Disease. *J Crohns Colitis* 2016; **10**: 619-625 [PMID: 26763722 DOI: 10.1093/ecco-jcc/jjw007]
- 61 **Paul S**, Del Tedesco E, Marotte H, Rinaudo-Gaujous M, Moreau A, Phelip JM, Genin C, Peyrin-Biroulet L, Roblin X. Therapeutic drug monitoring of infliximab and mucosal healing in inflammatory bowel disease: a prospective study. *Inflamm Bowel Dis* 2013; **19**: 2568-2576 [PMID: 24013361 DOI: 10.1097/MIB.0b013e3182a77b41]
- 62 **Papamichael K**, Van Stappen T, Vande Casteele N, Gils A, Billiet T, Tops S, Claes K, Van Assche G, Rutgeerts P, Vermeire S, Ferrante M. Infliximab Concentration Thresholds During Induction Therapy Are Associated With Short-term Mucosal Healing in Patients With Ulcerative Colitis. *Clin Gastroenterol Hepatol* 2016; **14**: 543-549 [PMID: 26681486 DOI: 10.1016/j.cgh.2015.11.014]
- 63 **Ungar B**, Levy I, Yavne Y, Yavzori M, Picard O, Fudim E, Loebstein R, Chowers Y, Eliakim R, Kopylov U, Ben-Horin S. Optimizing Anti-TNF- α Therapy: Serum Levels of Infliximab and Adalimumab Are Associated With Mucosal Healing in Patients With Inflammatory Bowel Diseases. *Clin Gastroenterol Hepatol* 2016; **14**: 550-557.e2 [PMID: 26538204 DOI: 10.1016/j.cgh.2015.10.025]
- 64 **Colombel JF**, Reinisch W, Mantzaris GJ, Kornbluth A, Rutgeerts P, Tang KL, Oortwijn A, Bevelander GS, Cornillie FJ, Sandborn WJ. Randomised clinical trial: deep remission in biologic and immunomodulator naïve patients with Crohn's disease - a SONIC post hoc analysis. *Aliment Pharmacol Ther* 2015; **41**: 734-746 [PMID: 25728587 DOI: 10.1111/apt.13139]
- 65 **Barnes EL**, Allegretti JR. Are Anti-Tumor Necrosis Factor Trough Levels Predictive of Mucosal Healing in Patients With

- Inflammatory Bowel Disease?: A Systematic Review and Meta-Analysis. *J Clin Gastroenterol* 2016; **50**: 733-741 [PMID: 26535480 DOI: 10.1097/MCG.0000000000000441]
- 66 **Baert F**, Kondragunta V, Lockton S, Vande Casteele N, Hauenstein S, Singh S, Karmiris K, Ferrante M, Gils A, Vermeire S. Antibodies to adalimumab are associated with future inflammation in Crohn's patients receiving maintenance adalimumab therapy: a post hoc analysis of the Karmiris trial. *Gut* 2016; **65**: 1126-1131 [PMID: 25862647 DOI: 10.1136/gutjnl-2014-307882]
 - 67 **Chiu YL**, Rubin DT, Vermeire S, Louis E, Robinson AM, Lomax KG, Pollack PF, Paulson SK. Serum adalimumab concentration and clinical remission in patients with Crohn's disease. *Inflamm Bowel Dis* 2013; **19**: 1112-1122 [PMID: 23584130 DOI: 10.1097/MIB.0b013e3182813242]
 - 68 **Zittan E**, Kabakchiev B, Milgrom R, Nguyen GC, Croitoru K, Steinhart AH, Silverberg MS. Higher Adalimumab Drug Levels are Associated with Mucosal Healing in Patients with Crohn's Disease. *J Crohns Colitis* 2016; **10**: 510-515 [PMID: 26783345 DOI: 10.1093/ecco-jcc/jjw014]
 - 69 **Vermeire S**, Noman M, Van Assche G, Baert F, D'Haens G, Rutgeerts P. Effectiveness of concomitant immunosuppressive therapy in suppressing the formation of antibodies to infliximab in Crohn's disease. *Gut* 2007; **56**: 1226-1231 [PMID: 17229796 DOI: 10.1136/gut.2006.099978]
 - 70 **Baert FJ**, Lockton S, Hauenstein S, Singh S, Gils A, Vermeire S. Sa1247 antibodies to adalimumab predict inflammation in Crohn's patients on maintenance adalimumab therapy. *Gastroenterology* 2014; **146**: S-242 [DOI: 10.1016/S0016-5085(14)60851-9]
 - 71 **Brandse JF**, Mathôt RA, van der Kleij D, Rispens T, Ashruf Y, Jansen JM, Rietdijk S, Löwenberg M, Ponsioen CY, Singh S, van den Brink GR, D'Haens GR. Pharmacokinetic Features and Presence of Antidrug Antibodies Associate With Response to Infliximab Induction Therapy in Patients With Moderate to Severe Ulcerative Colitis. *Clin Gastroenterol Hepatol* 2016; **14**: 251-258. e2 [PMID: 26545802 DOI: 10.1016/j.cgh.2015.10.029]
 - 72 **Tighe D**, Jeyarajah SK, Hall B, Sinead Smith S, McNamara D. Evaluation of the clinical efficacy of Anti-TNF- α drug trough and antibody levels in an Irish Inflammatory bowel disease cohort. ISG Winter Meeting, Dublin, 2015
 - 73 **Amiot A**, Hulin A, Belhassan M, Andre C, Gagniere C, Le Baleur Y, Farcet JP, Delchier JC, Hüe S. Therapeutic drug monitoring is predictive of loss of response after de-escalation of infliximab therapy in patients with inflammatory bowel disease in clinical remission. *Clin Res Hepatol Gastroenterol* 2016; **40**: 90-98 [PMID: 26138132 DOI: 10.1016/j.clinre.2015.05.019]
 - 74 **Papamichael K**, Vande Casteele N, Gils A, Tops S, Hauenstein S, Singh S, Princen F, Van Assche G, Rutgeerts P, Vermeire S, Ferrante M. Long-term outcome of patients with Crohn's disease who discontinued infliximab therapy upon clinical remission. *Clin Gastroenterol Hepatol* 2015; **13**: 1103-1110 [PMID: 25478919 DOI: 10.1016/j.cgh.2014.11.026]
 - 75 **Yanai H**, Lichtenstein L, Assa A, Mazor Y, Weiss B, Levine A, Ron Y, Kopylov U, Bujanover Y, Rosenbach Y, Ungar B, Eliakim R, Chowers Y, Shamir R, Fraser G, Dotan I, Ben-Horin S. Levels of drug and antidrug antibodies are associated with outcome of interventions after loss of response to infliximab or adalimumab. *Clin Gastroenterol Hepatol* 2015; **13**: 522-530. e2 [PMID: 25066837 DOI: 10.1016/j.cgh.2014.07.029]
 - 76 **Frederiksen MT**, Ainsworth MA, Brynskov J, Thomsen OO, Bendtzen K, Steenholdt C. Antibodies against infliximab are associated with de novo development of antibodies to adalimumab and therapeutic failure in infliximab-to-adalimumab switchers with IBD. *Inflamm Bowel Dis* 2014; **20**: 1714-1721 [PMID: 25069030 DOI: 10.1097/MIB.0000000000000138]
 - 77 **Van Assche G**, Vermeire S, Ballet V, Gabriels F, Noman M, D'Haens G, Claessens C, Humblet E, Vande Casteele N, Gils A, Rutgeerts P. Switch to adalimumab in patients with Crohn's disease controlled by maintenance infliximab: prospective randomised SWITCH trial. *Gut* 2012; **61**: 229-234 [PMID: 21948942 DOI: 10.1136/gutjnl-2011-300755]

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