Introduction

Several classes of medications are available for treatment of inflammatory bowel disease (IBD). The rate of clinical and endoscopic response varies greatly between and within the medication classes. Measurement of levels of the medications (or its metabolites) and anti-drug antibodies (ATI) may in many cases provide insight into the mechanism of the evolving loss of response (LOR), as well as suggest a possible salvage strategy. In this review, we will focus on therapeutic monitoring of two main classes of IBD medications, i.e. thiopurines, including azathioprine (AZA) and 6-mercaptopurine (6-MP), and tumor necrosis factor (TNF)-α inhibitors, including infliximab (IFX), adalimumab (ADA), certolizumab pegol (CZP) and golimumab (GLM).

TNF-α inhibitors

Anti-TNFs have been the mainstay of anti-inflammatory treatment in IBD for the past decade. Four agents are currently available in the US (IFX, ADA, CZP, and GLM), and three in Europe and Canada (IFX, ADA, and GLM). In addition, biosimilars are emerging and will provide additional therapeutic options in the near future. Assays for assessment of serum levels of IFX and ADA as well as corresponding anti-drug antibodies (ATI) are available commercially. The majority of clinical experience in therapeutic drug monitoring stems from IFX data, reviewed below in greater detail.

Definitions of LOR

Primary non-response is generally defined as a failure to achieve clinical response following an induction phase of treatment [1]. Importantly, some patients may take longer to achieve initial response. Primary non-response occurs in approximately one third of the patients started on anti-TNFs [2]. Secondary loss of response occurs at any point during the treatment after an initial response has occurred. However, there is no consensus regarding the criteria identifying response. While the European Crohn’s and Colitis Organization guidelines suggest a definition based on clinical scores (Crohn’s disease activity index, CDAI [3], multiple different definitions varying from endoscopic healing to a need for dose intensification have been proposed. The incidence of secondary loss of response is variable. For IFX, an annual risk of loss of response of 13% was reported [4]. However, the risk is not distributed evenly, with roughly 2/3 of the patients developing LOR with the first 12 months, and the rest losing response at a significantly slower pace. For ADA, annual LOR incidence of 10%-24% has recently been reported [5,6].
Risk factors for primary non-response

Several factors have been demonstrated to be associated with a primary loss of response to anti-TNF in IBD, including longer (>2 years) disease duration, extensive small bowel disease in CD, smoking and normal C-reactive protein (CRP) on initiation of treatment [7,8]. Polymorphisms in the apoptosis-related genes such as FAS-L and Caspase 9, as well as in the IBD5 locus, were also associated with an increased risk of primary LOR in CD [8,9]. In UC, increased age, anti-neutrophil cytoplasmic antibody-positive status and anti-Saccharomyces cerevisiae antibody-) negative status, and prior anti-TNF exposure were identified as risk factors for primary nonresponse [10].

Risk factors for secondary LOR

Although immunogenicity is by far the most studied, it is not the sole mechanism responsible for loss of response. Multiple additional etiologies, including non-adherence, fecal drug loss, non-immune clearance and non-TNF-driven disease, have been implicated in the pathogenesis of secondary LOR (Fig. 1, adapted from Ben-Horin et al).

Several risk factors are associated with increased risk of loss of response, including episodic treatment, non-inflammatory symptoms, symptomatic stricture and smoking [2]. In randomized controlled trials, concomitant treatment with AZA was shown to be protective against LOR [11]. In contrast, concomitant treatment with methotrexate failed to improve clinical response rates, possibly owing to a large proportion of patients being on corticosteroids at treatment onset in this particular trial [12]. Nonetheless, the latter study found higher trough levels of IFX and lower rates of antibody formation with methotrexate co-treatment [12].

Multiple studies have confirmed a correlation between clinical response and trough serum levels of anti-TNF medications [13-17]. Moreover, such correlation was recently established not only for clinical response but for endoscopic outcomes (mucosal healing) and decline of inflammation markers [18-21].

Currently, there is no clear consensus on the trough level values that correspond to clinical response. Recently, a cut-off trough level of 3 μg/mL has been suggested to have the optimal discriminatory accuracy for response to IFX in CD [22]. Trough levels of 3-7 μg/mL [23] and 5-10 μg/mL [24] have recently been suggested as target levels for maintenance therapy for both UC and CD. In addition, serum levels at non-trough time points have also correlated with clinical response. For example, a serum level of IFX of 12.0 μg/mL at 4 weeks from the last infusion was independently correlated with clinical response [15]. For ADA, a cut-off drug level of 5.85 μg/mL yielded optimal sensitivity, specificity and positive likelihood ratio for prediction of clinical response [26].

Importantly, identification of a uniform target level for serum IFX is challenging as the detection assays vary significantly between different centers. It also remains to be determined whether the trough levels associated with optimal response are similar for CD and UC.

Antibodies

ATI directed against the FAB fragment of the molecule [27] develop against both chimeric and fully humanized anti-TNFs. ATI interfere with the biologic activity by inhibiting the binding of the TNF-α inhibitors to both serum and membrane-bound

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**Figure 1** TDM based algorithm for management of loss of response to TNFα inhibitors. Adapted from Ben Horin, et al [87]

LOR, loss of response; CRP, C-reactive protein; FCP, fecal calprotectin; VCE, videocapsule endoscopy; BOG, bacterial overgrowth; BSD, bile salt diarrhea; ATI, anti-drug antibodies
Evaluation of serum levels and ATIs

Several techniques are available for the purpose of measurement of serum levels and antibodies. The most common is a solid phase double-antigen ELISA, in which IFX serves as both the capture antigen and the detection antibody. This technique is relatively reproducible and inexpensive [34]. This method has some important drawbacks, the main one being an inability to detect ATI in the presence of IFX in the serum. These results, reported as “inconclusive”, are very commonly reported. For instance, 72% of measurements of ATI in the SONIC trial were deemed inconclusive [11]. In addition, this method is also incapable of detection of immunoglobulin 4 (IgG4) ATI. A modified ELISA, employing anti-human λ antigen detection antibody (AHLC), has an improved capacity for detection of ATI in presence of IFX, as well as for detection of IgG4 ATI [35]. Such “double positive” results were reported in 9% of patients, and were associated with a trend towards a higher future risk of development of LOR [36]. An opposite state of “double negativity” (IFX-ATI-), frequently reported by Farrell et al [33].

Therapeutic drug monitoring (TDM) for primary non-response

In the majority of cases, primary non-response is not associated with subtherapeutic IFX levels [44]. In specific clinical situations, such as acute UC, increased clearance of IFX through fecal loss and accelerated formation of ATI [45,46] result in low serum levels of IFX accompanied by non-response. A strategy based on an accelerated dosing scheme based on patient’s clinical response for patients with acute UC has been suggested; however it is yet unclear how the immunopharmacological data should be incorporated in such algorithm [47]. Patients with a primary loss of response may still respond to another anti-TNF [9].

TDM for secondary LOR (Fig. 1)

When drug levels are adequate and there are no signs of active inflammation (CRP, fecal biomarkers, ileocolonoscopy, capsule endoscopy), alternative explanation for the symptoms (underlying IBS, bacterial overgrowth, bile salt diarrhea, infection, etc) should be sought.

When active inflammation is present and drug levels are adequate, the patient is unlikely to respond to dose escalation, and a switch to a different medication class should be considered [2,48].
In the presence of active inflammation with absent or low IFX levels, the determination of ATI should be undertaken. Initial reports had suggested that in presence of detectable ATI, dose escalation was generally ineffective (86% in patients without ATI vs. 17% in patients with detectable ATI, P=0.001 [49]. However, this study utilized a double-antigen assay ELISA unable to simultaneously detect IFX and ATI. Thus, all patients with detectable ATI had no detectable serum IFX. In a French study utilizing a modified ELISA, 6/10 patients with ATIs responded to dose escalation [50]. In a recent study evaluating the utility of IFX and ADA levels ATIs for prediction of response to intervention in LOR, only high-level antibodies (antibodies-to-adalimumab >4 mcg/mL-eq and antibodies-to-IFX >9 mcg/mL-eq) were 90% specific for failure to respond to dose intensification [51]. This study utilized an AHLC ELISA that permits the simultaneous detection of IFX/ADA and ATI. It is likely that an emergence of ATI is not an “all-or-none” phenomenon but rather a continuous phenomenon, with higher levels associated with a lower risk of a successful dose escalation.

An additional, relatively underexplored therapeutic option is the addition of an immunomodulator in patients on anti-TNF monotherapy. Combination therapy with IFX and AZA in the SONIC study was associated with a superior clinical response, as well as higher 4-week IFX levels and lower prevalence of antibodies [11]. A combination of IFX with methotrexate resulted in a significantly lower prevalence of ATI and a trend for higher serum IFX levels, although without a significant difference in clinical efficacy [12]. In these studies, an immunomodulator was initiated simultaneously with and anti-TNF. In a recent report in CD patients who have developed LOR to IFX accompanied by ATI, the addition of an immunomodulator in patients on monotherapy (AZA in 3 patients and methotrexate in 2 patients) resulted in a gradual restoration of clinical response, decrease in ATI titers and augmentation of IFX levels [52].

When clinical LOR appears in the setting of inadequate anti-TNF levels and low/undetectable ATI, dose escalation is suggested after verification of adherence. No consensus regarding the optimal escalation strategy exists. A pharmacokinetic modeling study in patients with rheumatoid arthritis suggested that interval reduction would provide a more effective drug level AUC compared to the equivalent dose increase [53]. However, retrospective studies comparing dose augmentation with shortening of the interval did not demonstrate any significant difference in the rates of regained response in CD [36,54] or UC [55].

In patients with controlled IBD activity on IFX, dose adjustment can also be beneficial. In the TAXIT study, CD patients in clinical remission were randomized to 2 strategies: 1) dose adjustment to trough levels of 3-7 μg/mL; 2) dose optimization as a response to LOR. Patients on the level-optimized strategy had superior disease control without an increase in a total cost [23].

**Thiopurines**

Thiopurine medications (6-MP and AZA) have long constituted the mainstay of IBD immunomodulator therapy. A randomized controlled trial in 1980 by Present et al [56] observed a 67% response rate to 6-MP (1.5 mg/kg) in CD. A meta-analysis by Pearson et al [57] reported an odds ratio of 3.09 for response to therapy over placebo. In the more recent meta-analyses conducted for the Cochrane database, a pooled efficacy of 54% and 71% for induction and maintenance of CD remission was reported for AZA [58,59]. Although the data are less robust for UC, AZA was shown to be superior for the maintenance of remission compared to placebo (failure to maintain remission: OR 0.41; 95% CI 0.24 to 0.70) [60]. Thiopurine treatment is associated with a decreased risk of surgery in CD patients [61]. Recent reviews suggest that approximately 50% of all IBD patients are treated with a thiopurine [62,63].

Currently recommended dosing of the thiopurines is 1.5-2.5mg/kg/day for AZA or 0.75-1.5mg/kg/day for 6-MP, according to the European Crohn’s and Colitis Organization guidelines [64]. The American Gastroenterology Association (AGA) guidelines recommend 2.0-3.0 mg/kg/day for AZA and 1-1.5 mg/kg/day for 6-MP [65].

Thiopurine treatment is associated with adverse effects in 15-39% of patients, potentially leading to their discontinuation [63,66]. At least 9% of IBD patients are primary non-responders to thiopurines [67]. In addition, treatment with thiopurines is associated with loss of response over time in some cases. In a recent retrospective study including 363 IBD patients followed up for up to 8 years, the proportion of patients still using thiopurines at 3, 6, 12, 24, and 60 months was 73%, 69%, 63%, 51% and 42%, respectively [68]. The main reported reasons for discontinuation reported were refractoriness and adverse effects.

The therapeutic benefit and toxicity of thiopurines are mediated by the levels of their principal intracellular metabolites (Fig. 2). The purine analogue 6-TGN is the primary biologically active metabolite that is incorporated into cellular nucleic acids, resulting in the inhibition of lymphocyte proliferation and T-cell apoptosis. In addition, 6-TGN was reported to inhibit TNF-related apoptosis-inducing ligand, TNF receptor superfamily member 7, and α4-integrin in activated T-lymphocytes [69]. The slow onset of the action of the (up to 3 months) may be explained by the depletion of antigen-specific memory cells [70]. 6-methylmercaptopurine metabolites (6-MMP) are associated with hepatotoxicity [71], but may also exert an independent anti-proliferative effect on T-lymphocytes [63].

**Thiopurine metabolism**

Once absorbed, AZA is converted to 6-MP by a non-enzymatic pathway. 6-MP can be metabolized through 3 main enzymatic pathways: to 6-thiouric acid (6-TU) by xanthine oxidase (XO), activated to 6-MMP by the key enzyme thiopurine methyltransferase (TMPT), or to the biologically active 6-thioguanine nucleotides (6-TGN) by hypoxanthine phosphoribosyl transferase (HPRT), inosine monophosphate dehydrogenase (IMPDH), and guanosine monophosphate synthetase (GMPS) [72]. The gene for
TPMT is located on the short arm of chromosome 6. Several variant alleles (genotypes) have been reported to decrease TPMT activity. These polymorphisms result in a trimodal distribution of TPMT activity in the general population, with absent to very low activity in 0.3% of individuals with a homozygous mutation. Intermediate enzyme activity among heterozygotes is seen in 11% and normal or high activity is observed in about 89%. Patients with low or intermediate TPMT activity phenotypes who are treated with standard doses of AZA or 6-MP are at risk of myelosuppression caused by excess accumulation of 6-TGN [73]. Intermediate or low TPMT activity is most frequently associated with TPMT*2, TPMT*3A or TPMT*3C alleles in Caucasians [73], and TPMT*3C in African-Americans [74].

In patients with homozygous normal phenotype or normal enzymatic activity thiopurines can be initiated at a normal dose, while in heterozygotes or in patients with intermediate enzymatic activity the initial dose should be reduced by 30-70%; in the rare cases of mutant homozygotes with low or absent enzymatic activity, this class of medications should be avoided [75].

**Clinical utility of TPMT assessment**

TPMT can be assessed either by identification of mutated alleles (genotype) or by measuring the biologic activity (phenotype), usually in erythrocytes [76]. An important limitation of the genotyping is that less frequent mutations mostly relevant to the non-Caucasian population will be missed on standard testing; on the other hand, phenotypical assessment is not reliable (up to 90 days) after a recent blood transfusion [76].

FDA recommendations suggest assessment of TPMT (either genotype or phenotype) before initiation of thiopurines [65]. However, this is not mandated by the European guidelines [64]. TPMT assessment is associated with several benefits in addition to avoiding rare but potentially fatal severe bone marrow suppression [77]. When TPMT genotype/phenotype is unknown, a common practice is to initiate thiopurine treatment at a low dose with a graduate escalation to a therapeutic dosing range. However, this practice is both unsafe and impractical, as it may take up to 6 months to achieve therapeutic metabolite levels. This “start low go slow” strategy is unnecessary in the majority of patients with normal TPMT activity. Guiding the dosing by TPMT and thiopurine metabolite levels will lead to a faster onset of response and reduction of costs [78]. TPMT deficiency explains approximately 25% of all the cases of myelosuppression on thiopurine treatment [60]; it does not account for all the possible factors contributing to the risk of leukopenia and does not preclude the need for continuous blood count monitoring. Moreover, TPMT testing does not prevent long-term myelosuppression that may occur at any time, appearing after one year of treatment in 25% of the patients [79]. In addition, TPMT testing does not predict the risk of idiosyncratic adverse events such as fever, arthralgias, hepatitis or pancreatitis [76]. An association between TPMT activity and response to thiopurines has been suggested. TPMT activity below 35 pmol/h/mg of hemoglobin correlated with a greater chance of clinical response (81% vs. 43%; P<0.001) [81]. In an additional study TPMT activity below 15.3 U/mL RBC is associated with a six fold higher response rate to AZA [82]. The risk of resistance is increased in patients with high TPMT activity (over 14 U/mL RBC; OR, 0.21; 95% CI, 0.06-0.71; P=0.009) [63].

**Thiopurine metabolites for monitoring of therapy in IBD**

6-TGN levels have been significantly and independently associated with therapeutic response in IBD. The initial assessment of thiopurine metabolites should be performed at least 3 weeks after initiation of treatment in patients with normal TPMT activity. Dubinsky and colleagues first reported that patients with 6-TGN level between 235 and 450 pmol/8×10^8 erythrocytes are 5-times more likely to be in clinical remission in comparison to patients with lower 6-TGN levels; in the same patient cohort, 6-MMP levels above 5,700 pmol/8×10^8 erythrocytes were associated with a threefold risk of hepatotoxicity [71,80]. A meta-analysis showed that the pooled odds ratio for achieving therapeutic response for a 6-TGN level of ≥235 pmol/8×10^8 erythrocytes was 3.3 (95% confidence interval - 1.7-6.3) in comparison to levels <235 (P<0.001) [81]. 6-TGN levels exceeding 450 pmol/8×10^8 erythrocytes are associated with myelotoxicity [82]. Several additional cut-off 6-TGN values have been proposed to optimize therapeutic response [63]. In patients without clinical response and...
patients, characterized by low (<100 pmol/8×10⁸) levels and thiopurine metabolites can easily identify non-compliant 6-MMP/6-TGN levels usually exceeding 24 [76]. In addition, low 6-TGN levels in presence of high 6-MMP levels, with thiopurines

Laboratory monitoring for patients treated with thiopurines

CBC and transaminases should be assessed before onset of treatment and at 2, 4 and 8 weeks after initiating therapy, irrespective of TPMT status. Baseline and follow-up pancreatic enzymes should also be followed, as in some cases elevated amylase and lipase may precede the clinical presentation of pancreatitis [76]. Blood counts should then be repeated every 3 months, or 2 weeks after dose adjustment. Thiopurine metabolite levels can be determined after 2-3 weeks on therapy or after dose adjustment; the levels should be reassessed when facing a loss of response, adverse effect or when a medication with a potential effect on the thiopurine metabolism (such as 5-ASA, allopurinol, furosemide etc) is added. In addition, it is advisable to assess metabolite levels twice yearly for the purposes of routine monitoring and verification of adherence [76].

Concluding remarks

A large body of evidence supports the clinical utility of therapeutic drug monitoring in IBD for patients treated with thiopurines or TNF-α inhibitors. Timely assessment of drug/metabolite levels and anti-drug antibodies may result in an improved clinical outcome and minimization of preventable complications. In cases of loss of response, therapeutic drug monitoring can guide the selection of the appropriate management strategy, combined with clinical, laboratory and endoscopic data.

References


47. Gibson D, Heetun Z, Byrne D. An accelerated infliximab dosing strategy for rescue therapy in acute severe colitis is associated with reduced early colectomy rate. *J Crohns Colitis* 2014;8:S51, S55-S56.


