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Alimentary Tract

Infliximab trough levels and persistent *vs* transient antibodies measured early after induction predict long-term clinical remission in patients with inflammatory bowel disease

Giorgia Bodini[a,*], Edoardo G. Giannini[a], Vincenzo Savarino[a], Lorenzo Del Nero[a], Sara Lo Pumo[a], Matteo Brunacci[a], Nicola De Bortoli[b], Anjali Jain[c], Salvatore Tolone[d], Edoardo Savarino[e]

[a] *Gastroenterology Unit, Department of Internal Medicine, University of Genoa, Genoa, Italy*
[b] *Gastroenterology Unit, Department of Internal Medicine, University of Pisa, Pisa, Italy*
[c] *Department of Research and Development, Prometheus Laboratories Inc., San Diego, CA, USA*
[d] *Department of Surgery, Second University of Naples, Naples, Italy*
[e] *Gastroenterolgy Unit, Department of Surgery, Oncology and Gastroenterology, University of Padua, Padua, Italy*

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ABSTRACT

Background: The use of therapeutic drug monitoring has been proposed as a useful tool in the management of patients with loss of response to biological therapy in patients with inflammatory bowel disease.
Aims: To evaluate whether early, post-induction anti-tumor necrosis factor trough levels and the presence of different types of anti-drug antibodies may impact long-term clinical remission in patients with inflammatory bowel disease.
Methods: We prospectively assessed anti-tumor necrosis factor trough levels and both persistent and transient anti-drug antibodies. The Harvey–Bradshaw Index and the partial Mayo score were evaluated at each visit or in case of relapse.
Results: At week 14, median infliximab trough levels were significantly lower in patients who experienced loss of response at week 48 as compared to patients in stable remission (1.3 mcg/mL [range 0–10.2 mcg/mL] *vs*. 10.1 mcg/mL[range 0–42.8 mcg/mL], $P < 0.0004$). ROC curve identified an infliximab trough levels of 6.2 mcg/mL as the cut-off value with the highest accuracy (*c*-index = 0.864) for loss of response at week 48. At week 14 we observed a correlation between anti-drug antibodies concentration and infliximab trough levels ($r_s = -0.513$, $P = 0.04$).
Conclusions: The results highlight the usefulness of assessing early biological TL in order to predict patients' long-term outcome.

1. Introduction

Loss of response (LOR) in inflammatory bowel disease (IBD) patients treated with anti-tumor necrosis factor (TNF) therapy is a common problem. During the first year, up to 40% of patients on biological therapy may experience a LOR. The annual rate of LOR for infliximab (IFX) and adalimumab (ADA) has been estimated to be as high as 13% and 24%, respectively [1–3]. Recently, the use of therapeutic drug monitoring (TDM) by means of anti-TNF trough levels and the detection of anti-drug antibodies (AAA) has been proposed as a useful tool in the management of patients who have LOR [4,5]. Indeed, it has been shown that low drug trough levels (TL) and presence of AAA represent the most important cause of secondary LOR during anti-TNF therapy [6,7]. Other possible causes of LOR are episodic treatment, non-inflammatory symptoms, symptomatic stricture and smoking, the presence of low serum albumin levels, high inflammatory burden or a switch of the pro-inflammatory cytokine pattern of disease [8,12]. For example, it has been hypothesized that – in predisposed subjects – anti-TNF therapy may cause a cytokine imbalance resulting in an up-regulation of dendritic cells and an increase of IL-23 [9].

The presence of AAA is not always correlated with low TLs and worse outcome, and this finding can be explained by considering the existence of two different types of AAA: transient AAA (t-AAA)

* Corresponding author at: Gastroenterology Unit, Department of Internal Medicine, University of Genoa, Viale Benedetto XV, 6, 16132 Genoa, Italy.
E-mail address: giorgia.bodini@edu.unige.it (G. Bodini).

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and persistent AAA (p-AAA). P-AAA do not disappear over time, while the presence of t-AAA seems to be inconsistent and likely does not affect both drug trough levels and patients' clinical outcome. However, there is poor evidence of the role of P-AAA due to the difficulties of detecting and measuring them [10]. Indeed, the majority of studies evaluating the role of AAA have been performed with ELISA kits, which, in most cases, are not able to detect AAA in case of drug presence [11].

However, there is a lack of information regarding the possibility to predict early the long-term outcome of patients treated with anti-TNF drugs. Therefore, in this prospective study our aim was to evaluate whether post-induction anti-TNF trough levels and the presence of different types of AAA (p-AAA and t-AAA) may have an impact on long-term clinical remission in IBD patients treated with biological therapy.

2. Patients and methods

2.1. Patients

This prospective study was performed at our tertiary referral center between December 2012 to December 2014. The study included patients with IBD who were treated with anti-TNF drugs and fulfilled the following inclusion criteria: male and female patients between the ages of 18 and 75; diagnosis of Crohn's Disease (CD) or Ulcerative Colitis (UC) confirmed by endoscopic, radiologic and histologic evaluation, primary responders to anti TNF-drugs evaluated by means of a Harvey–Bradshaw Index (HBI) <5 or at least a decrease of 3 points in CD patients and a partial Mayo score <2 or at least a decrease of 2 points in UC patients [13,14]. Exclusion criteria were: colectomy, ileostomy, extensive small bowel resection (as determined by the investigator) or a short bowel syndrome, patients who were currently receiving total parenteral nutrition, and history of cancer, positive *Clostridium difficile* stool assay, *Listeria*, human immunodeficiency virus, central nervous system demyelinating disease, or untreated tuberculosis. Moreover, before starting anti-TNF drugs, all patients were evaluated to exclude latent tuberculosis (QuantiFERON-TB Gold®, chest radiography, and careful history taking), hepatitis C and B infections.

The study was performed according to the Declaration of Helsinki after approval by the local IRB. All patients were asked to give written informed consent before the start of the study.

2.2. Methods

All subjects who agreed to participate in our investigation underwent careful history taking, physical and clinical examination (including current medication, height and weight, tobacco use, alcohol and coffee consumption). The Montreal classification was used for assessing UC and CD location and behavior [15].

After the induction phase that consisted of ADA 160/80 mg at week 0 and week 2 or IFX 5 mg/kg at weeks 0–2–6, patients who achieved clinical remission as above-mentioned were included in a prospective follow-up program to evaluate the maintenance of clinical and biochemical remission. Blood samples were collected at weeks 14, 22 and 30. We evaluated drugs trough levels early after induction (*i.e.*, week 14) and the AAA presence and concentrations every 8 weeks from week 14 to week 30 (*i.e.*, weeks 14, 22 and 30) and evaluated both p-AAA and t-AAA. The HBI and partial Mayo score were evaluated at each scheduled visit or in case of relapse. A relapse was defined by a HBI >5 or a partial Mayo score >2 for 2 consecutive weeks. C-reactive protein (CRP) was considered normal if lower than 5 mg/L.

Anti-TNF trough levels and AAA presence were assessed using a homogeneous mobility shift assay developed by Prometheus Laboratories Inc., San Diego, California [16,17]. P-AAA were defined as the presence of AAA for at least 3 consecutive time points, while t-AAA were defined as the presence of inconsistent AAA positivity (*e.g.*, negative-positive-negative; positive-negative-negative).

Table 1
Baseline demographic and clinical features of the 45 patients included in the study.

Gender, male (n, %)	28 (62.2)
Age, years (median, range)	40 (21–70)
Type of disease, Crohn's Disease (n, %)	32 (71.1)
Age at diagnosis <40 years (n, %)	34 (75.6)
Body mass index, kg/m^2 (median, range)	23 (16–36)
Disease duration, years (median, range)	7 (1–31)
Montreal classification	
Ulcerative Colitis (n, %)	
E1-Ulcerative proctitis	0 (0)
E2-Left side UC	4 (30.8)
E3-Extensive UC	9 (69.2)
Crohn's Disease localization (n, %)	
L1-Terminal Ileum	19 (59.4)
L2-Colon	3 (9.4)
L3-Ileocolon	10 (31.2)
L4-Upper GI	0 (0)
Crohn's Disease behavior (n, %)	
B1, non-constricting/non-penetrating	14 (43.8)
B2, stricturing	11 (34.3)
B3, penetrating	7 (21.9)
Extra-intestinal manifestation (n, %)	10 (22.2)
Patients with previous use of azathioprine (n, %)	18 (40.0)

2.3. Statistical analysis

Continuous data are presented as median and range, while categorical data are shown as absolute value and percentage. Continuous data are assessed using the Mann–Whitney *U*-test, while categorical data are compared using the Fisher's exact test. The receiver operating characteristic curve (ROC curve) was applied in order to find the best sensitivity and specificity cut-off value of anti-TNF trough levels at week 14 for the prediction of LOR at week 48. Moreover, ROC curve was used to identify the cut-off prevalence-adjusted negative and positive predictive value for LOR. The validity of the model was measured by means of the concordance (c)-statistic (equivalent to the area under ROC curve) [11]. A model with a c above 0.7 is considered useful, while a c between 0.8 and 0.9 indicates excellent diagnostic accuracy. The correlation between AAA and drug TL was assessed by means of Spearman's rank correlation test (r_s). A *P*-value <0.05 in a two-tailed test was considered statistically significant.

3. Results

3.1. Baseline characteristics

The flow of patients within the study is shown in Fig. 1. Briefly, in our cohort of 102 IBD patients treated with anti-TNF drugs (75 CD patients and 27 UC patients, 59 patients treated with IFX and 43 treated with ADA), we included 45 patients who fulfilled the inclusion criteria for this study. Thirty-three patients (73.3%) were treated with IFX (21 CD and 12 UC patients) and 12 patients (26.7%) were treated with ADA (12 CD).

The baseline characteristics of the study population are shown in Table 1. Patients were mostly males (n = 28, 62.2%), their median age was 40 years (range, 21–70 years), and more than two-thirds of patients received their IBD diagnosis before the age of 40. Among CD patients the prevalent localization was ileal (n = 19, 59.4%) and ileocolonic (n = 10, 31.2%), while the most frequent disease behaviour was non-constricting/non-penetrating (n = 14. 43.8%). UC patients

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