**CONCURRENT IMMUNOMODULATOR THERAPY IS ASSOCIATED WITH HIGHER ADALIMUMAB TROUGH LEVELS DURING SCHEDULED MAINTENANCE THERAPY**

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**Abstract**

**Introduction**

Combination therapy with infliximab and immunomodulators is superior to monotherapy, resulting in better outcomes and higher trough levels of infliximab. The role of concurrent immunomodulatory therapy on adalimumab trough levels has not been adequately investigated. Therefore we evaluated the impact of concomitant immunomodulation on trough levels of scheduled maintenance adalimumab therapy.

**Method**

We conducted a prospective observational, cross-sectional study of all inflammatory bowel disease patients on maintenance therapy who had adalimumab trough levels measured between Jan 2013-Jan 2016. Drug level and anti-drug antibody measurements were performed on sera using a solid phase assay. Pairwise comparison of means was used to compare trough levels in patients with and without concomitant immune modulator therapy.

**Results**

In total 79 patients were included. Twenty-three patients (29.1%) were on weekly dosing whereas 56 (70.9%) were on alternate weeks. Median adalimumab trough levels were comparable in patients with and without clinical remission (6.8 µg/ml (IQR 5.6-8.1) (6.7 µg/ml (IQR 3.9-8.1), respectively. Patients with an elevated faecal calprotectin >250 µg/g had lower adalimumab trough levels (median 6.7, IQR 3.9-8) compared to patients with faecal calprotectin <250 µg/g (median 7.7, IQR 6.1, 8.1) though this did not achieve statistical significance, (p=0.062). Median adalimumab trough levels among patients on concurrent immunomodulators was 7.2 µg/ml (IQR 5.7-8.1) whilst those not on concurrent immunomodulators had levels of 6.1 µg/ml (IQR 2.7-7.7, p=0.0297).

**Conclusion**

Adalimumab trough levels were significantly higher in patients on concurrent immunomodulators during maintenance therapy. There was a trend towards a lower adalimumab trough level in patients with elevated calprotectin.

**Introduction**

The anti-tumour necrosis factor (anti-TNF) agents, infliximab and adalimumab are effective in the treatment of inflammatory bowel diseases (IBD), Crohn’s disease and ulcerative colitis. Infliximab is a chimeric and adalimumab is a fully humanised monoclonal immunoglobulin G1 (IgG1) antibody against TNF. The two agents appear comparable in terms of clinical efficacy[1,2] but the structural difference between the two anti-TNF agents may have particular implications for combination therapy with immune modulators (IM), such as thiopurines and methotrexate.

The superiority of combination therapy with thiopurines is well established for infliximab with evidence of efficacy in achieving steroid free remission for both ulcerative colitis[3] and Crohn’s disease[4]. In addition to a clinical benefit, concomitant IM therapy exerts a beneficial effect on the pharmacokinetics and immunogenicity of infliximab. Several studies[5,6] including a randomised trial[4], have shown a beneficial effect of concomitant IM therapy in increasing trough levels of infliximab and reducing immunogenicity[7,8]. The beneficial effect of thiopurines on infliximab trough levels may last beyond the induction phase. In a randomised trial of withdrawal of immunosuppression in stable patients on combination therapy, infliximab trough levels were higher at 2 years in patients on combination therapy compared to patients on infliximab monotherapy alone[9]. Relatively fewer data exist on the efficacy of concomitant IM therapy and its effect on trough levels of adalimumab. In a meta-analysis of data from randomised trials, there was a small clinical benefit to combination therapy for induction but not maintenance therapy[10] but prospective randomised data are lacking. Currently available data, though limited, suggests that combination therapy with IM are unlikely to augment trough levels of adalimumab[11] in the same way as infliximab. In light of the relative paucity of data for concomitant IM therapy for adalimumab, we investigated the effect of combination therapy on clinical efficacy and trough levels of adalimumab.

**Methods**

We conducted a single-centre observational study of IBD patients treated with ADA at the Royal Liverpool University Hospital between January 2013 and February 2016. Patients who had concurrent measurements of serum trough and anti-drug antibody levels were eligible for inclusion. Trough and antidrug antibody levels were obtained within 24 h of scheduled ADA administration. Only patients on scheduled maintenance therapy were included in the study, and testing was undertaken at the discretion of the treating clinician, based upon clinical need or out of clinical interest. Concurrently, clinical information including immunomodulator therapy, body mass index (BMI), site of disease, duration of disease and anti-TNF therapy, smoking status, prior anti-TNF exposure, and disease activity [Harvey Bradshaw Index for Crohn’s disease (CD) and simple clinical colitis activity index for ulcerative colitis (UC)] and any dose escalation was recorded. Clinical remission was defined as a Harvey Bradshaw Index of up to 5 and a simple clinical colitis activity index of up to 2. Biochemical variables, including serum albumin and CRP levels, obtained at the time of trough level measurement were recorded. All participants who had a trough level measured outside of clinical indication provided written informed consent. The study was approved by Northwest Liverpool East ethics committee (12/NW/0274).

*Drug level and anti-drug antibody assay*

Drug level measurements were performed on sera using the LISA-Tracker ADA indirect ELISA kits (Theradiag, Marne-la-Vallée, France). These assays enable quantitative determination of free-drug levels 0.1–8.0 µg/ml. Antidrug antibodies were quantitated using the LISA-Tracker anti-adalimumab double antigen ELISA kits (Theradiag). The assays detect free antidrug antibodies, and a level greater than 10 ng/ml was considered positive.

*Statistical analysis*

Categorical variables have been summarized as frequency (%) and continuous variables as median (interquartile range, IQR), and were compared between remission or treatment groups using Pearson’s chi-square test (or Fisher’s Exact test, when expected frequencies were low) and Mann Whitney U test, respectively. All analyses were carried out using Stata v13 software (Stata Statistical Software, Release 13; StataCorp LP, College Station, Texas, USA).

**Results**

A total of 79 patients were included in the study; an additional patient was tested but had anti-drug antibody and was therefore excluded from the analysis. The remaining patients all had undetectable anti-drug antibodies (<10ng/ml). The baseline characteristics of the included subjects are summarised in Table 1.

*Clinical and biochemical remission in monotherapy versus combination therapy groups*

The proportion of patients in clinical remission was comparable in patients on monotherapy (n=17, 48.6%) and combination therapy (n=20, 45.5%). Ten patients (23.8%) on combination therapy had CRP >5mg/dl compared to 14 (41.2%) in the monotherapy group (p=0.105). Twenty patients (60.6%) on combination therapy had an elevated faecal calprotectin compared to 14 patients (56.0%) in the monotherapy group (p=0.724).

*Adalimumab trough levels in patients with clinical and biochemical remission*

Adalimumab trough levels were similar in patients with clinical remission (6.8 µg/ml (5.6-8.1, n=37) than those without (6.7 µg/ml (3.9-8.1, n=42), Figure 1A. Patients with an elevated CRP >5mg/dl (n=24) had lower adalimumab trough levels (median 6.4, IQR 5.6, 8.1, range 0.3, 8.1) compared to patients with a normal CRP (n=52) (median 6.9, IQR 6.9 (5.6, 8.1) range 0.1, 8.1) but this was not statistically significant (p=0.131), Figure 1B. Similarly, patients with an elevated faecal calprotectin >250 µg/g (n=34) had lower adalimumab trough levels (median 6.7, IQR 3.9-8) compared to patients with faecal calprotectin <250 µg/g (n=24) (median 7.7, IQR 6.1, 8.1) though this also did not achieve statistical significance, (p=0.062), Figure 1C.

*Concurrent immunosuppression is associated with higher adalimumab trough levels*

Median (IQR) ADA trough levels among patients on concurrent IM was 7.2 µg/ml (5.7-8.1) whilst patients not on concurrent IM had levels of 6.1 µg/ml (2.7-7.7, P=0.0297)

**Discussion**

We report that patients on concurrent IM therapy had higher trough levels of adalimumab during scheduled maintenance therapy. Our finding of a higher trough level in patients on concurrent IM during maintenance therapy is broadly consistent with that reported by Chiu et al[12]. In an analysis of serum samples from patients enrolled to CLASSIC-I and II studies, a higher concentration of adalimumab was reported at weeks 24 and 56 in patients with concurrent IM therapy. Despite a similar magnitude of difference to that noted in our study (median difference of 2 µg/ml), their findings did not achieve statistical significance. To the contrary, other studies[11,13] have reported a lack of effect of concurrent IM on adalimumab trough levels including a previous report from a smaller cohort included in this study[14]. Several factors including duration of therapy, type of assay, timing of sampling and baseline disease activity, all of which influence trough levels[15] could account for the observed differences.

It is unclear whether or not the observed increase in adalimumab trough levels on concurrent IM is clinically relevant. The optimal cut-off value of adalimumab trough level associated with clinical remission and mucosal healing is still under evolution. Two previous studies suggested that an adalimumab trough level above 5.85 µg/ml[16] and 4.9 µg/ml[17] correlated with clinical remission and mucosal healing respectively. However, recent studies suggested cut-off levels of 8.14 µg/ml[18] and 7.1 µg/ml[19] for prediction of mucosal healing. Nonetheless, several lines of evidence suggest a possible clinical benefit for combination therapy. A meta-analysis showed higher clinical remission rates after induction therapy with concurrent IM compared to adalimumab alone but this difference was not observed during maintenance therapy[10]. A further retrospective study showed that combination therapy was associated with a lower risk of induction therapy failure and therapy beyond 6 months was associated with fewer semesters with flares and a lower need for dose escalation[20]. This is consistent with other observations of a longer time to dose escalation with combination therapy[13]. Overall, these findings, in conjunction with our observations, suggest a possible benefit of combination therapy for both induction and maintenance. However, it is difficult to draw definitive conclusions about the merits of combination therapy with adalimumab in the absence of an adequately powered randomised trial to address the efficacy of combination therapy. Moreover, the possible benefits need to be tempered against an increased risk of lymphoma[21], non-melanoma skin cancers[22] and urinary tract cancers[23] associated with thiopurine therapy.

We noted comparable trough levels of adalimumab in patients with and without clinical remission whereas previous studies[12,16] reported higher trough levels in patients with clinical remission. We noted a trend towards higher trough levels in patients with lower calprotectin levels but this did not achieve statistical significance. Our data is consistent with previous findings of a higher trough level in patients with endoscopic remission[17,18] but the data on calprotectin is limited. Moreover, the calprotectin value that best correlates with mucosal healing is not fully established. We chose a calprotectin cut-off of 250 µg/g[24] but there is a wide range of correlation describing the association between faecal markers and endoscopic disease activity (*r*-values ranging from 0.32 to 0.87, *P*-values ranging from < 0.0001 to 0.7815)[25]. One potential reason for lower adalimumab trough levels being associated with higher CRP is faecal loss. Faecal drug loss has been shown to be greater with greater degrees of mucosal inflammation, of which a higher CRP is a surrogate[26].Further studies are required to establish therapeutically relevant trough levels of adalimumab using calprotectin as a surrogate for mucosal healing as this would be preferable to invasive endoscopic assessment.

Surprisingly, the incidence of anti-drug antibodies in our study was relatively low compared to previously reported rates of 9.2%-30%[11,13,27]. In addition to differences in patient population, the differences may also be explained by indications for testing and the different assays used across the studies. The assay used in our study was not a drug tolerant assay which could partly explain the low incidence of antibodies. Moreover, there is no standardised unit for comparison of antibody titres across various assays. Finally, a higher proportion of patients in our study were on combination therapy which reduces the likelihood of anti-drug antibodies.

Our study has some notable strengths and limitations. All patients had their sample drawn within 24 hours of scheduled administration whereas previous studies[18] had used a wider sampling window which may not be truly reflective of trough adalimumab levels. The upper limit of quantitation was 8µg/ml for adalimumab trough levels which could have missed clinically relevant differences as adalimumab levels up to 12 µg/ml are associated with an increased rate of mucosal healing[19]. Such a difference between the two groups may not have been detected as a consequence. Finally, due to the cross-sectional nature of our study, we were unable to establish if the observed differences in trough levels resulted in better clinical outcomes.

In conclusion, we report higher adalimumab trough levels in patients with concurrent IM on scheduled maintenance therapy. Our findings do not necessarily support the use of combination therapy and the benefits and risks of such a strategy compared to adalimumab monotherapy for induction and maintenance needs to be established in a randomised clinical trial.

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Conflicts of interest: AB, SD, TS and GF report no conflicts of interest. SS has received speaker fee from MSD, Actavis, Abbvie, Dr Falk pharmaceuticals, Shire and received educational grant from MSD, Abbvie, Actavis and is an advisory board member for Abbvie, Dr Falk pharmaceutics, Janssen and Vifor pharmaceuticals.

Author contributions: AB, TS and GF were involved in data collection and drafting of the manuscript. SD was involved in data analysis, drafting and final revision of the manuscript. SS was involved in study design, data collection, analysis, drafting and revision of the manuscript.

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**Figure Legends:**

**Figure 1A-C: Adalimumab trough levels (median ± IQR) in patients with and without clinical remission (1A), with and without biochemical remission as defined by CRP (1B), and faecal calprotectin (1C)**

**Figure 2: Adalimumab trough levels (median, IQR) in patients with and without concomitant immunomodulatory therapy**