CLINICAL STUDY

Predictive value of infliximab trough levels in maintenance therapy for 5-year sustained clinical remission in patients with inflammatory bowel disease

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ABSTRACT

BACKGROUND: Despite long-term use of infliximab (IFX) in IBD treatment, its optimized use is unclear due to its complicated pharmacokinetics/dynamics. Hence, the predictive value of IFX trough levels (TL) is important in treatment management.

METHODS: We performed a prospective, cross-sectional, observational study with 74 IBD patients treated with IFX (mean 9.1 years, SD ± 3). TL was measured during maintenance therapy, in which maintenance of remission was followed for 5 years.

RESULTS: TL > 3 µg/ml during maintenance therapy was a significant predictor of clinical remission in 5 years in UC patients (82 % vs 62 %, $p < 0.001$). Concomitant treatment with azathioprine (AZA) was significantly associated with TL > 3 µg/ml during maintenance therapy in a cohort of IBD patients ($p = 0.05$). Deviations in percentage of remission and fraction of relapses in TL categories were insignificant in a cohort of CD patients (85 % vs 74 %, $p > 0.05$).

CONCLUSIONS: TL > 3 µg/ml during maintenance therapy is a strong predictor of sustained clinical remission for 5 years in UC patients. The use of combination therapy with AZA, due to its significant association with high TL, may have a practical benefit in achieving better clinical outcomes in UC patients *(Tab. 2, Fig. 10, Ref. 20)*. Text in PDF *www.elis.sk*

KEY WORDS: trough levels, inflammatory bowel disease, ulcerative colitis, Crohn's disease, infliximab, therapeutic drug monitoring.

Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) are the two main forms of inflammatory bowel disease (IBD). Despite their shared characteristics, UC and CD can be distinguished by different genetic predispositions and risk factors as well as clinical, endoscopic, and histological features. The aetiology of IBD remains unknown; however, genetically susceptible individuals often have a dysregulated mucosal immune response to commensal gut flora, which results in bowel inflammation.

Since its introduction as a biologic therapy for IBD, infliximab (IFX), an anti-tumour necrotic factor alpha (TNF) antibody, has been widely used in clinical practice. However, because of its complex pharmacokinetic and pharmacodynamic properties, the best method for use of IFX is still unclear. Despite the introduction of new molecules in recent years, namely integrin antagonists

and interleukin inhibitors for the treatment of moderate to severe IBDs, the use of IFX has remained unchanged due to its fast response in controlling IBD symptoms.

In the treatment of patients, specialists must consider several important determining clinical variables, each of which affect the outcome of the disease: patient age and gender, patient age at the time of first diagnosis of the disease, patient lifestyle and comorbidities, along with the mode of treatment, concomitant combination therapy with immunosuppressors or immunomodulators, choice of medication, routes of administration, intolerance to treatment and its side effects. Due to these complexities, many studies have aimed to identify a united, acceptable regimen of treatment and identify the predictors of success or failure of treatment.

One debatable strategy proposed for achieving optimal biologic therapy is therapeutic drug monitoring (TDM). TDM is a practice of measuring serum drug concentration levels (known as trough level, TL) to determine the presence or absence of anti-drug antibodies (ADAbs) and gain predictive value regarding short- and long-term outcomes. TDM assumes that a systematic and algorithmic assessment of drug TL and ADAbs can objectively identify potential reasons for failure in therapy and define the next steps in disease management and/or provide insight into the aetiology of undesired outcomes. However, most clinicians currently assert

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that the low availability and low-cost effectiveness of controlling for TL and ADAbs in everyday clinical settings make TDM a less attractive and less practical option. Furthermore, changing the treatment intensity or medication type based on TL and ADAbs alone in otherwise symptom-free patients has not been shown to have a significant difference in clinical outcomes in several clinical trials, and may cause unnecessary complications in some cases as compared with the classical adjustment of treatment based on the patient's clinical presentation. Despite many clinical trials designed to support or refute the usefulness of TDM, there is currently insufficient evidence from such studies to draw conclusions about this strategy. Hence, more studies are needed to provide evidence on the optimization of IFX therapies, and to consider important coexisting variables in clinical treatment, especially in regard to long-term outcomes. The aim of the current study is to evaluate the predictive value of TL, ADAbs, and concomitant treatment during maintenance therapy for long-term disease outcomes.

Methods

Study design and cohort of patients

In this prospective, cross-sectional, observational study, we gathered data from a cohort of 74 IBD patients diagnosed with moderate-to-severe UC or CD based on clinical presentation as well as endoscopic, histopathologic, and radiologic examinations, who were under biologic treatment with IFX. The patients started IFX treatment between January 2005 and December 2017. All patients had control IFX TL measured in the second half of 2017 during maintenance therapy (as the point of inclusion in the study). A long-term follow-up was completed in December 2021 with 71 patients; 3 patients were omitted from the study after December 2019 because they failed to attend their regular follow-ups.

Inclusion criteria

All patients given scheduled treatment after initiation of induction therapy met the following criteria: patients were at least 18 years old at the time their first IFX treatment; were naïve to biologic treatment; were treated as ambulatory patients; were steroid-dependant or intolerant (steroid dependency was defined as more than 8 weeks of oral treatment with > 10 mg/day of prednisone during the last 12 weeks or at least 3 months of such a dosage during the last 6 months) and/or on concomitant immunomodulatory (namely azathioprine, AZA) treatment or AZA intolerant.

Treatment and data collection protocol

Patients were treated with IFX as induction therapy at a dose of 5 mg/kg body weight at weeks 2–6 and then every 8 weeks for maintenance therapy. If the treatment was intensified due to the clinical course of the disease, dosages were increased to 10 mg/kg body weight or the intervals between infusions were shortened. In such cases, we recorded the date, reason for treatment intensification, and duration. To maintain safety data, we documented severe post-infusion adverse events. Treatment discontinuation was defined as an interval of more than 4 months between IFX infusions; for each case of discontinuation, we recorded the reason for discontinuation and any concurrent treatment (corticosteroids/AZA). Data on previous and concomitant medication were recorded, including 5-aminosalicylic acid (5-ASA), corticosteroids, and AZA.

Estimation of clinical outcomes

The extent of disease for both CD and UC at the time of inclusion in the study was recorded using the Montreal classification. We recorded demographic data on age, gender, height, and weight (at the time of inclusion in the study), duration of biologic treatment, and laboratory data values such as C-reactive protein (CRP), white blood cell count, and F-calprotectin. Harvey-Bradshaw Index (HBI), partial Mayo (pMayo) scores, and small Inflammatory Bowel Disease Questionnaire (sIBDQ) (Slovak version) scores were collected retrospectively. The pMayo score was chosen over the full Mayo score because of its greater availability to patients and omission of invasive endoscopy. HBI, pMayo, sIBDQ scores, and the laboratory data values, were gathered twice a year (in 6-month periods during patients' check-up appointments) throughout the study. The disease activity indexes, sIBDQ scores, and laboratory data were collected again if patients were given intensification of treatment, switched or swapped to another treatment (loss of response, LOR), or hospitalized because of IBD complications.

The following outcome measures were examined:
1) Clinical remission at follow-ups (from 2017 to 2021) and clinical remission at the end of the study
2) LOR, switch or swap of treatment, hospitalization, surgery, death

Measurement of TL and anti-drug antibodies

Data on trough levels (µg/mL) and presence or absence of ADAbs (if available) were categorized according to Table 1. IFX was detected using Rida screen assays. UC and CD patients were evaluated separately.

Ethical considerations

Clinical data were retrieved and anonymised before analysis. In accordance with local legislation and institutional requirements, neither ethical review and approval nor informed consent for participation were required for this observational study.

Statistical evaluation

We used Microsoft Office Excel for collection of data and Analyse-it software for statistical evaluation. Categorical variables are presented as percentages. Frequencies and differences between groups were determined using chi-squared tests when appropriate for calculating the p value (p value was set as $p \leq 0.05$). Association of TL value and clinical response was evaluated by Kaplan-Meier

Tab. 1. Classification of outcomes based on TL value.

TL µg/mL	TL µg/mL	TL µg/mL	TL µg/mL
0–1	1–3	3–7	> 7

TL: trough level

Tab. 2. Demographic information and clinical characteristics of patients.

	CD patients n= 48	UC patients n= 26
Number of patients	48/74 (64.8%)	26/74 (35.1%)
Male/female proportion	31/17 (64.5%/35.4%)	16/10 (61.5%/38.4%)
Age of patients (mean)	43.6 (SD±11.2)	47.6 (SD±13.5)
Age at start of biologic treatment (mean)	33.0 (SD±10.6)	38.1 (SD±11.3)
Duration of treatment with IFX, years (mean)	9.77 (SD±3.5)	8.6 (SD±3)
Duration of follow-up after measurement of TL, years	5	5
Extent/location of disease (based on Montreal classification)	L1: 7 (14.5%) L2: 10 (20.8%) L3: 29 (60.4%) L4: 2 (4.1%) B1: 19 (39.5%) B2: 9 (18.7%) B3: 20 (41.6%) P: 27 (56.2%)	E1: 0 E2: 8 (30.7%) E3: 18 (69.2%)
Number of patients on concomitant treatment with IFX: (at the time of inclusion in the study)		
Azathioprine + 5-ASA	13 (27.0%)	13 (50.0%)
Only azathioprine	16 (33.3%)	3 (11.5%)
Only 5-ASA	7 (14.5%)	8 (30.7%)
Neither	12 (25.0%)	2 (7.6%)
Number of patients treated with oral steroid treatment at time of starting biologic treatment	38 (79.1%)	18 (69.2%)

5-ASA: 5-aminosalicylic acid, CD: Crohn's disease, IFX: infliximab, SD: standard deviation, UC: ulcerative colitis

survival curves for each category of patients. Association of the presence of concomitant treatment (immunosuppressive treatment with AZA) was analysed using Wilcoxon-Mann-Whitney U tests (with level of significance p = 0.05).

Definitions

Remission was defined as a lasting control of disease activity and persistent improvement of symptoms as assessed during follow-ups.

Results

Baseline demographic and clinical characteristics of the cohort

Of the 74 patients in the study, 48 patients (64.8 %) were diagnosed with CD and 26 patients (35.1 %) were diagnosed with UC. Baseline demographics and clinical characteristics of patients are presented in Table 2. Most patients with CD were treated for ileocolonic complications (60.4 %), and most patients with UC were treated for the pancolitis form of the disease (69.2 %). Of the 74 patients, 44 (59.4 %) were on concomitant treatment and 30 (40.5 %) had prior treatment with AZA at the time of inclusion in study. The main reason for discontinuing AZA was intolerance 28/30 (93.3 %) (usually skin reactions followed by leukopenia and pancreatitis), and only in a minority of cases 2/30 (6 %) as a result of non-response. Fifty-six patients (75.6 %) were on concomitant oral steroid treatment at the time of initiation of biologic treatment. For 3 patients, oral steroid treatment pulses were reintroduced in short episodes after starting biologic treatment due to relapse of disease. The follow-up period after baseline TL measurement was 5 years.

TL values and absence or presence of anti-drug antibodies

In total, 17/74 (22.9 %) patients had TL < 1 μg/mL, 15/74 (20.2 %) patients had TL of 1–3 μg/mL, 18/74 (24.3 %) patients had TL of 3–7 μg/ml and 24/74 (32.4 %) patients had TL > 7 μg/mL. In 32 patients, the presence or absence of ADAbs was recorded at the time of sampling IFX TL. The presence of ADAbs was significant (> 400 ng/mL) in only 2/32 patients; both patients were females diagnosed with UC and TL < 2 μg/mL. The median time between the initiation of therapy and measurement of TL was 6 years (IQR: 4–8). Eight out of 74 patients had TL measured within the first year after initiation of biologic therapy (but not during induction therapy, meaning weeks 0, 2, and 6). In 38/74 patients (51.3 %, CD n = 22/38, 57.8 %, UC n = 16/38, 42.1 %), we recorded treatment intensification (intervention) by means of shortening infusion administration to 6- or 4-week intervals. Intensification was decided based on patients' clinical presentation, not on TL values. Thirty-four out of 38 patients (89.4 %, CD: n = 19/22, 86.3 %, UC n = 15/16, 93.7 %) were still on an intensified regimen by the end of the study and 4 patients were put back on a normal 8-week interval regimen.
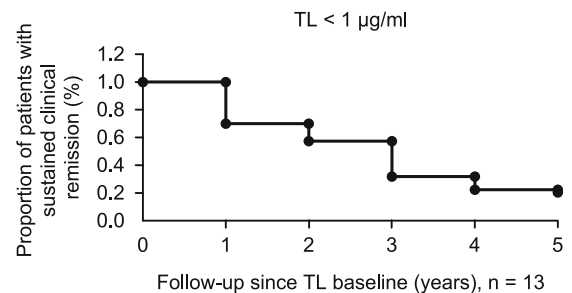

Fig. 1. Sustained clinical remission of IFX in CD patients with baseline TL < 1 μg/ml over 5 years of follow-up. TL: trough level.
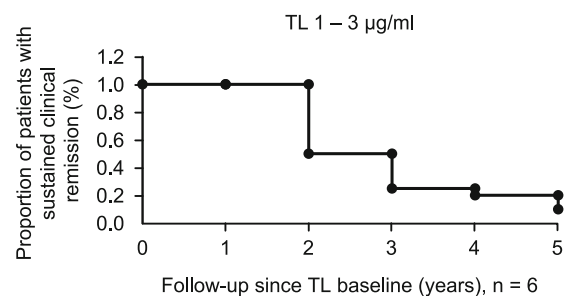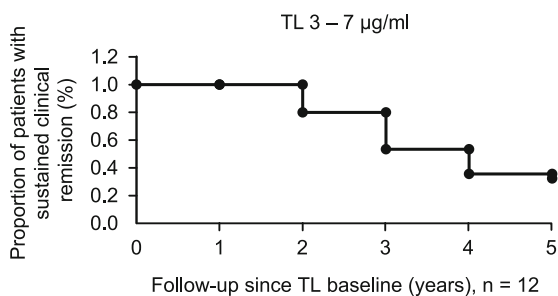

Fig. 2. Sustained clinical remission of IFX in CD patients with baseline TL of 1–3 μg/ml over 5 years of follow-up. TL: trough level.

Five out of 38 patients had intensification of treatment after sampling of TL (anytime between 2018 and 2021). There was a significant correlation between intensification of treatment and TL > 7 μg/mL, (14/33 42.4 %, p = 0.035,). Such correlation, however, was not found for TL values of 3–7 μg/ml (p = 0.28) or lower.

Association of TL values and long-term clinical remission in CD patients

The percentage of remissions within 5 years of follow-up for each baseline TL category in CD patients is presented in Figures 1

to 5. The accumulated 5-year average of percentage of remissions in CD patients for the baseline TL category < 1 μg/ml was 80 %; TL 1–3 μg/ml was 76.6 %, TL 3–7 μg/ml was 85 %, and TL > 7 μg/ml was 68.1 %, respectively. Besides a slightly better accumulated 5-year average percentage of remissions, the separate yearly percentage of remissions for the category TL 3–7 μg/ml was comparatively higher relative to the percentage of remissions in the same years in the other three categories of CD patients. Despite these relatively better outcomes for CD patients in TL category 3–7 μg/ml), the association of these TL values and remission at 5 years in comparison to other TL categories was not shown to be statistically significant (85 % vs 74 %, p > 0.05). Baseline maintenance TL was not shown to have a predictive value for 5-year outcomes in our cohort of CD patients. Deviations in percentage of remission and fraction of relapses in different TL categories were insignificant (< 17% deviation in percent of remission and fraction of relapse) (Fig. 5). Interestingly, not only were higher TLs (> 7 μg/ml) not correlated with better clinical outcomes at 5 years in CD patients (Fig. 4), but the percentage of remission of patients in this category was also higher in comparison to the other three categories (including two TL categories with TL < 3

Fig. 3. Sustained clinical remission of IFX in CD patients with baseline TL of 3–7 μg/ml over 5 years of follow-up. TL: trough level.
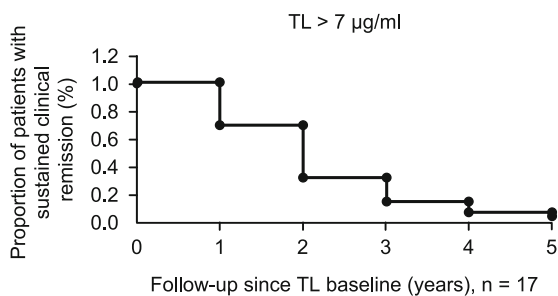

Fig. 4. Sustained clinical remission of IFX in CD patients with baseline TL > 7 μg/ml over 5 years of follow-up. TL: trough level.
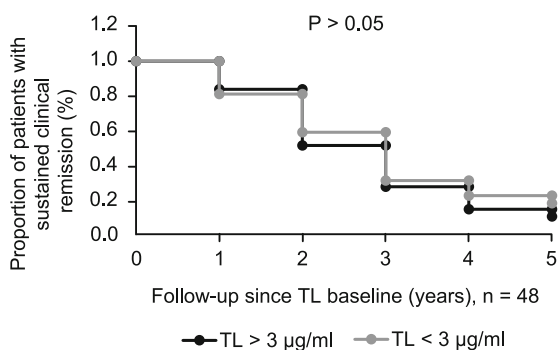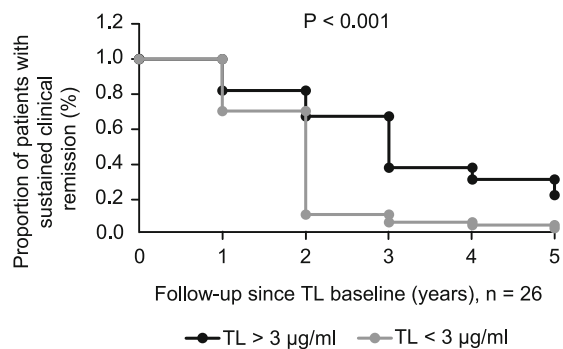

Fig. 5. Comparison of sustained clinical remission of IFX in CD patients with baseline TL < 3 vs > 3 μg/ml over 5 years of follow-up. TL: trough level.

Fig. 6. Comparison of sustained clinical remission of IFX in UC patients with baseline TL < 3 vs > 3 μg/ml over of 5 years of follow-up. TL: trough level.
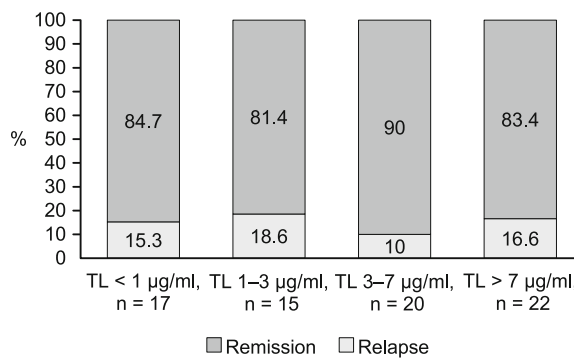

Fig. 7. Accumulated number of relapses within 5 years for different TL values (%). Corrected based on category population cofactor. TL: trough level.

µg/ml). Due to such a low percentage of remission, the overall 5-year outcomes of patients in categories of TL > 3 µg/ml (plotted on a Kaplan – Meier survival estimate curve) (Fig. 5) showed inferior results in comparison with the category of patients with a baseline TL < 3 µg/ml. This finding could be partially explained by the significant correlation between intensification of treatment and TL > 7 µg/mL (14/33, 42.4 %, p = 0.035) in this category of patients, which was not found for TL values of 3–7 µg/ml (p = 0.28) or lower, indicating that patients in this TL category are initially patients with a worse clinical presentation of the disease and hence worse outcome.

Association of TL values and long-term clinical remission in UC patients

Due to low numbers of UC patients in some TL categories and insufficient statistical power of the sample size, the collected data were analysed in two categories of baseline TL: above and below 3 µg/ml (Fig. 6).

Baseline TL values > 3 µg/ml in UC patients were associated with a higher percentage of remission (72% vs 27 % within 5 years). The difference in percentage of remission in each follow-up year (except for the first year) was higher in the category TL > 3 µg/ml than in the category TL < 3 µg/ml (Fig. 6). Association of TL values > 3 µg/ml with remission of clinical symptoms was statistically significant and baseline TL values > 3 µg/ml during maintenance therapy was a significant predictor of clinical remission in 5 years (average percentage of remission in 5 years of follow-up in TL > 3 µg/ml: 82 %, vs TL < 3 µg/ml: 62 %, p < 0.001).

Association of TL values and percentage of relapses in IBD patients

Lower numbers of relapses (%) within 5 years were recorded for patients with TL values of 3–7 µg/ml (10 %) (Fig. 7). The highest percentage of relapses (18.6 %) was recorded for patients with TL values of 1–3 µg/mL, followed by 16.6 % for patients with TL values > 7 µg/ml and 15.3 % for patients with TL values < 1 µg/mL. The percentage of relapses was calculated based on the total number of observed relapses and corrected based on the given category population cofactor of both groups of patients (CD and UC).

Association of TL values and loss of response and hospitalization in IBD patients

LOR was recorded in 5 cases (CD: 1x, UC: 4x). Of the 4 UC patients, 2 had baseline TL values > 7 µg/ml, with undetectable ADAbs at the time of TL sampling, suggesting mechanistic LOR. LOR in these two patients was recorded at a median of a year (IQR: 1–2) after TL sampling. One of these patients had intensification of treatment for a period of 4 years prior to sampling of TL. The other 2 UC patients both had baseline TL values < 3 µg/ml (1x: baseline TL of < 1 µg/ml, 1x: 1–3 µg/ml, who had prior intensification of treatment for a 1-year period before TL sampling). In both patients, the presence of ADAbs was detected at the time of TL sampling (in both cases, ADAb levels were > 500
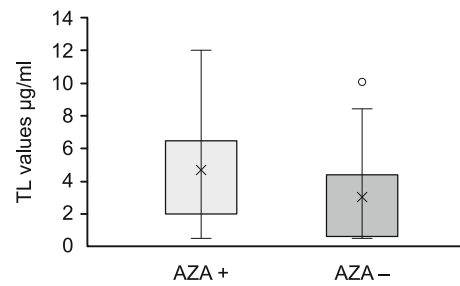

Fig. 8. Association between presence or absence of immunosuppressive concomitant treatment (AZA) on IFX TL values during maintenance therapy in a cohort of IBD patients. AZA: azathioprine, TL: trough level.

ng/ml), which suggests immune-mediated LOR. One of the two patients had further LOR to a swapped treatment. The only CD patient with LOR had a baseline TL value of 1–3 µg/ml, with no detectable ADAbs at the time of TL sampling, which suggests a non-immune pharmacokinetic failure. Despite dose intensification, the clinical symptoms in this patient did not improve and the patient underwent a swap of treatment for a period of 4 years. LOR to the swapped treatment was recorded during this patient's last follow-up. We recorded 5 cases (6.7 %, CD: n = 3x: all L3/B3, UC: n = 2x, including the UC patient with immune-mediated LOR described above, both E3) of hospitalization for IBD complications (relapse of symptoms and worsening of the patients' clinical state). Three out of the 5 patients had TL values < 2 µg/ml (less than one year from the time of TL sampling to hospitalization), and 2/5 had TL values > 5 µg/ml (average of 2 years from the time of TL sampling to hospitalization). No operations or deaths due to IBD complications were recorded during this study.

Association of concomitant treatment with TL values and percentage of relapses during maintenance therapy

The association of presence or absence of AZA as concomitant immunosuppressive therapy to IFX treatment in IBD patients on the drug's TL value was evaluated using the Wilcoxon-Mann-Whitney U test. IBD patients were divided into two groups: AZA+ and AZA –, indicating the presence or absence of concomitant therapy with AZA. The analysed data were further plotted using a box and whisker plot (Fig. 8). Analysis of data as separate cohorts of CD and UC patients, due to insufficient statistical power of the sample size, was abandoned. Concomitant treatment with AZA was significantly associated with TL values > 3 µg/ml during maintenance therapy in the cohort of IBD patients with a median of 10 years (IQR: 7–12) (U-statistic: 305, critical values for the Mann-Whitney U test: 307, n1 = 30, n2 = 30, p = 0.05).

Five-year outcome on the number of relapses per year (%) in CD and UC patients on concomitant treatment with single AZA therapy, single 5-ASA therapy, or combination of both vs no concomitant therapy is shown in figures 9–10. In both categories of patients, there were fewer relapses in the group of patients on concomitant treatment vs no concomitant treatment. The 5-year
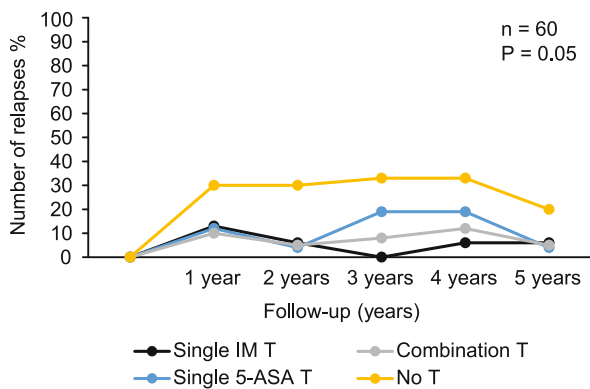
Fig. 9. Five-year outcomes on percentage of relapses/year in concomitant treatment with immunomodulator (AZA) – 5-ASA – combination therapy vs no concomitant treatment in CD patients. T: therapy, 5-ASA: 5-aminosalicylic acid, IM: immunomodulator treatment.
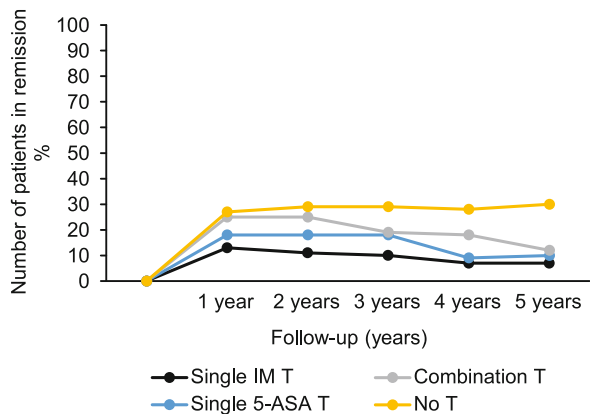

Fig. 10. Five-year outcomes on percentage of relapses/year in concomitant treatment with immunomodulator (AZA) – 5-ASA – combination therapy vs no concomitant treatment in UC patients. T: therapy, 5-ASA: 5-aminosalicylic acid, IM: immunomodulator treatment.

percentage of relapses for patients on concomitant single AZA treatment vs no AZA treatment was 6.2 % compared with 20.4 % for CD patients and 9.6 % compared with 21.6 % for UC patients, respectively. The presence of AZA concomitant treatment was a significant predictor of a lower percentage of relapses within 5 years in both groups of patients (p = 0.001 in cohort of CD patients, n = 48; p = 0.03 in cohort of UC patients, n = 26). The 5-year percentage of relapses for patients on concomitant single 5-ASA treatment vs no 5-ASA treatment was 12 % compared with 19 % for CD patients and 14.6 % compared with 19.3 % for UC patients, respectively. The presence of 5-ASA concomitant treatment was not a significant predictor of a lower percentage of relapses within 5 years in either CD patients or UC patients (p = 0.23 in cohort of CD patients, n = 48; p = 0.10 in cohort of UC patients, n = 26). The combination concomitant therapy (both 5-ASA and AZA) was superior to no concomitant therapy in terms of a lower

percentage of relapses during 5 years in CD patients (8 % vs 30 %, p = 0.0001, n = 48). The combination therapy, however, was not superior to single 5-ASA or single AZA concomitant treatment in CD patients (8 % degree of accumulated relapse at 5 years in combination therapy vs 7 % in single AZA therapy vs 11.6 % in single 5-ASA therapy, p > 0.05, n = 48).

Discussion

Predictive value of TL in maintenance therapy for 5-year sustained clinical remission

Baseline TL values > 3 µg/ml during maintenance therapy was significantly associated with sustained clinical remission within 5 years in our cohort of UC patients (average percentage of remissions at 5-year follow-up in TL > 3 µg/ml: 82 %, vs TL < 3 µg/ml: 62 %, p < 0.001). Hence, TL values > 3 µg/ml in UC patients could be a good predictor of favourable outcomes within 5 years. Despite the available data published on the association between higher TL values and favourable clinical outcomes in UC patients, the benefit of proactive TDM dose adjustment for maintaining targeted TL in UC patients is not clear (mostly due to the retrospective nature and lack of causality in those studies). The data collected from such studies with UC patients suggest that therapeutic decision-making through proactive TDM is more likely to achieve a sustained clinical remission than clinically-guided decisions alone (1, 2). International guidelines (such as ECCO) so far do not recommend for or against the use of proactive TDM in everyday practice in the management of UC patients during maintenance therapy (2).

A TL value of 3–7 µg/ml during maintenance therapy was not significantly associated with a higher percentage of remission and lower number of relapses at 5-year follow-up in CD patients (percentage of remission in CD patients for baseline TL category of < 1 µg/ml was 80 %; TL 1–3 µg/ml was 76.6 %, TL 3–7 µg/ml was 85 %, and TL > 7 µg/ml was 68.1 %, respectively). Although percentages of remission in the two TL categories < 3 µg/ml (namely < 1 µg/ml and 1–3 µg/ml) were lower than the TL category of 3–7 µg/ml, during 5 years of follow-up, the difference between the categories was not statistically significant (85 % vs 74 %, p > 0.05). Despite the expected slightly more favourable outcomes for percentage of sustained clinical remission within 5 years in the group of patients with TL values of 3–7 µg/ml, the TL value > 3 µg/ml is not a good predictor of clinical remission. Despite the published studies on the positive predictive value and association of higher TL with favourable long-term outcomes (5–7), our study found that TL values > 7 µg/ml during the maintenance therapy was not associated with a higher percentage of remission at 5 years; in contrast, high TL values showed worse outcomes in comparison with the other three categories (see above). This result can be explained by the significant association of TL > 7 µg/ml and intensification of treatment during the maintenance therapy due to clinical relapse (42.4 %, p = 0.035, median of 3 years (IQR: 1–5) from start of intensification to TL sampling). Such associations have been shown in other studies as well (8). Since a large portion of patients in our cohort with TL values >

7 μg/ml were in fact patients on intensified treatment due to relapse, a meaningful association between high TL values and clinical remission was not found. This result jeopardizes the overall evaluation of TL value > 3 μg/ml in CD patients and represents a limitation of this study. In a prospective cohort study, Kennedy et al. showed that low drug concentrations are highly associated with anti-TNF failure and immunogenicity (9). TL values of 1–3 μg/ml and < 1 μg/ml were associated with higher accumulated percentage of relapses at 5-year follow-up. However, differences in outcomes between these two categories were not significant (accumulated rate of relapse within 5 years in TL 1–3 μg/ml 18.6 % vs 15.3 % in TL < 1 μg/ml). LOR was recorded in only one CD patient in our cohort during the study (with TL < 3 μg/ml, no detected ADAbs), rendering the statistical evaluation of TL value and association with LOR useless. However, it is noteworthy to mention the possible importance of reactive TDM in decision-making for the management of LOR. All three of the possible different mechanisms of LOR were detected in as few as 5 recorded cases in our study (2x mechanistic LOR, 2x immune-mediated LOR, and 1x non-immune pharmacokinetic LOR), emphasizing the complex aetiologies of treatment failure. The maintenance TL value was not a predictor of LOR in our cohort of IBD patients.

Effect of concomitant therapy with AZA on TL

Concomitant treatment with AZA was significantly associated with TL values > 3 μg/ml in our cohort of IBD patients (U-statistic: 305, critical values for the Mann-Whitney U-Test: 307, n1 = 30, n2 = 30, p = 0.05). Several studies have shown a positive effect of concomitant AZA therapy on TL value (11-13). It has been suggested that this association is attributed to an improved pharmacokinetic profile of IFX when combined with AZA and it seems to be dose dependent (12). Polakovicova et al reported that the proportion of patients with IFX TL > 3 μg/ml increases with increasing doses of AZA due to a metabolic shift towards 6-thioguanine nucleotide (6-TGN) levels and independent from AZA monotherapy's effect on disease activity and/or production of ADAbs (12). To date, the complete mechanism of action of AZA's effect on TL value is not yet clearly understood.

Effect of concomitant therapy with 5-aminosalicyclic acid and AZA on percentage of relapses

Concomitant single AZA treatment was significantly associated with lower percentage of relapses within 5 years in our cohort of IBD patients (P = 0.001 in CD patients, n = 48, p = 0.03 in UC patients, n = 26). This result is in accordance with published data confirming the superiority of concomitant IFX treatment with AZA in CD patients and UC patients (14–16). To date, two large randomized controlled trials, SONIC and SUCCESS-UC, are the most prominent studies suggesting the benefit of a combination therapy with IFX and AZA over IFX treatment alone (11, 17). Fraser et al. found that the combination therapy with AZA in both categories of IBD patients is associated with the achievement of sustained remission with a median of 5 years (16). In another recent study conducted with 11,000 IBD patients on anti-TNF treatment, Targownik et al. states that the use of concomi-

tant immunosuppressive treatment with AZA is associated with a statistically significant reduction in the "likelihood of treatment failure" (15). The use of immunosuppressive treatment based on such results is recommended in UC and CD patients as a combination therapy during the maintenance treatment by international guidelines such as ECCO; however, due to safety concerns, this should be considered carefully, particularly in patients aged over 65 (2, 18). Although the percentages of relapses were slightly lower in the group of patients on single concomitant treatment with 5-ASA in both cohorts of patients, there was no significant association between the use of this medication and percentages of relapses (p = 0.23 in cohort of CD patients, n = 48; p = 0.10 in cohort of UC patients, n = 26). This result is in accordance with the data published by Singh et al on a large cohort of UC patients, stating that a concomitant use of 5-ASA was not associated with better clinical outcomes (adjusted OR, 0.67 (95% CI, 0.45–1.01), p = 0.06 (19). Similarly, a recent study on a cohort of 5,697 CD patients published by Bernstein et al found no beneficial impact on the outcomes of patients by adding 5-ASA to the combination therapy with IFX and AZA (20).

Conclusions

TL values > 3 μg/ml during the maintenance therapy is a strong predictor of sustained clinical remission within 5 years in UC patients. The use of combination therapy with AZA, due to its significant association with TL values > 3 μg/ml and lower percentage of relapses, seems to have practical benefits in achieving better clinical outcomes in UC patients.

TL values during the maintenance therapy is not a strong predictor of sustained clinical remission within 5 years in CD patients. There is no significant difference in percentage of remissions in CD patients with TL values < 3 μg/ml in comparison with CD patients with TL values > 3 μg/ml within 5 years of maintenance therapy.

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