Digestive and Liver Disease 52 (2020) 400–407

Contents lists available at ScienceDirect

Digestive and Liver Disease

journal homepage: www.elsevier.com/locate/dld

Alimentary Tract

Effects of anti–TNF-alpha therapy on hemoglobin levels and anemia in patients with inflammatory bowel disease

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ARTICLE INFO

Article history:
Received 11 September 2019
Accepted 22 November 2019
Available online 28 December 2019

Keywords:
Anemia
Crohn's disease
Inflammatory bowel disease
Ulcerative colitis

ABSTRACT

Background: Tumor necrosis factor-α (TNF-α) is involved in inducing inflammatory anemia. The potential effect of anti–TNF-α agents on anemia in inflammatory bowel diseases (IBD) is still unknown.
Methods: Analytical data and disease characteristics from 362 IBD patients [271 CD/91UC) treated with anti-TNF-α drugs were retrospectively collected. Effects on disease activity, blood markers and prevalence of anemia were assessed after 6 and 12 months of therapy.
Results: 29.3% patients presented anemia at baseline, and significantly reduced to 14.4% and 7.8% after 6 and 12 months of therapy, respectively. Mean ± SD Hb levels increased significantly at month 6, and this increase was sustained at 12 months. Serum markers of iron metabolism increased significantly compared to baseline, as disease activity measured by C-reactive protein (CRP) was reduced. All these effects were observed independently for CD and UC, and were independent of iron supplementation during treatment. Anemia at baseline (OR 4.09; 95%CI 1.98–8.45) and elevated CRP (OR 3.45; 95CI 1.29–9.22) were independently associated with risk of persistent anemia, as well as iron replacement during therapy (OR 4.36; 95%CI 2.07–9.16).
Conclusions: Controlling disease activity with anti-TNF- α therapy significantly and independently associated with resolution of anemia in IBD, with no relevant role for iron replacement therapy.

1. Introduction

Anemia is the most common systemic complication and extraintestinal manifestation in inflammatory bowel disease (IBD) [1,2] and significantly affects health-related quality of life [3–5]. Anemia determines long-term disease outcomes, including the amount of treatment needed, hospital admissions [6], and the need for surgery in IBD populations [7,8], all of which lead to substantial increases in health-care costs [9]. Anemia in IBD is pathogenically complex, with several factors contributing to it, the most common types of anemia being those caused by iron deficiency and anemia of chronic disease (ACD), which often overlap. ACD occurs in patients with acute or chronic immune activation [10] and is associated with the production of pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α [11,12]. The inflammatory activity of the disease has been identified as an independent risk factor for anemia in this population [13–15]. Treatment of anemia in IBD should be therefore directed at both correcting the underlying mucosal inflammation and providing adequate iron and vitamin supplementation [16].

Anti-TNF-α inhibitors, including infliximab (IFX), adalimumab (ADA) and golimumab (GOL), were the first antibody-based drug therapies available for IBD patients and they are generally used as a first-line biological options in patients with Crohn's disease

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https://doi.org/10.1016/j.dld.2019.11.019
1590-8658/

(CD) and ulcerative colitis (UC). These drugs are able to induce and maintain disease remission in the long term in a high proportion of patients [17,18] and they also appear to be effective for certain IBD-associated extra-intestinal manifestations [19], just as they are in several rheumatic and dermatologic diseases.

Regarding anemia, there is evidence that anti-TNF-α treatment improves hemoglobin (Hb) levels in patients with rheumatoid arthritis [20,21], psoriatic arthritis, and ankylosing spondylitis [22], but the potential effect of this drug on anemia or Hb levels in IBD is still unknown: while recent research in pediatric CD documented the benefit of anti-TNF-α therapy on Hb levels [23], conflicting results have been provided for adults [24,25].

In this study, we evaluated the effects of treatment with a TNF-α inhibitor on Hb levels and anemia in a large series of patients with CD and UC and naïve to biological therapy. Changes in iron metabolism and disease activity induced by therapy were also evaluated.

2. Methods

2.1. Study design and data source

Between January 2018 and December 2018 we undertook a cross-sectional, multicenter study within the Ciudad Real province IBD working group, which represents all IBD units in this region of Spain [26]. Patients of all ages on electronic databases of the participating hospitals with a diagnosis of CD or UC, established according to standard clinical, endoscopic, histological, and radiological criteria [27,28], were identified. Epidemiological and clinical data obtained included patient age, sex, type and location of the disease according to the Montreal classification system [29], smoking habits at diagnosis, and the presence of extra-intestinal manifestations at disease onset. Patients with indeterminate colitis were excluded.

Only naïve patients to biological therapy, with information available on hematological parameters recorded within the 3 months before starting treatment with an anti-TNF-α inhibitor, were included; these patients must have received anti-TNF-α therapy for at least 6 or 12 months, and had analytical assessments at months 6 and/or 12. Prospectively collected demographic, clinical, and laboratory data from visits to IBD clinics were used. Analytical parameters included Hb; hematocrit; mean corpuscular volume (MCV); mean corpuscular Hb(MCH); mean corpuscular Hb concentration (MCHC); serum ferritin, serum transferrin, serum iron levels; and transferrin saturation (TfS), wherever possible. Disease activity was measured in terms of C-reactive protein (CRP) serum concentration. Clinical and analytical information was obtained by electronic medical record–based computer searches and manual confirmation of information; details on anti-TNF-α therapy and intravenous iron administration was obtained from electronic registries of the pharmacies of each hospital; oral iron supplement prescriptions for all participants were sought from the regional system of electronic pharmaceutical prescriptions and all cross-checked with patients' electronic clinical charts.

Anemia was considered as a binary outcome variable (yes/no) and defined according to sex- and age-specific Hb and hematocrit cut-offs established by the WHO for Caucasian populations [30]. Thus, the minimum normal Hb and hematocrit levels for non-pregnant adult women were 12 g/dL and 36%, respectively, while those for adult men were 13 g/dL and 39%, respectively. The same criteria were followed for pediatric patients, regardless of sex: children below 5 years of age were considered anemic when Hb concentration was <11 g/dL; for those aged between 5 to 11 years old anemia was defined as Hb <11.5 g/dL; and for children between 12 to 14 years old a level of Hb <12 g/dL was considered anaemic [30]. The prevalence of anemia was calculated as the ratio of the number of anemic patients over the total number of patients included in the study. Patients with Hb <10 g/dL, irrespective of age and gender, were considered to have severe anemia, as classified in previous research [13,31–33].

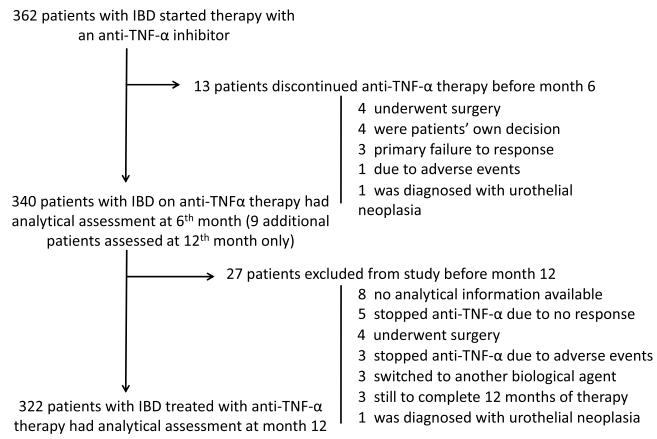

Fig. 1. Study population flow chart. Patients with IBD who started treatment with anti-TNF-α inhibitors and completed 6 and 12 months of treatment, together with the reasons for exclusion from the study.

2.2. Statistical analyses

Results for continuous variables are expressed as the mean and SD or as the median and interquartile range (IQR); qualitative variables are presented as absolute and relative frequencies. The Shapiro–Wilk test was used to verify the normal distribution of data. The $\chi 2$-test (Fisher's exact test, where appropriate) or Student's *t*-test were used to compare qualitative and quantitative variables, respectively. Paired nominal data were evaluated by *t*-test for paired samples, ANOVA or McNemar tests. Correlation with Pearson's r (r) was used for assessing the relationship between the change in CRP and Hb levels or iron metabolism indices. Logistic regression was performed to adjust for potential confounders. Odds ratios (OR) with 95%CIs were calculated for significant variables. Tests were 2-sided, and a significance level of 0.05 was used throughout. Analyses and summaries were carried out with PASW (v18.0; SPSS Inc., Chicago, Illinois).

2.3. Ethical considerations

This study was conducted in accordance with the Declaration of Helsinki principles. The registries supporting this study were approved by the local ethics or research committees at the participating centers.

3. Results

3.1. Subject characteristics

A total of 362 IBD patients (271 CD, 91 UC) who received anti-TNF-α treatment and had analytical information before starting therapy, were included. Among them, 340 patients continued anti-TNF-α treatment for at least 6 months with complete laboratory data available and 322 continued anti-TNF-α after 1 year and had an annual blood analysis available. Fig. 1 shows patient flow.

Anti-TNF-α inhibitors administered to these patients overall included IFX in 175 (48.3%), ADA in 182 (50.3%), and GOL in only 5 (1.4%) patients. ADA was used more frequently than IFX in patients with CD (56.1% vs. 43.9; p = 0.006); in contrast, patients with UC

Table 1
Demographic and clinical characteristics of IBD patients treated anti-TNF-α included in the study.

		IBD overall (n = 362)	Crohn's disease (n = 271)	Ulcerative colitis (n = 91)	p
Age at recruitment [Mean (SD) (range)]		35.1 (15.4; 8–83)	33.9 (15.5; 8–83)	38.6 (14.6; 11–77)	0.012
Sex [male (%)/female (%)]		221 (61)/141 (39)	166 (61.3)/105 (38)	55 (60.4%)/36 (39.6)	0.890
Age at diagnosis (A) [n (%)]	A1	24 (6.6)	16 (5.9)	8 (8.8)	0.093
	A2	210 (58)	166 (61.3)	44 (48.4)	
	A2	128 (35.4)	89 (32.8)	39 (42.9)	
Disease location (L) [n (%)]	L1	–	97 (35.8)	–	–
	L2	–	49 (18.1)	–	
	L3	–	119 (43.9)	–	
	L4	–	6 (2.2)	–	
Disease behavior (B) [n (%)]	B1	–	150 (55.8)	–	–
	B2	–	73 (27.1)	–	
	B3	–	46 (17.1)	–	
Disease extension (E) [n (%)]	E1	–	–	9 (9.9)	–
	E2	–	–	38 (41.8)	
	E3	–	–	44 (48.4)	
IBD complications [n (%)]		104 (31.6)	85 (34.6)	19 (22.9)	0.048
Type of complication [n (%)]	Abscesses	32 (9.9)	31 (12.7)	1 (1.3)	0.003
	Cutaneous	21 (6.5)	18 (7.5)	3 (3.7)	0.232
	Osteo-articular	75 (23.6)	55 (23.1)	30 (25)	0.730
History of Surgery [n (%)]		41 (12.7)	38 (15.7)	3 (3.8)	0.005
Smoking habit [n (%)]	Never	118 (52.4)	83 (47.7)	35 (68.6)	0.027
	Active	68 (30.2)	59 (33.9)	9 (17.6)	
	Former smoker	39 (17.3)	32 (18.4)	7 (13.7)	
Anti-TNFα drug [n (%)]	Infliximab	175 (48.3)	119 (43.9)	56 (61.5)	<0.001
	Adalimumab	182 (50.3)	152 (56.1)	30 (33)	
	Golimumab	5 (1.4)	–	5 (5.5)	
Dose intensification [n (%)]		94 (26.8)	69 (26.4)	25 (27.8)	0.804
Anemia at IBD diagnosis [n (%)]		152 (53.3)	116 (54)	36 (51.4)	0.713

were treated more frequently with IFX (61.5% vs. 33%; p = 0.002). The age of the patient did not influence the choice of drug.

Patient age at the moment of starting anti-TNF-α therapy was 35.1 years (SD 15.4; range 8–83). CD patients were younger [33.9 (15.5; 8–83)] than UC [38.6 (14.6; 11–77)] (p = 0.012) at the point of inclusion; 24 children (16 CD, 8 UC) were also included. Demographic and clinical data of the IBD study patients are presented in Table 1.

Iron replacement was administered to 120 patients (33.1%) including 67 women and 53 males. Overall, 67/141 women received iron supplements (47.5%) compared to 53/168 males (24%) (p < 0.001), with no differences between CD and UC (34.3% vs. 29.7%, respectively; p = 0.415). This included oral in 77 (67.5%), IV iron sucrose in 19 (16.7%) and IV iron carboxymaltose in 18 (15.8%). No significant differences in iron preferences were found according to type of IBD.

The prevalence of anemia was significantly higher among patients who required iron replacement therapy; these patients also had a significantly lower hemoglobin concentration at all evaluation points, compared to those who did not receive iron. At any one point, markers for iron metabolism were also significantly worse among patients treated with iron supplements (Table 2). No patients were treated with erythropoiesis-stimulating agents.

3.2. Hematological profile and prevalence of anemia in patients with IBD and changes along TNF-α treatment

Before starting anti-TNF-α therapy, the mean ± SD Hb concentration for the whole series was 13.2 ± 1.9 (range 6.4–17.3) g/dL, slightly higher in CD (13.4 ± 1,8 g/dL) than in UC (12.8 ± 2,1 g/dL) (p = 0.072). After 6 months of treatment, Hb levels increased up to 14 ± 1.7 (range 8.2–17.6) g/dL, with a higher increase also for CD (14.1 ± 1.7; range 9.8–17.6 g/dL) than for UC 13.7 ± 1.8; range 8.2−17 g/dL). All changes were statistically significant from the baseline (p < 0.001 for all comparisons).

After 12 months of therapy Hb levels continued to increase, but with no significant difference to the results at month 6 (being 14.1 ± 1.6 g/dL, 14.1 ± 1.6 and 13.9 ± 1.7 g/dL for the whole IBD series, CD and UC, respectively). Fig. 2 represents changes in Hb concentrations over the time.

At baseline, 106 IBD patients overall (29.3%) presented with anemia, with higher prevalence found for UC (35.2%) than for CD patients (27.3%) (p = 0.154). Proportionally, more women than men presented with anemia at the start of anti-TNF-α therapy (36.9% vs. 24.4%, respectively; p = 0.008). After 6 months, only 49 patients (14.4%) remained anemic (including 35 with CD [13.8%] and 14 with UC [16.3%]. The proportion of anemic patients progressively decreased at month 12, when only 25 patients (7.8%) remained anemic (including 16 patients with CD [6.6%] and 9 with UC [11.1%]. Reductions in the prevalence of anemia were statistically significant for all disease groups (p < 0.05). Table 3 compares the changes on hematologic laboratory data and prevalence of anemia in our cohort of IBD patients treated with anti-TNF-α agents, from baseline to months 6 and 12.

Among patients treated with iron supplements, both oral and parenteral routes led to significant improvements in hemoglobin concentration and the frequency of anemia from baseline to month 12. Patients treated with parenteral iron showed a non-significant trend towards a lower prevalence of anemia at the end of treatment (Table S1). Anemia also improved significantly in the subgroup of patients who were not treated with iron supplements, from 21.5% at baseline to 7% at 6 months (p < 0.001), and to 2.8% after 1 year of treatment (p < 0.01 from baseline) (Table 2).

Finally, the prevalence of severe anemia (Hb <10 g/dL) was 5%, at the point of starting anti-TNF-α therapy; at month 6 it reduced to 1.9%, and remained at 1.9% at month 12 of treatment.

3.3. Changes in disease activity and Iron metabolism

Serum CRP values (mean ± SD) were elevated at baseline with no differences between CD and UC (2.05 ± 3.7 vs. 1.9 ± 3.5 mg/dL, respectively). Treatment with anti-TNF-α agents resulted in a significant reduction in disease activity in terms of CRP serum levels for the whole IBD series, after 6 months (from 2.01 ± 3.9 to

Table 2
Demographic characteristics of a cohort of patients with IBD who received and did not iron replacement therapy during treatment with anti-TNF-α inhibitors. Changes on hemoglobin levels, hematologic parameters, iron metabolism markers and disease activity are shown at baseline, month 6 and month 12.

	IBD overall (n = 362)	Not supplemented with iron (n = 242)	Supplemented with iron (n = 120)	p
Age [Mean (SD)]	35.1 (15.4; 8–83)	35.1 (14.2)	35.1 (17.7)	0.982
Sex [male (%)/female (%)]	221 (61) / 141 (39)	168 (69.4) / 74 (30.6)	53 (44.2) / 67 (55.6)	<0.001
CD (n[%]) / UC (n[%])	271 (74.9) / 91 (25.1)	178 (73.6) / 64 (26.4)	93 (77.5) / 27 (22.5)	0.415
Anemia at IBD diagnosis [n (%)]	152 (53.3)	86 (45.5)	66 (68.8)	<0.001
Treatment				
Infliximab (n [%])	175 (49)	115 (48.5)	60 (50)	0.792
Adalimumab (n [%])	182 (51)	122 (51.5)	60 (50)	
Baseline				
Anemia at recruitment(n [%])	106 (29.3)	52 (21.5)	54 (45)	<0.001
Severe anemia (Hb<10 g/dL) (n [%])	18 (5)	4 (1.6)	14 (11.7)	0.012
Hemoglobin (g/dL) [mean (SD)]	13.24 (1.9)	13.77 (1.7)	12.28 (1.8)	<0.001
Serum iron (μg/dL) [mean (SD))]	64.44 (36.8)	70.74 (38)	50.81 (30.1)	<0.001
Serum ferritin (μg/L) [mean (SD)]	125.49 (169.1)	136.34 (170.1)	84.61 (117.8)	0.002
Serum transferrin (mg/dL) [mean (SD)]	242.84 (49.3)	242.31 (52.4)	252.72 (54.69)	0.223
Transferrin saturation (%) [mean (SD)]	23.37 (12.7)	26.11 (13.7)	16.92 (9.2)	<0.001
C-reactive protein (mg/dL) [mean (SD)]	2.01 (3.9)	1.72 (2.9)	2.39 (4.7)	0.164
Month 6				
Anemia (n [%])	49 (14.4)	16 (7)	33 (29.2)	<0.001
Severe anemia (Hb<10 g/dL) (n [%])	7 (1.9)	1 (0.4)	6 (5)	0.413
Hemoglobin (g/dL) [mean (SD]	13.97 (1.7)	14.44 (1.5)	13.08 (1.7)	<0.001
Serum iron (μg/dL) [mean (SD)]	78.4 (39.9)	87.7 (41)	59.27 (29.5)	<0.001
Serum ferritin (μg/L) [mean (SD)]	101.14 (162.4)	108.63 (155.1)	76.94 (133.8)	0.086
Serum transferrin (mg/dL) [mean (SD)]	263.02(44.3)	259.46 (38.8)	271.39 (53.4)	0.158
Transferrin saturation (%) [mean (SD)]	25.48 (13.5)	28.66 (14.6)	18.74 (9.1)	<0.001
C-reactive protein (mg/dL) [mean (SD)]	0.81 (2.2)	0.93 (2.7)	0.66 (0.9)	0.196
Month 12				
Anemia (n [%])	25 (7.8)	6 (2.8)	19 (17.6)	<0.001
Severe anemia (Hb<10 g/dL) (n [%])	7 (1.9)	–	7	0.137
Hemoglobin (g/dL) [mean (SD)]	14.07 (1.6)	14.48 (1.4)	13.22 (1.8)	<0.001
Serum iron (μg/dL) [mean (SD)]	79.6 (41.2)	83 (36.3)	73.3 (50)	0.061
Serum ferritin (μg/L) [mean (SD)]	104.35 (165.4)	108.1 (147.4)	81.7 (161.3)	0.171
Serum transferrin (mg/dL) [mean (SD)]	271.04 (44.6)	265 (39.4)	275.2 (51.9)	0.209
Transferrin saturation (%) [mean (SD)]	23.74 (11.2)	26.58 (11.7)	22.64 (11.3)	0.079
C-reactive protein (mg/dL) [mean (SD)]	0.44 (0.7)	0.39 (0.7)	0.57 (0.84)	0.077

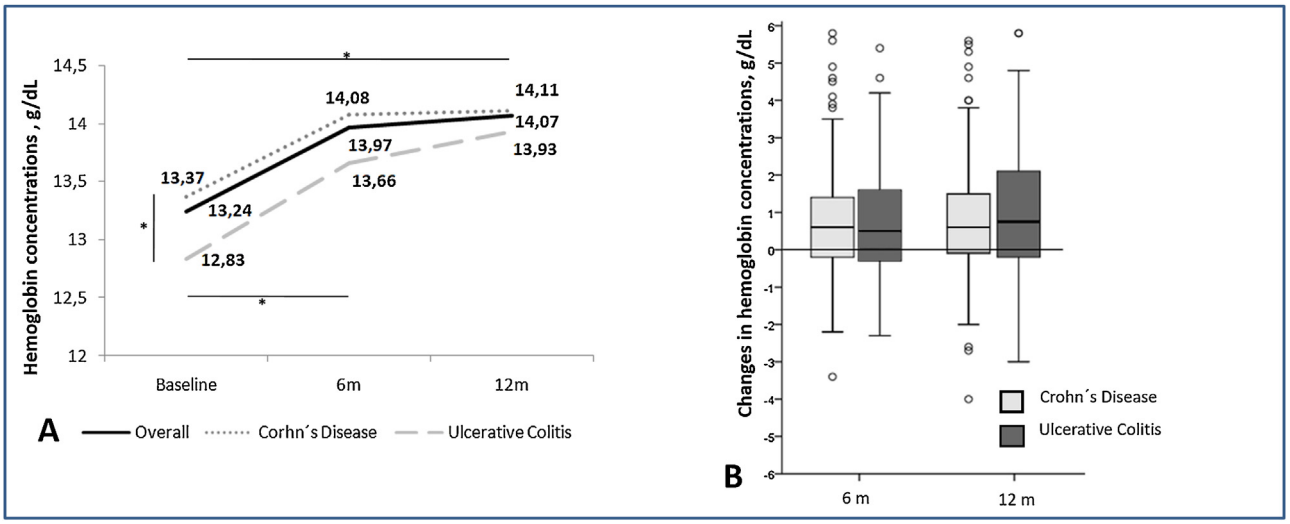

Fig. 2. Mean changes from baseline to months 6 and 12 in hemoglobin levels (g/dL) in our series of IBD patients treated with anti-TNF-α inhibitors (A) and changes over the baseline induced by therapy in Crohn's disease and ulcerative colitis (B). *p < 0.05; ** p < 0.007.

Table 3
Changes in hemoglobin levels, hematologic parameters, iron metabolism markers and disease activity indices in a cohort of IBD patients treated with anti-TNF-α agents at baseline, month 6 and month 12.

	IBD overall (n = 362)			Crohn's disease (n = 271)			Ulcerative colitis (n = 91)		
	Baseline	Month 6	Month 12	Baseline	Month 6	Month 12	Baseline	Month 6	Month 12
Hemoglobin (g/dL) [mean (SD)]	13.24 (1.9)[a]	13.97 (1.7)	14.07 (1.6)	13.37 (1.8)[a]	14.08 (1.7)	14.11 (1.6)	12.83 (2.1)[a]	13.66 (1.8)	13.93 (1.7)
Hematocrit (%)[mean (SD)]	39.65 (5.5)[a]	41.61 (4.8)	41.72 (4.8)	40.01 (5.3)[a]	41.91 (4.7)	41.84 (4.8)	38.57 (6.1)[a]	40.72 (5.2)	41.35 (4.7)
Anemia (n [%])	106 (29.3)[a]	49 (14.4)[c]	25 (7.8)	74 (27.3)[a]	35 (13.8)[c]	16 (6.6)	32 (35.2)[a]	14 (16.3)[c]	9 (11.1)
Serum iron (μg/dL) [mean (SD))]	64.44 (36.8)[a]	78.4 (39.9)	79.6 (41.2)	65.41 (38.1)[a]	76.35 (40)	77.73 (38.5)	61.19 (35.3)[a]	83.54 (38.9)	79.78 (30.6)
Serum transferrin (mg/dL) [mean (SD)]	242.84 (49.3)[a]	263.02 (44.3)	271.04 (44.6)	236.21 (51)[a]	260.63 (45.7)	267.86 (45.6)	264.32 (36.8)	270.76 (39)	281.32 (40)
Transferrin saturation (%) [mean (SD)]	23.37 (12.7)	25.48 (13.5)	23.74 (11.2)	23.78 (12.9)	25.57 (14.4)	23.86 (11.5)	22.02 (12.2)	25.17 (10.6)	23.3 (10.7)
Serum ferritin (μg/L) [mean (SD)]	125.49 (169.1)	101.14 (162.4)	104.35 (165.4)	128.6 (165.9)[b]	96.22 (114.4)	106.57 (155.1)	115.19 (180.6)	117.17 (265.4)	97.1 (196.9)
C-reactive protein (mg/dL) [mean (SD)]	2.01[a] (3.9)	0.81 (2.2)[c]	0.44 (0.7)	2.05 (4)[a]	0.9 (2.3)[c]	0.5 (0.7)	1.90 (3.5)[a]	0.57 (2)	0.32 (0.6)

[a] Denotes statistically significant differences regarding to both 6 and 12 months.
[b] Denotes statistically significant differences regarding to month 6.
[c] Denotes significant differences compared to month 12.

0.81 ± 2.2 mg/dL, respectively, p < 0.001) and also after 12 month of therapy (0.44 ± 0.7 mg/dL, p < 0.001 compared to baseline and p = 0.022 compared to month 6). Reductions were significant for both CD and UC. Accordingly, the number of IBD patients with elevated CRP serum concentration (defined as CRP > 2 mg/dL [34]) reduced significantly over the study period, from 91 (27%) at baseline to 30 (9.6%) at month 6, and to only 14 (4.8%) at month 12.

Iron serum levels increased from baseline (mean ± SD: 64.4 ± 36.8; 7–248 μg/dL) to months 6 (78.4 ± 39.9; 1–272 μg/dL; p < 0.001) and 12 (79.6 ± 41.2; 0.2–389 μg/dL; p < 0.001 compared to baseline). A parallel increase was also noted for serum transferrin (mean ± SD being 242.84 ± 49.3 mg/dL before therapy; 263.02 ± 44.3 mg/dL at month 6; and 271.04 ± 44.6 mg/dL at month 12; p < 0.001 with regard to baseline for all comparisons). No differences were found for CD and UC in both markers. Ferritin serum levels decreased non-significantly from baseline (125.49 ± 169.1 μg/L) to month 6 (101.14 ± 162.4 μg/L), but no further at month 12 (104.35 ± 165.4 μg/L). Serum CRP concentration correlated with Hb levels (Pearson's r=-0.29), ferritin (r = 0.25), iron (r=-0.32) and transferrin (r = −0.36) serum levels (p < 0.001 for all correlations) (Table 3).

When only the 242 patients who did not received iron supplements during anti-TNF-α therapy were considered, results were quite similar: Baseline serum iron levels increased from 70.74 ± 38 μg/dL to 87.7 ± 41 μg/dL at month 6 (p < 0.001), and were maintained at month 12 (83.0 ± 35.3 μg/dL; p = p < 0.001 with regard to baseline), thus confirming this effect as solely dependent on the control of inflammation mediated by TNF-α. In addition, serum transferrin increased from 242.3 ± 52.4 mg/dL to 259.46 ± 38.8 at month 6 (p = 0.001) and to 265 ± 39.4 mg/dL (p < 0.001 compared to baseline and p = 0.017 compared to month 6) in patients who did not receive iron supplements. It was also observed in the overall IBD series that ferritin serum levels decreased significantly from baseline (136.3 ± 170 μg/L) to month 6 (108.6 ± 155 μg/L; p = 0.027), but with no further decrease at month 12 (108.1 ± 147 μg/L).

3.4. Effects of the different anti-TNF-α agents on hemoglobin levels and anemia

Patients treated with IFX showed a greater frequency of anemia at baseline than those who received ADA (34.3% vs. 24.7%; p = 0.047); this resulted in a higher rate of patients treated with IFX remaining anemic at month 6 compared to those treated with ADA (18.6% vs. 10.3%; p = 0.031), but not at month 12 (8.8% vs. 6.9%; p = 0.521).

In contrast, changes in Hb concentration induced by each of the drugs were not different after 6 months (mean increase for IFX and ADA respectively being 0.71 and 0.72 g/dL; p = 0.944) and 12 months (0.91 vs. 0.78, p = 0.310) (Fig. 3).

No differences in serum ferritin, serum transferrin or iron levels were observed between patients treated with either drug (Table S2). Due to the limited number of patients included in the study with UC and treated with GOL, no analysis of them was undertaken; besides, no significant changes in Hb levels from baseline were documented over the treatment period (median ± IQR being 13.9 ± 4.6; 13.4 ± 3.1 and 13.8 ± 1 g/dL for assessments at baseline, month 6 and month 12, respectively.)

3.5. Determinants of resolution of Anemia with Anti-TNF-α therapy

Bivariate analyses demonstrated a significantly higher proportion of anemia in IBD women compared to men, irrespective of disease type or age. A higher disease activity, defined as serum CRP concentrations ≥2 mg/dL [34], was significantly more common among anemic patients in IBD overall, CD and UC (p < 0.05 for all comparisons). Anemia was also significantly more common among patients who received iron replacement therapy. No differences in the prevalence of anemia were observed when small intestine location (L1 + L3) was compared to exclusive colonic location (L2) in CD (13.8% vs. 13,3%, respectively; p = 0.935, nor when CD patients with a penetrating (B3) compared to a non-penetrating (B1 + B2) disease behavior were compared (15.3% vs. 9.3% respectively; p = 0.39. For UC, the prevalence of anemia did not significantly change with disease extension, being 11.1% for left-sided colitis; 18.9% in extensive disease and 15% for proctitis (p = 0.813).

A logistic regression was performed to determine the contribution of various factors in the persistence of anemia after 6 months of anti-TNF-α therapy. Anemia at baseline (OR 4.09; 95%CI 1.98–8.45; p < 0.001) and elevated serum CRP (OR 3.45; 95CI 1.29–9.22; p = 0.013); were identified as independently associated with the risk of persistent anemia. In addition, iron replacement during therapy was identified associated to persistent anemia 6 months after starting anti-TNF-α therapy (OR 4.36; 95%CI 2.07–9.16; p < 0.001). Type of disease (CD or UC), smoking status, age and female gender

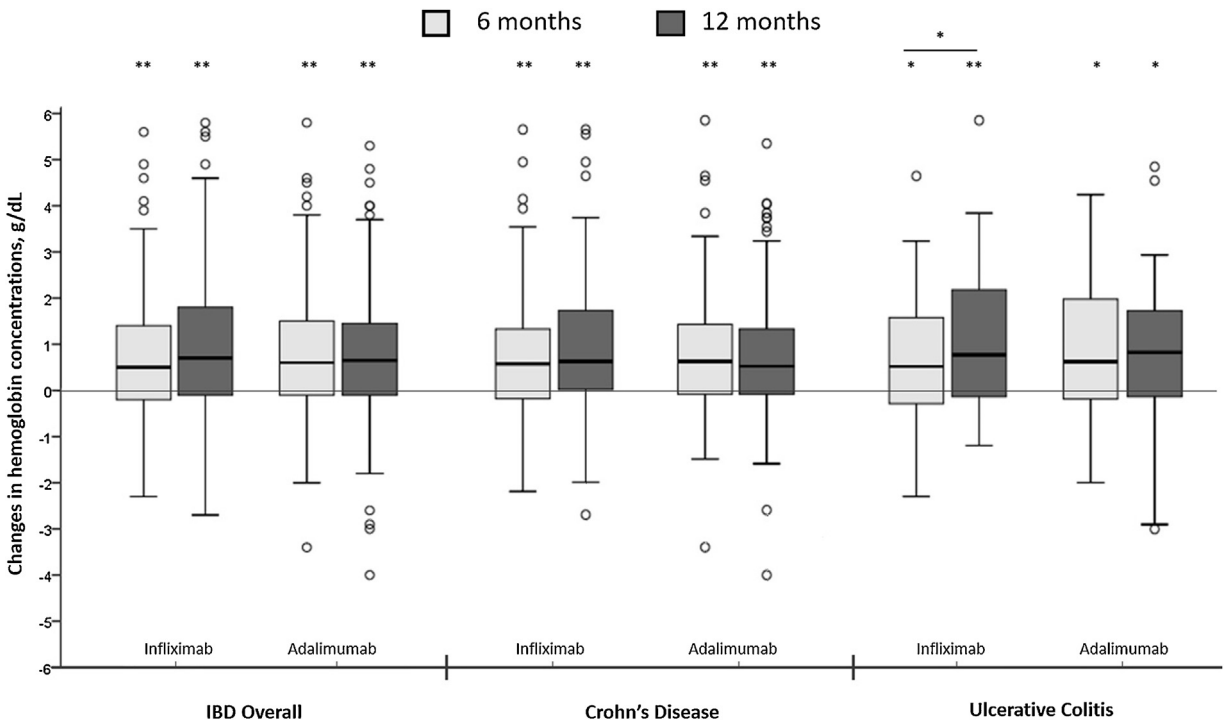

Fig. 3. Changes from baseline to months 6 and 12 in mean ± SD hemoglobin level (g/dL) in patients with IBD, treated with Infliximab and Adalimumab. *p < 0.05; **p < 0.001.

were not found to be significantly associated with the persistence of anemia.

4. Discussion

This study provides evidence for the first time that TNF-α inhibition is an effective treatment for anemia in patients with IBD, as it significantly increases Hb concentration and serum markers of iron metabolism, as the inflammatory activity of the disease is reduced. This effect was observed independently for patients with CD and with UC, and was not determined by iron supplementation during treatment.

Despite the abundant data on the therapeutic effects of TNF-α inhibitors in IBD [35], to date no study had unequivocally shown the impact of this therapy in reversing anemia in adult patients with CD and UC. Our study demonstrated a hematopoietic response characterized by a significant increase in Hb concentration already at 6 months after the onset of treatment, and a progressive reduction in the ratio of anemic patients from baseline (29.3%) to month 6 (14.4%) and month 12 (7.8%). The ability of anti-TNF-α therapy to improve Hb, iron and transferrin serum levels occurred in parallel with a reduction in the inflammatory activity of the disease, measured by CRP.

The first evidence of anti-TNF-α therapy as effective for IBD patients with refractory anaemia was provided by Domènech et al. in 2005 [36]: A 24-yr-old patient with CD and persistent iron-deficiency anemia, who required repeated blood transfusions after failure of IV iron supplements and EPO treatment, completely recovered Hb and blood markers of iron deposits after five infusions of IFX. Later, Bergamaschi et al. reported in 2010 (25) rapid hematologic responses in 12 out of 18 anemic patients with CD treated with the same drug. Although a relatively large number of studies had documented the effectiveness of anti-TNF-α agents in improving Hb levels and reversing anemia in patients with several rheumatic inflammatory diseases [37–40], the same effect could not be subsequently demonstrated in patients with IBD [24], where anemia affects up to a third of patients [2].

Our research established the presence of anemia at baseline, just before starting anti-TNF-α therapy, together with the inflammatory activity of the disease, as the major determinants for the persistence of anemia in IBD patients under anti-TNF-α therapy. Sex, type of disease, its extension, location and behavior, and smoking status had no influence on the response to therapy, which is contrary to their reported role in relation to anemia at the point of IBD diagnosis [13,41]. Iron supplementation also had no significant effects in improving anemia; subgroup analyses showed that patients who did not receive iron orally or intravenously improved Hb concentration and iron metabolism markers equally compared to the whole series of IBD patients. In addition, iron supplementation was found to be an independent determinant for the persistence of anemia at month 6 of anti-TNF-α therapy in multivariate analysis, therefore iron replacement was prescribed to patients with more severe anemia at baseline. In this sense, although intestinal iron loss is proposed as the most frequent etiology of anemia in IBD, together with reduced absorption [42], chronic inflammation plays a major role in most patients. Several proinflammatory cytokines involved in IBD such as IFN-γ, IL-1, IL-6, and TNF-α have systemic effects on bone marrow stem cells and are responsible for an inadequate iron delivery from plasma to the bone marrow, inhibition of erythropoiesis, and erythropoietin production [43]. Hepcidin has been recognized as an essential regulator of iron metabolism. This acute phase protein released by the liver inhibits the function of ferroportin-1, expressed by macrophages and enterocytes; thus, high levels of hepcidin favor iron storage in the reticuloendothelial system and reduce iron absorption from the gut, promoting the development of anomia [44]. Hepcidin expression is mainly induced by IL-6 and its synthesis is down-regulated by erythroferrone (ERFE) [45]. Prior studies demonstrated that serum levels of hepcidin were elevated in adult and pediatric patients with IBD and positively correlated with disease activity and serum concentrations of CRP and IL-6 [23,37–39]. Anti-TNF-α therapy has been demonstrated recently to improve iron metabolism in IBD by reducing IL-6 levels and hepcidin, with no changes in the ERFE pathway [40]. Our findings are in line with the results of these studies and provide evidence of the

close association between the control of inflammatory activity and the improvement in iron availability for hematopoiesis.

Our study has several strengths, one of which is the use of a large, all age and homogeneous series of patients with CD and UC undergoing their first-line of biological therapy. Patient recruitment was undertaken through the gastroenterology/IBD units of participant public regional hospitals that have share clinical management protocols, common clinical and laboratory records and electronic prescription monitoring systems. This guaranteed the traceability of treatments throughout the first year of anti-TNF-α therapy. The recovery of comprehensive data regarding the iron status of IBD patients at baseline and during therapy allowed us to investigate the changes in the type of anemia during the study period, thus overcoming the limitations of previous studies [24]. Still, the external validity of our results should be studied further as we cannot assume our region is representative of the entire IBD population.

Some important limitations should be also acknowledged for our study. Despite recovering archived well identified analytical data, the retrospective nature of the collection, reduced subjectivity. However, by assessing widely used analytical values, the risk of bias in the results obtained was reduced. Disease activity was measured exclusively by CRP, since clinical activity indexes were not available or could not be accurately calculated for every patient. The potential effects of concomitant therapies used on our patients were not assessed. Due to the small number of patients who received iron supplements intravenously in our series (37 patients), no significant advantages over the oral route could be demonstrated, although a trend towards better outcomes was documented with the first, in agreement with current literature [46]. Finally, our study provides scarce data on pediatric onset IBD, and the results therefore may not be completely representative of this patient group. Finally, the paucity of patients treated with GOL prevented the assessment of the potentially different effect this drug might have on anemia.

In conclusion, this research provides evidence that anti-TNF-α therapy improves Hb levels and reduces the rates of anemia in patients with IBD in the short term. This effect coincided with reduction in disease activity measured by CRP and was independent from iron supplementation during therapy.

Disclaimers

None.

Conflict of interest

None declared.

Grant support

None declared.

Acknowledgements

EJ Laserna-Mendieta is recipient of a Rio Hortega grant (CM17/00003) from Instituto de Salud Carlos III (ISCIII), Spanish Ministry of Health, Social Services and Equality, which is partly funded by the European Social Fund (period 2014-2020).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.dld.2019.11.019.

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