Prevalence and pathogenesis of anemia in inflammatory bowel disease. Influence of anti-tumor necrosis factor-α treatment

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The online version of this article has a Supplementary Appendix.

ABSTRACT

Background

Anemia is a common complication of inflammatory bowel disease, but its epidemiology may be changing due to earlier diagnosis and improved treatments. We investigated the prevalence and pathogenesis of anemia in patients with inflammatory bowel disease.

Design and Methods

In a cross-sectional study 263 out-patients with inflammatory bowel disease (165 with Crohn's disease, 98 with ulcerative colitis) were investigated. The influence of time from diagnosis, disease activity, inflammation and the status of iron and hematinic vitamins on the level of hemoglobin and prevalence of anemia were evaluated. In a second group of 27 patients with Crohn's disease, undergoing anti-tumor necrosis factor-α treatment with infliximab because of refractory or fistulizing disease, we determined the effects of infliximab on disease activity, hemoglobin, serum erythropoietin levels, iron status and inflammation.

Results

In all, 104 of the 263 patients with inflammatory bowel disease were anemic. Age, gender and azathioprine treatment had no influence on anemia. The prevalence of anemia was highest at diagnosis (65%), decreased during the first 4 years after disease onset, and was stable thereafter. Active disease was associated with higher rates of anemia. At diagnosis most anemic patients had anemia of chronic disease; during follow-up iron deficiency and multifactorial forms of anemia became more prevalent. Eighteen of 27 patients undergoing treatment with infliximab were anemic; most of them had anemia of chronic disease. Infliximab reduced disease activity and improved anemia in 12 patients. This was mediated by an increased production of erythropoietin for the degree of anemia. *In vitro* infliximab increased the growth of erythroid progenitors from the peripheral blood of patients with active disease.

Conclusions

Anemia is a common problem in out-patients with inflammatory bowel disease; the prevalence and severity of anemia are related to the activity of the bowel disorder. The pathogenesis of anemia changes during the course of the disease, with anemia of chronic disease having a major role at diagnosis and iron deficiency and multifactorial forms of anemia during follow-up. In patients requiring anti-tumor necrosis factor-α treatment, response to therapy improves erythropoiesis.

Key words: anemia, inflammatory bowel, iron deficiency.

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Introduction

Crohn's disease and ulcerative colitis are idiopathic inflammatory bowel diseases. Symptoms of inflammatory bowel disease include abdominal pain, diarrhea and fever, but extraintestinal manifestations and involvement of several organ systems are frequently observed. Anemia is a common hematologic complication of inflammatory bowel disease, occurring in 6% to 74% of patients.[1] The wide range in the reported prevalence of anemia is related to differences in study design (e.g. investigation of patients at presentation or during the course of the disease, in-patients or out-patients), in the criteria used to define anemia, and to the increasing awareness of the disease and improved treatments, leading to earlier diagnosis and lower rates of anemia observed in more recent studies. Although the actual prevalence of anemia in inflammatory bowel disease may be lower than previously suggested, for many patients anemia remains a significant problem that negatively affects their quality of life and contributes to the social burden of the disease.[2]

Iron deficiency, due to chronic blood loss and/or reduced iron absorption, and anemia of inflammation (or anemia of chronic disease, ACD) are the most common types of anemia in inflammatory bowel disease, although cobalamin or folate deficiencies, as well as treatment-induced myelosuppression, sometimes have a role.[3] In many cases the pathogenesis of anemia is multifactorial, since blood loss, inflammation, malabsorption of several nutrients and use of potentially myelotoxic drugs may occur in the same patient.[4] The importance of anemia in inflammatory bowel disease is confirmed by the inclusion of hematocrit and manifestations such as blood in stools or rectal bleeding in scoring systems commonly used to evaluate disease activity in both Crohn's disease[5] and ulcerative colitis.[6,7]

Tumor necrosis factor (TNF)-α is a pro-inflammatory cytokine that plays a pathogenic role in immune-mediated diseases including Crohn's disease, ulcerative colitis, rheumatoid arthritis, psoriasis, and ankylosing spondylitis.[8-11] This led to the development of anti-TNF-α agents for treatment of the above-mentioned conditions. Raised TNF-α levels have been found in the serum of patients with Crohn's disease, and recent evidence has implicated TNF-α in the pathogenesis of ACD.[12] TNF-α may cause anemia through the inhibition of erythroid progenitors (BFU-E) and through effects on iron metabolism, the latter characterized by iron retention within macrophages and inhibition of intestinal iron absorption.[13-15]

In the present work we evaluated the prevalence and pathogenesis of anemia in a series of patients with inflammatory bowel disease, mainly focusing on mechanisms related to inflammation and iron deficiency. We also investigated the changes in factors related to iron metabolism and erythropoiesis in a group of patients with Crohn's disease undergoing anti-TNF-α treatment.

Design and Methods

Patients

The prevalence and pathogenesis of anemia were investigated in 263 out-patients attending the inflammatory bowel disease out-patient clinics of our Institutions during 2006 and 2007. The study was approved by the institutional ethics committees on human studies according to criteria established by the modified Helsinki Declaration (1983), and patients provided informed consent. In addition, we investigated 27 patients with a diagnosis of Crohn's disease who were candidates for treatment with the anti-TNF-α antibody infliximab (Remicade, Schering Plough, Milan, Italy) either because they were refractory to treatment with steroids (n=10) or because they were affected by fistulizing disease (n=17). None of the patients in this group had been treated with cyclosporine or methotrexate, or received blood transfusions, iron supplementation or erythropoiesis-stimulating agents. No patients had asymptomatic or symptomatic stenoses, strictures, or abscesses at the time of the study.

The diagnoses of Crohn's disease and ulcerative colitis were based on the usual clinical criteria.[16,17] Disease activity was assessed by the Crohn's Disease Activity Index (CDAI),[5] and by the Clinical Activity Index (CAI).[6] A CDAI score of more than 150 in Crohn's disease and a CAI score of more than 4 in ulcerative colitis corresponded to active disease. The presence of renal failure, malignancy, connective tissue diseases or hematologic conditions that could cause anemia, other than ACD or nutritional, were criteria for exclusion from the study.

Treatment with infliximab and assessment of patients

The 27 Crohn's disease patients undergoing treatment with infliximab received three consecutive infusions of the antibody administered at weeks 0, 2, and 6 at a dose of 5 mg/kg. Concomitant treatment with 5-aminosalicylic acid or sulfasalazine was continued. In the ten steroid-refractory patients steroids were tapered and then discontinued. The CDAI score was determined within 1 week before the first infusion and 14 weeks after beginning therapy. Clinical response was defined as a decrease in CDAI by 70 points or more. Complete remission was defined as a CDAI score of 150 points or less.

Definition of anemia

Anemia was defined by hemoglobin levels lower than 13.0 g/dL for men and 12.0 g/dL for women.[18] According to the WHO criteria mild anemia corresponded to a hemoglobin level of 9.5 g/dL or more, moderate anemia to a hemoglobin of 8 g/dL or more, but less than 9.5 g/dL, and severe anemia to a hemoglobin of less than 8.0 g/dL. The diagnosis of ACD required a low transferrin saturation (< 16%) with normal or increased serum ferritin concentration (> 100 μg/L). Iron-deficiency anemia was characterized by the presence of anemia associated with low serum ferritin (<10 μg/L for females, <15 μg/L for males) or with a transferrin saturation less than 16% together with serum ferritin levels less than 30 μg/mL. The association of iron-deficiency anemia and ACD was characterized by a transferrin saturation of less than 16% and a serum ferritin between 30 and 100 μg/mL inclusive.[19,20] These diagnostic criteria were previously suggested and validated by several authors.[19-21] In this work we did not use the soluble transferrin receptor to log serum ferritin ratio as an indicator of iron-deficiency anemia in the presence of inflammation. However, given the different behavior of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) in these groups of patients, and the intermediate behavior of patients with the association of iron-deficiency anemia and ACD, we believe that the number of possible misdiagnoses was low and did not influence the general results of our study.

In patients treated with infliximab, a major hematologic

response was defined as the normalization of the hemoglobin level or a hemoglobin increase of 1.0 g/dL or more; a minor hematologic response was represented by a hemoglobin increase of 0.5 to 0.9 g/dL.

Laboratory investigations

All patients underwent clinical evaluation and determination of complete blood cell counts, body iron status, serum levels of folate and vitamin B12, CRP and ESR. In a subgroup of 39 unselected patients (22 with Crohn's disease and 17 with ulcerative colitis) and in 29 healthy controls serum prohepcidin concentration was determined. In patients treated with infliximab serum erythropoietin concentration was determined immediately before the first infusion and at week 14 of therapy. The following commercial kits were used for these determinations: the EPO ELISA—Medac (Hamburg, Germany) and the pro-Hepcidin ELISA Kit from DRG (Heidelberg, Germany).

In vitro assays were performed to evaluate the effect of infliximab on the growth of peripheral blood BFU-E from ten patients with untreated active Crohn's disease and ten age-matched healthy volunteers. For these assays, 5×10^5 peripheral blood mononuclear cells were seeded in 30 mm plastic dishes in 1 mL methylcellulose (StemCell Inc., Vancouver, Canada) containing 30% fetal bovine serum (HyClone, Logan, UT, USA), 10 ng/mL interleukin-3, 10 ng/mL granulocyte-monocyte colony-stimulating factor, 50 ng/mL stem cell factor, and 1 U/mL human erythropoietin (all from PeproTech EC Ltd., London, UK) and cultured with infliximab, 100 μg/mL, its isotype-matched control (human IgG1, Sigma-Aldrich, Poole, UK) or 10 ng/mL recombinant human TNF-α (R&D Systems, Abingdon, UK). After 14 days of incubation at 37°C in 5% CO_2, the number of BFU-E was scored according to standard criteria.

Statistical analysis

Continuous variables were compared by the Student's t-test, the F test (one-way analysis of variance), the Mann-Whitney U test, the Wilcoxon test and the Kruskal-Wallis test. For categorical variables the χ^2 test and Fisher's exact test were used. Correlations between continuous variables were expressed by Pearson's correlation coefficient or Spearman's R test. Data are reported as means ± 1 SD. *P* values less than 0.05 are considered statistically significant.

Results

Prevalence and pathogenesis of anemia in inflammatory bowel disease

The study population investigated for determination of the prevalence and etiology of anemia in out-patients with inflammatory bowel disease included 165 subjects with Crohn's disease and 98 with ulcerative colitis. The general features of these patients are reported in Table 1. Seventy-one patients with Crohn's disease (43%) were anemic compared to 33 patients with ulcerative colitis (34%), but the difference was not statistically significant. Anemia was mild in 85 patients, moderate in 15, severe in 4, with no differences in severity between patients with Crohn's disease and those with ulcerative colitis. Age, gender and concurrent therapy with azathioprine had no influence on the prevalence of anemia, although females had mean hemoglobin levels lower than males (11.9±1.8 g/dL *versus* 13.4±1.9 g/dL, $P<0.001$). Anemia was more common at diagnosis than during follow-up (65% and 35%, respectively, $P=0.001$). During the first 4 years after diagnosis rates of anemia gradually decreased

Table 1. Summary of demographic and clinical characteristics of patients with inflammatory bowel disease in the anemia evaluation cohort.

Condition	Crohn's disease		Ulcerative colitis		
	Non-anemic	Anemic	Non-anemic	Anemic	Reference values
N. of patients	94	71	65	33	
*Patients at diagnosis	6	16	7	8	
Age, years	38.9±15.1	39.9±14.4	40.9±15.1	40.2±12.7	
Time from diagnosis, years	8.0±6.6	9.0±8.8	7.5±6.6	7.4±6.7	
Males/females	53/41	36/35	44/21	17/16	
*Hemoglobin, g/dL	13.8±1.2	10.8±1.4	14.2±1.2	10.8±1.3	13.0-17.5 (M) 12.0-16.5 (F)
*Mean corpuscular volume, fL	88±6	82±12	89±5	80±11	82-98
*CDAI score	137±9*	206±94			≤ 150
*CAI score			4.7±3.3	8.0±3.0	≤ 4
*C-reactive protein, mg/dL	1.53±2.56	3.29±4.27	1.02±1.44	3.72±3.72	< 0.8
*Erythrocyte sedimentation rate (mm/hour)	19±17	40±26	16±16	42±24	1-15
*Transferrin saturation, %	30±16	16±10	29±11	12±5	15-45
Serum ferritin, μg/L	169±664	171±499	76±68	85±106	15-250 (M) 10-150 (F)
°Prohepcidin, ng/mL	113±7	94±8	91±45	70±35	54±29
Azathioprine: n. of patients on treatment	13	15	3	3	

*Values correspond to number (N.) of patients or to means ± 1 SD. * denotes statistically significant differences between the anemic and non-anemic groups; ° denotes a significant difference between patients (22 with Crohn's disease, 17 with ulcerative colitis) and a reference group of 29 healthy controls. CDAI: Crohn's disease Activity Index; CAI: Clinical Activity Index.*

(*P*=0.007) and hemoglobin concentration was directly related to the time since diagnosis (r=0.301, *P*<0.001; Figure 1), remaining stable thereafter (*data not shown*). In both Crohn's disease and ulcerative colitis, anemia was associated with low mean corpuscular volume, reduced transferrin satruation and increased ESR, CRP and CDAI or CAI scores (Table 1). Disease activity scores showed an inverse correlation with hemoglobin level (Figure 2). When the CDAI score was calculated removing the effect of hematocrit (modified CDAI), the correlation with hemoglobin was still present, but at a lower degree of significance. The modified CDAI score did, however, remain significantly higher in active (195±78) than in quiescent disease (49±33, *P*<0.001) and correlated with ESR and CRP (*data not shown*). In Crohn's disease anemia was not related to either disease location or behavior (*data not shown*); in contrast, in ulcerative colitis the proportion of patients with pancolitis was higher in the anemic subgroup (61% *vs.* 34%, *P*=0.029, Table 3), and patients with pancolitis had lower hemoglobin levels than those with left-sided colitis/proctitis (12.4±2.3 g/dL and 13.5±1.8 g/dL, respectively, *P*=0.017).

Most anemic patients had either ACD or iron-deficiency anemia (Table 2 and *Online Supplementary Table S1*), followed by vitamin deficiencies and multifactorial forms of anemia. ACD was associated with higher ESR and CRP values than iron-deficiency anemia, although no differences were observed in disease activity scores between patients with ACD and iron-deficiency anemia (*Online Supplementary Table S1*); patients with the combination of iron deficiency and ACD had intermediate ESR and CRP values. At diagnosis most cases of anemia (54%) were isolated ACD, compared with 21% during the follow-up period (*P*=0.002). The difference was more significant in Crohn's disease. Diagnosis was also associated with higher levels of ESR (44±27 *versus* 24±21, *P*<0.001), CRP (5.02±4.75 *versus* 1.67±2.65 mg/dL, *P*<0.001) and serum ferritin (148±130 *versus* 134±512 µg/L, *P*=0.021) than follow-up, and the difference was more significant when patients anemic at diagnosis were compared to patients observed during the follow-up (*data not shown*). Multifactorial forms of anemia were more common during follow-up than at diagnosis (21 out of 80 cases of anemia at follow-up, 1 out of 24 cases at diagnosis, *P*=0.026). In Crohn's disease the frequency of iron deficiency as a cause of anemia increased during follow-up with respect to that at diagnosis (42% and 12%, respectively, *P*=0.030).

The serum level of prohepcidin (the precursor of hepcidin, a negative regulator of iron absorption that contributes to abnormal iron homeostasis in anemia of inflammation) was

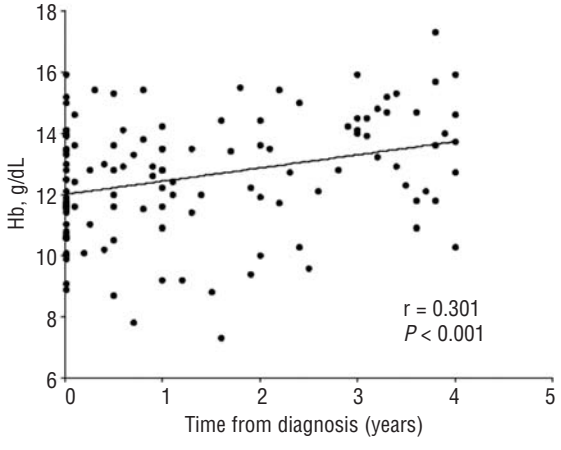

Figure 1. Temporal trend of hemoglobin (Hb) concentration in patients with inflammatory bowel disease during the first 4 years from diagnosis. Hb increases with time and the prevalence of anemia decreases.

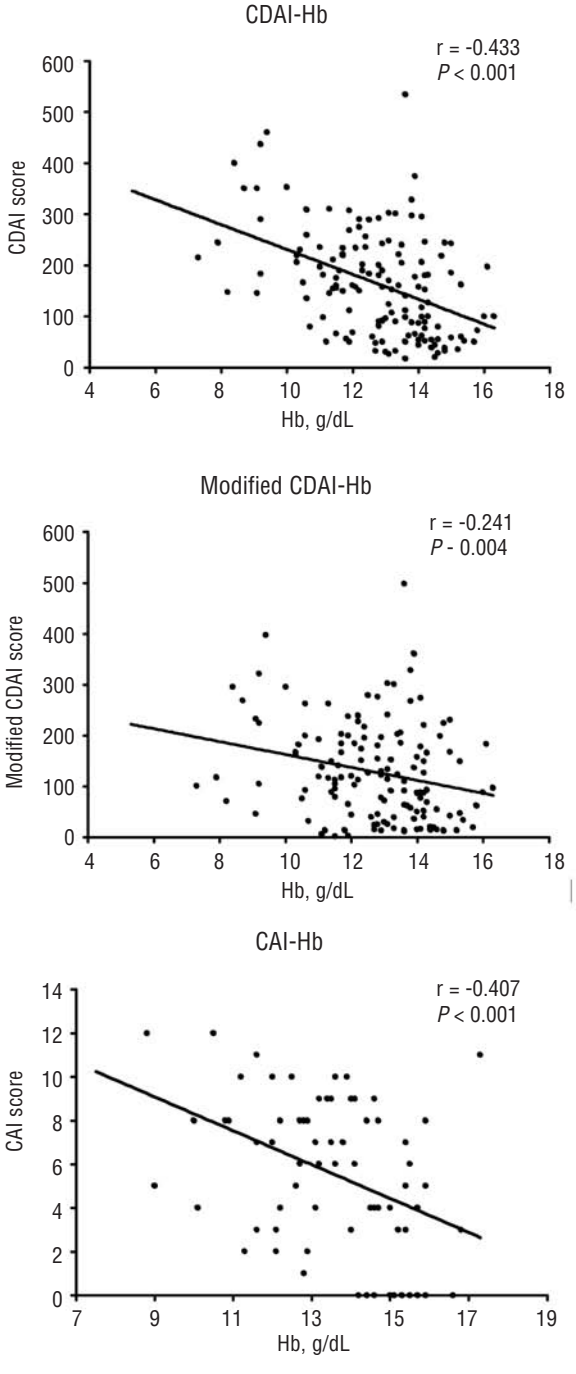

Figure 2. Correlations between disease activity indices and hemoglobin in patients with inflammatory bowel disease. The correlation between CDAI and hemoglobin is less significant when the contribution of hematocrit to the score is removed (modified CDAI, central panel).

higher in patients with inflammatory bowel disease than in healthy controls (96±53 compared with 54±29 ng/mL, *P*=0.007) and correlated with CRP (r=0.427, *P*=0.007). Among the patients with Crohn's disease, prohepcidin levels were higher in those with active disease (123±64 compared with 66±17 ng/mL in patients with quiescent disease, *P*=0.033) and were correlated with serum ferritin (r=0.4293, *P*=0.038). Crohn's disease patients with ACD had higher prohepcidin concentrations than those with iron-deficiency anemia (136±75 compared with 72±35 ng/mL), but the difference was not statistically significant.

Infliximab treatment of patients with Crohn's disease

The clinical features and treatment results for the 27 patients with Crohn's disease undergoing treatment with infliximab are shown in Table 3. Eighteen patients were anemic before starting infliximab and most of them (n=12) had ACD. Nineteen of the 27 patients had a clinical improvement after 14 weeks of treatment: 18 had a complete response and one had a partial response (Table 3). Hematologic responses were observed in 12 patients (10 major responses and 2 minor responses), and were already apparent 2 weeks after the first infusion of infliximab. In all, the mean CDAI value was significantly reduced after 14 weeks of treatment and, in responsive patients, this was associated with increased hemoglobin concentration and reduced serum ferritin and ESR (Table 3). The log of serum erythropoietin [log(Epo)] concentration negatively correlated with hemoglobin (r=-0.576, *P*=0.0001; regression equation: log(Epo)=-0.107× Hb+2.421), although the difference between erythropoietin values before and after treatment with infliximab was significant only in responsive patients. When the ratio between the observed log(Epo) and the log(Epo) predicted according to the regression equation was considered (Epo O/P ratio), higher post-treatment values were observed but, again, the difference was statistically significant only for responding patients (0.85±0.26 before treatment, 1.08±0.15 following treatment with infliximab, *P*=0.022; Table 3).

Table 2. Causes of anemia in inflammatory bowel disease.

Type of anemia	Crohn's disease		Ulcerative colitis	
	diagnosis	follow-up	diagnosis	follow-up
Iron deficiency anemia	2	23	4	9
Anemia of chronic disease	10	12	3	5
Cobalamin/folate deficiency	2	3	0	3
IDA + ACD	0	9	1	5
Cobalamin/folate deficiency + IDA or ACD	0	4	0	2
Undetermined	2	4	0	1
Total n. of patients with anemia	16	55	8	25

IDA: iron-deficiency anemia; ACD: anemia of chronic disease.

Table 3. Data on iron status, disease activity and inflammation in 27 patients with Crohn's disease treated with infliximab.

Groups of patients (N.)	Responding patients (n=19)		Non-responding patients (n=8)	
	wk 0	wk 14	wk 0	wk 14
Hemoglobin, g/dL	12.3±1.6	12.9±1.5	10.8±1.2	11.8±1.4
Transferrin saturation, (%)	18±11	23±15	15±8	18±11
Serum ferritin, µg/L	126±103	53±43	79±85	83±91
ESR, mm/hour	31±11	16±11	49±43	48±30
CRP, mg/dL	2.62±3.00	1.19±2.06	5.70±6.35	3.84±2.76
CDAI	220±79	108±41	218±64	217±56
Erythropoietin (mU/mL)	9.52±6.00	12.14±5.97	15.76±3.17	18.95±11.3
Epo O/P ratio	0.85±0.26	1.08±0.15	1.04±0.11	1.07±0.33

The reported results, expressed as mean ± 1 SD, correspond to data obtained immediately before (wk 0) and at the end (wk 14) of the treatment.wk: week; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; CDAI: Crohn's disease Activity Index. Response to treatment with infliximab was associated with significant changes in hemoglobin levels (P=0.0056), ESR (P<0.0001), CRP (P=0.0004), serum ferritin (P=0.0059), erythropoietin (P=0.0385) and Epo O/P ratio (P=0.0216).

In vitro *evaluation of erythropoiesis*

To assess the influence of infliximab on erythropoiesis, we cultured hematopoietic progenitors from the peripheral blood of ten patients with active Crohn's disease and evaluated the effect of infliximab on the number of BFU-E after 14 days of culture in semisolid medium. As shown in Figure 3, the mean number of BFU-E in the presence of infliximab (97.2±14.4) was significantly higher than that of colonies cultured with a control IgG1 (51.5±12.7; *P*=0.010). In contrast, TNF-α significantly reduced the number of BFU-E (17.6±6.2; *P*<0.001). BFU-E growth and response to *in vitro* treatment with infliximab were not different between healthy volunteers and patients with active Crohn's disease (*data not shown*).

Discussion

In this study we investigated the distribution and pathophysiology of anemia in a cohort of out-patients with inflammatory bowel disease attending two Institutions in northern

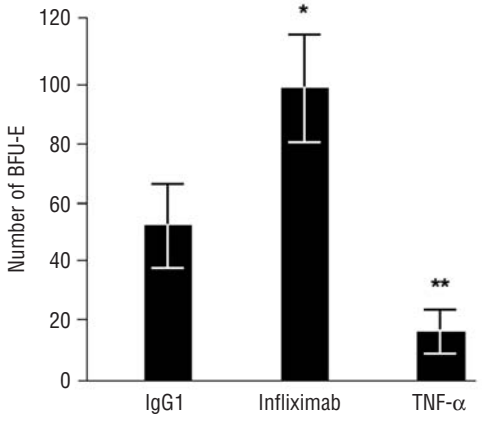

Figure 3. Growth of peripheral blood erythroid progenitors (BFU-E) from untreated patients with active Crohn's disease (n=10). The effect of TNF-α, infliximab and its isotype control (human IgG1) is shown. *indicates a difference between the IgG1 and the infliximab samples (*P*=0.010); **indicates a difference between the TNF-α and the other samples (*P*<0.001).

Italy. A cross-sectional study does not provide information about the mean duration of episodes of anemia and response to treatment. However, analysis of these patients does offer an overview of the burden of anemia in a population of inflammatory bowel disease patients during the natural history of the disease. Overall we found a 40% prevalence of anemia and confirmed that anemia is a common complication of inflammatory bowel disease. In most cases anemia was mild (hemoglobin ≥9.5 g/dL) but, since the study population included only out-patients, we probably missed some cases of more severe anemia that required hospitalization.

We observed a peculiar temporal trend in the prevalence and pathogenesis of anemia, characterized by higher rates of anemia at diagnosis followed by a progressive decrease during the subsequent 4 years. In the same period of time there was a mild increase in the mean hemoglobin level. According to our diagnostic criteria, inflammation was the main cause of anemia at diagnosis, and this is in agreement with the observation that diagnosis was characterized by higher indices of inflammation. The increasing frequency of iron-deficiency anemia during follow-up, associated with a reduced incidence of ACD, may be related to chronic intestinal blood loss and/or iron malabsorption which can take months to years in order to induce iron deficiency and anemia.

Inflammation is known to inhibit iron absorption, and subjects with active Crohn's disease have impaired oral iron absorption associated with increased urinary excretion of hepcidin,[22] the liver-derived peptide that inhibits iron absorption.[23] We measured serum prohepcidin concentration as a substitute for hepcidin; prohepcidin levels were higher in patients with inflammatory bowel disease than in normal subjects and correlated with CRP and the CDAI score. Although the prohepcidin assay has been considered a poor indicator of hepcidin synthesis, these correlations suggest a link between abnormal iron homeostasis, inflammation and disease activity in inflammatory bowel disease. We, therefore, suggest that, in addition to directly causing ACD, recurrent flares of inflammation over a period of years substantially reduce iron absorption in patients with inflammatory bowel disease and lead to an iron deficiency that may persist during periods of disease remission.

Since the main site of iron and folate[24] absorption is the duodenum, malabsorption and deficiencies of these nutrients could be a consequence of upper gastrointestinal involvement. Upper gastrointestinal involvement in inflammatory bowel disease is considered a rare complication occurring in 0.5 to 4% of patients,[25] but some pediatric studies report gastro-duodenal involvement in up to 40% of patients with Crohn's disease, often in a subclinical form.[26,27] Thus, compared to the situation at diagnosis, when anemia is usually due to isolated ACD or iron-deficiency anemia, the course of inflammatory bowel disease is characterized by an impairment of erythropoiesis due to a variety of mechanisms, as suggested by the increasing frequency of cases of anemia due to different combinations of iron-deficiency anemia, ACD and vitamin deficiencies observed during the follow-up. We do not routinely treat inflammatory bowel disease patients with iron and/or vitamin supplements, unless the diagnostic work-up performed following a diagnosis of anemia shows a specific hematinic deficiency, since the safety of iron supplementation for the prevention of anemia in inflammatory bowel disease is still a matter of discussion. This may partially explain the trend we observed in the prevalence of iron-deficiency anemia and the higher prevalence of anemia in our study than in others.[28-30]

In the present study anemia was related to disease activity in both Crohn's disease and ulcerative colitis (Figure 2). Greater disease activity in anemic patients with Crohn's disease has been previously reported.[28] Since the CDAI value is influenced by hematocrit, this result is not unexpected. However, using a modified CDAI (the CDAI score calculated without considering the hematocrit), the correlation with hemoglobin was preserved, but only in ten patients (11% of the population with active disease) did anemia have a determinant role for the classification of patients within the active disease group. Thus, in most cases the definition of active disease does not depend on the presence of anemia which, however, remains an expression of more active and severe disease.

Anti-TNF-α therapy is being increasingly used in inflammatory bowel disease to treat patients with steroid refractory or fistulizing disease (at least in Crohn's disease); the treatment specifically targets one of the main inflammatory pathways involved in the pathogenesis of tissue damage.[10,11] Since TNF-α is a negative regulator of erythropoiesis and contributes to ACD,[12] we investigated how TNF-α neutralization with infliximab affects anemia and iron status parameters *in vivo* and modulates the *in vitro* growth of erythroid progenitor cells (BFU-E). ACD was the most common form of anemia in patients undergoing infliximab treatment. In these patients infliximab improved anemia through the control of inflammation and disease activity, as suggested by the reduction in ESR, serum ferritin and CRP in responsive patients. This was confirmed by the increased post-therapy Epo O/P ratio in responding patients, and indicates that infliximab makes erythropoietin production more adequate for the level of hemoglobin, thus allowing more efficient bone marrow stimulation.

Interleukin-6, rather than TNF-α, is a mediator of hepcidin production and abnormal iron homeostasis in inflammation; whether infliximab directly modulates interleukin-6 or hepcidin production, and through this pathway improves erythropoiesis in inflammatory bowel disease, is presently unknown. *In vitro* infliximab increased the growth of BFU-E from peripheral blood, as already shown for bone marrow-derived BFU-E in a study involving patients with rheumatoid arthritis.[13] This suggests that infliximab can modulate erythropoiesis at different levels. At variance with the study by Papadaki *et al.*[13] we found that infliximab enhanced the growth of BFU-E also from peripheral blood of normal subjects; further studies are needed to clarify the mechanism of BFU-E stimulation exerted by infliximab.

Authorship and Disclosures

GB was the principal investigator and takes primary responsibility for the paper; he co-ordinated the research and wrote the paper. ADS, GBP and GRC co-ordinated the research and wrote the paper, RA, EB and VR performed the laboratory work for this study. SA, PB, AC, PC, KM, and AM recruited the patients.

The authors reported no potential conflicts of interest.

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