Clinical Trial Protocol

Trial Title:	Clinical burden of anemia in inflammatory bowel disease: **R**ole of **I**ron **D**eficiency **A**nd iron **R**eplacement **T**herapy, observational study (RIDART I)
Protocol Version:	2.1

Chief Investigator:	Prof. Antonio Di Sabatino
CI Address:	Department of Internal Medicine, Fondazione IRCCS Policlinico San Matteo, Pavia
Telephone:	0382-501596; 0382.502095
Investigation Promoter:	The study is promoted by IG-IBD (Italian Group for the study of Inflammatory Bowel Diseases).

1 Protocol Signatures:

I have read the attached protocol entitled "Clinical burden of anemia in inflammatory bowel disease: Role of Iron Deficiency And iron Replacement Therapy" dated 02/11/2015 and agree to abide by all provisions set forth therein.

I agree to comply with the conditions and principles of Good Clinical Practice as outlined in the European Clinical Trials Directives 2001/20/EC and the GCP Directive 2005/28/EC.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor

I give my approval for the attached protocol entitled "Clinical burden of anemia in inflammatory bowel disease: Role of Iron Deficiency And iron Replacement Therapy" dated 22/02/2016

Chief Investigator

Name: Antonio Di Sabatino

Signature: _____

Date: 22/02/2016

Study Statistician

Name: Catherine Klersy

Signature: _____

Date: 22/02/2016

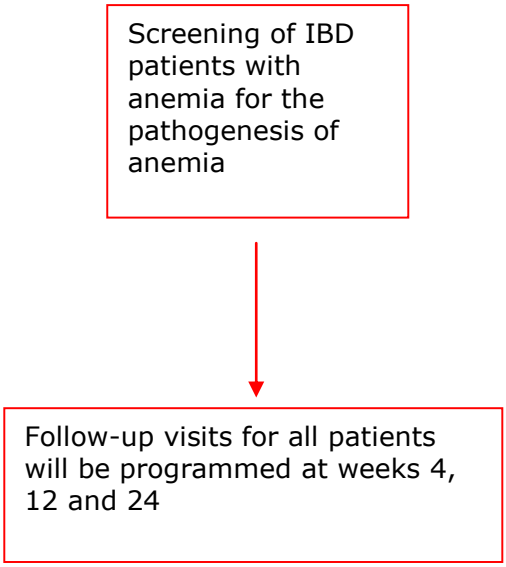
2 Trial Management Committee and Protocol Contributors

Prof. Antonio Di Sabatino (CI, TSC and TMG)
Dr. Gaetano Bergamaschi (TSC and TMG)
Dr. Paolo Giuffrida (Medico Specializzando in Gastroenterologia ed Endoscopia Digestiva, Fondazione IRRCS Policlinico S. Matteo, TMG)
Dr. Davide Grataroli (Medico Specializzando in Immunologia, Fondazione IRCCS Policlinico S. Matteo, TMG)
Dr. Marco Guerci (Medico Specializzando in Medicina Interna, Fondazione IRCCS Policlinico S. Matteo, TMG)
Dr.ssa Michela Masotti (Medico Specializzando in Medicina Interna, Fondazione IRCCS Policlinico S. Matteo, TMG)
Dr.ssa Chiara Salvatore (Medico Specializzando in Gastroenterologia ed Endoscopia Digestiva, Fondazione IRCCS Policlinico S. Matteo, TMG)
Dr.ssa Catherine Klersy, Servizio di Biometria e Statistica & Clinical Trial Center (CTC), Direzione Scientifica, Fondazione IRCCS Policlinico S. Matteo (statistical aspects)

Abbreviations

AI	Anemia of inflammation
CA	Competent Authority
CAI	Clinical activity index
CD	Crohn disease
CDAI	Crohn disease activity index
CRF	Case Report Form
CTQT	Clinical Trial Quality Team
DMC	Data Monitoring Committee
DSUR	Development Safety Update Report
GCP	Good Clinical Practice
GP	General Practitioner
Hb	Hemoglobin
IBDQ	Inflammatory bowel disease questionnaire
IDA	Iron deficiency anemia
IG-IBD	Gruppo Italiano per lo Studio delle Malattie Infiammatorie Croniche Intestinali
IMP	Investigational Medicinal Product
MHRA	Medicines and Healthcare products Regulatory Agency
NIMP	Non Investigational Medicinal Product
RA	Regulatory Agency
R&D	Research and Development
REC	Research Ethics Committee
SAE	Serious Adverse Event
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMG	Trial Management Group
TSC	Trial Steering Committee
UC	Ulcerative colitis
VAS	Visual analogue scale

3 Trial Flow Chart



4 Introduction

4.1 Background

Anemia is the most common extraintestinal manifestation of IBD, occurring in 6 to 74 percent of patients. This wide range in the prevalence of anemia is related to differences in study design, in the criteria used to define anemia and to the increasing awareness of anemia in these patients. According to a recent meta-analysis of European studies involving 2192 IBD patients, the overall prevalence of anemia in Europe was 27%, ulcerative colitis patients being less likely to develop anemia than those with Crohn's disease (Filmann et al, 2014). Therefore, anemia represents a significant clinical and social burden in IBD management; this is more evident in southern Europe where the prevalence of anemia is close to 40%.

4.2 Clinical Data

Most cases of anemia in IBD are due to iron deficiency (IDA) and to anemia of inflammation (AI) (Bergamaschi et al, 2010). Guidelines concerning the diagnosis and treatment of IDA in IBD have been published by the European Crohn's and Colitis Organisation (ECCO, Dignass et al, 2015). According to present guidelines iron supplementation should be started when IDA is present or even in the presence of iron deficiency without anemia. A meta-analysis of iron supplementation studies in IBD-associated IDA found that intravenous iron is more effective and better tolerated than oral iron supplementation, but the sample size of the included studies was small (Lee et al, 2012); absolute indications for intravenous iron include severe anemia (haemoglobin <10.0 g/dL) and intolerance or inadequate response to oral iron.
Although the ECCO diagnostic criteria for IDA are simple, and iron supplementation represents a cheap and usually effective treatment, many IBD patients with IDA are not properly treated. The inconsistent adherence, by many physicians, to treatment guidelines for IDA in IBD is often motivated by the belief that mild to moderate degrees of anemia may not have a significant impact on the patient's quality of life or do not represent the main clinical problem of the patient, that oral iron supplementation may adversely affect disease activity, and that parenteral iron administration may cause severe side effects.

4.3 Rationale for Investigation

In order to improve the diagnostic and therapeutic work-up of anemia in IBD, it is necessary to precisely define the prevalence and pathogenesis of anemia in the Italian IBD population and gain informations about how anemic patients, particularly those with IDA, are usually managed and followed-up. In addition, since IDA and AI are the most common forms of anemia in IBD, and both mechanisms often concur to the pathogenesis of anemia in the same subject, stringent diagnostic criteria to distinguish the three conditions (IDA, AI and coexistence of IDA and AI) are urgently needed. The differential diagnosis between IDA and AI is often difficult since inflammation modifies serum ferritin and transferrin saturation (TfSat) that become unreliable as indicators of iron status. Recently, serum hepcidin concentration has been identified as a new index of iron status that can be useful to differentiate IDA and AI in IBD (Bergamaschi et al, 2013; Mecklenburg et al, 2014). Hepcidin, a peptide produced by the liver in response to increased iron stores, inflammation and reduced erythropoietic activity, is the master regulator of body iron homeostasis and acts by inhibiting iron absorption and iron release from hepatocytes and macrophages to plasma (Ganz T, 2011). The potential role of serum hepcidin determination as a diagnostic tool in the differential diagnosis of anemia in IBD and in other inflammatory diseases must be clearly defined. The investigation of these topics is important since a more accurate pathogenetic diagnosis of anemia in IBD, while preventing the useless and potentially

harmful treatment of AI with iron supplementation, might allow the identification and appropriate treatment of IDA patients in the presence of inflammation.

5 Study Design

5.1 Statement of design

We will perform a longitudinal, prospective, observational study whose aim is the determination of the prevalence and pathogenesis of anemia in IBD patients. Anemia is defined according to WHO criteria: Hb <13.0 g/dL for males and Hb <12 g/dL for females (Blanc et al, 1968). Anemia work-up should be initiated whenever Hb concentration is below normal and will include the determination of the laboratory parameters reported in the CRF. A serum ferritin <100 µg/L and transferrin saturation <20% are required for the diagnosis of isolated IDA and for the association of IDA and inflammation.

5.2 Number of Centers

We plan to involve at least 60 IG-IBD Centers in this observational study.

5.3 Number of Subjects

We expect to include 2000 anemic patients in the study; approximately 80 to 90% of these patients will have some form of iron deficiency and/or AI. This will allow to evaluate differences in prevalence of IDA and AI according to gender, age, diagnosis, time from diagnosis, presence of inflammation, activity, extension and behaviour of the disease, and to evaluate variables associated with type and severity of anemia in both univariate and multivariate analysis.

5.4 Participants Study duration

Expected duration of subject participation to both the observational and the therapeutic trials will be 24 weeks; all anemic patients recruited in the study will undergo screening to investigate the mechanism of anemia, and follow-up evaluation will be performed at weeks 4, 12 and 24. At follow-up, informations about the treatment of anemia, fatigue and quality of life must be collected.

5.5 Study objectives

Table 1 summarizes study objectives and outcome measures.

6.5.1 Primary objective

Primary endpoint of this observational study is to determine the prevalence of anemia in Italian patients with IBD.

6.5.2 Secondary objectives

Secondary objectives of the study are a) to investigate the pathogenesis of anemia in IBD, with a particular focus on the differential diagnosis between IDA and AI, and how disease activity, extension or behavior influence the relative frequency of IDA and AI; b) to verify the adherence to ECCO guidelines for the treatment of IDA in IBD (the proportion of patients with IDA that receive adequate iron supplementation); c) to administer dedicated questionnaires to the patients in order to measure the influence of anemia on fatigue and quality of life among IBD patients.

5.6 Study Outcome Measures

5.6.1 Primary outcome measure

The primary outcome will be computed as the ratio of the number of anemic patients over the number of patients screened for anemia together with its 95% confidence interval.

5.6.2 Secondary outcome measure

1. Relative prevalence of IDA and AI in IBD will be computed as the ratio between the number of patients with IDA or with AI over the total number of anemic patients
2. Influence of anemia on fatigue and quality of life among IBD patients. Quality of life will be evaluated using the validated Italian translation of the Inflammatory Bowel Disease Questionnaire (IBDQ, Ciccocioppo et al, 2011), whereas the effect of anemia on fatigue will be measured using a Visual Analogue Scale.
3. Influence of anemia on hospitalizations, additional outpatient visits, number of endoscopic examinations and further treatments will be assessed through the analysis of correlations between the degree of anemia and the relevant parameters.

6 Selection and withdrawal of subjects

6.1 Inclusion Criteria and Exclusion

To be included in the trial the patient must:

- Have given written informed consent to participate
- Be aged 18 years and over
- Have IBD and anemia

Whenever the inclusion criteria are satisfied, the patient can be included in the study since there are no exclusion criteria.
Patients can be participating in other research, either, observational, interventional or other drug research concerning the treatment of IBD. Patients with IDA can also participate in the companion study RIRART II that will compare three iron supplementation regimens in terms of efficacy and safety.

6.2 Subject withdrawal criteria

At anytime during the study, participating subjects may ask to be withdrawn from the study. Withdrawn subjects can be followed up for their IBD at the same participating Center where they were initially enrolled. Withdrawn subjects will not be replaced.

7 Concomitant therapy

Nutritional supplementation with iron and/or vitamin integrators containing folic acid or vitamin B12, as well as any other treatment of IBD and relative comorbidities are allowed during the study.

8 Procedures and assessments

Laboratory assessments can be performed outside the participating Centers, and results faxed or emailed to the Investigators who will register data in the eCRF. Result notification to the trial team must be performed as soon as possible.

8.1 Screening evaluation

8.1.1 Screening Assessments

Study-related specific assessments will only be conducted after subjects have given written informed consent.

8.1.2 Subject Registration

Subjects will be registered locally and assigned a registration number/code. This number, together with the Center code, will constitute the unique patient identification code. In the trial documentation the patient must be always identified by means of this code, and only the patient physicians must be able to identify the patient from the assigned code.

8.2 Baseline assessments

All patients will have a full medical history taken and a clinical examination. The following data points are to be recorded:

a) Weight in Kg
b) Gender
c) Age and date of birth
d) Any significant past medical history
e) CDAI and CAI disease activity scores for Crohn's disease and ulcerative colitis respectively
f) Full blood count (including platelets and differential white cell count)
g) Biochemical series: including creatinine, serum iron, transferrin, serum ferritin, C reactive protein (PCR), folic acid, vitamin B12 (these are mandatory; further biochemical parameters useful for the study are reported in the CRF)
h) IBDQ score, VAS evaluation of fatigue
i) Actual treatment

8.3 Study assessments

8.3.1 Timing of assessments

Follow-up will be performed at weeks 4, 12 and 24 . The following data are to be recorded:

a) Physical examination
b) Adverse Event Review
c) Weight in Kg
e) Full blood count
f) Biochemical series
g) IBDQ score,VAS evaluation of fatigue
h) Actual treatment

8.4 Long-Term Follow-up Assessments

After the end of the study patients will be followed-up as usual for patients with their conditions. Telephone calls will be used to contact patients if visits or data collection time-points are missed; whenever data collection will be impossible, the patient will be identified as 'lost to follow-up'.

8.5 End of Study Participation

Patients will be expected to return to normal standard of care following their participation in the study.

8.6 Schedule of Assessments

A format of all study-related assessments is included in the CRF.

9 Evaluation of Results (Definitions and response/evaluation of outcome measures)

The primary end-point of the observational study (prevalence of anemia) will be evaluated at patient recruitment. Other informations concerning laboratory parameters, quality of life and clinical course will be collected at the scheduled follow-up visits.

10 Statistical methods and number of Subjects to be enrolled

If at least 60 IG-IBD Centers will participate to the study, 5000 to 8000 IBD patients will be screened for anemia within 6 to 12 months from the start of the study. Since anemia affects 25% to 40% of IBD patients, we expect to include 2,000 anemic patients for this observational study. For the first endpoint (the prevalence of anemia) the precision that can be obtained, based on this hypothesis, is summarized in Table 2. Approximately 80 to 90% of these patients will have some form of iron deficiency and/or AI. This will allow to evaluate differences in prevalence of IDA and AI according to gender, age, diagnosis, time from diagnosis, presence of inflammation, disease activity, extension and behavior, and to evaluate variables associated with type and severity of anemia in both univariate and multivariate analysis.
Multivariable models will be fitted to assess the role of anemia on the outcome, while adjusting for potential confounders. Linear regression, logistic regression or Poisson regression models will be used depending on the outcome distributions. Descriptive statistics will be computed overall and by anemia groups.

11 Definition of the end of the study

The competent authority and Ethics Committee will be notified of the end of the clinical study within 90 days of its completion. The end of study will be the date of the last patient's last visit.

12 Data handling and record keeping

13.1 CRF

All data will be transferred into a Case Report Form (CRF) which will be coded. The investigator will maintain separately a logbook with patient ID and corresponding code. All study data in the CRF will be extracted from and be consistent with the relevant source documents. The CRFs will be completed, dated and signed by the investigator or designee in a timely manner. It remains the responsibility of the investigator for the timing, completeness, legibility and accuracy of the CRF pages. The CRF will be accessible to study coordinators, data managers, the investigators, Auditors and Inspectors as required.
CRFs will be emailed to the study coordination centre (Fondazione IRCCS Policlinico San Matteo, Pavia).
The investigator will retain a copy of each CRF page at site. The investigator will also supply the study coordination Center with any required, anonymised background information from the medical records as required.
The investigators must ensure that the CRFs and other trial related documentation is sent to the trial coordination Center containing no patient identifiable data.
Completed CRF data should be entered into the database within 2 months of the pages being completed/or patient visit being completed.
The investigator will retain all copies of the CRF in the relevant sections of their Investigator Site File with any required coded background information from the medical records as required.
All CRF pages must be clear, legible and completed in black ink.

12.1 Source Data

The investigators agree to keep records in a logbook of all participating patients: sufficient information to link CRFs, hospital records and samples, all original signed informed consent forms and copies of the CRFs, Patient Medical Records, On-line test results.

12.2 Data Protection & Patient Confidentiality

All investigators and site staff involved in this study comply with the requirements of the Data Protection Act 1998 and Trust Policy with regards to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles.

13 Data Monitoring Committee/Trial Steering Committee

The study has been planned in Pavia by Prof. A. Di Sabatino, Dr. G. Bergamaschi and Prof. G.R. Corazza, from the Department of Internal Medicine, Fondazione IRCCS Policlinico San Matteo; they represent the Trial Steering Committee.
Remote data monitoring will be performed and will be coordinated by an employee of the Scientific Direction of the IRCCS Fondazione Policlinico San Matteo, with the support of members of the Steering Committee in Pavia.

14 Ethical & Regulatory considerations

14.1 Consent

The Informed Consent is in compliance with GCP, local regulatory requirements and legal requirements. Informed consent from each patient or the patient's legally acceptable representative will be obtained before any trial-specific activity is performed. The informed consent form used and any change made during the course of this trial, will be submitted to the REC. The investigator will retain the original of each patients signed informed consent form.
Should a patient require a verbal translation of the trial documentation by a locally approved interpreter/translator, it is the responsibility of the individual investigator to use locally approved translators.
Any new information which becomes available, which might affect the patient's willingness to continue participating in the study will be communicated to the patient as soon as possible. This may occur either verbally over the telephone, or at their next visit (if visits are close together).

14.2 Ethical Committee review

Before the start of the trial or implementation of any amendment we will obtain approval of the study protocol, protocol amendments, informed consent forms and other relevant documents e.g., advertisements and GP information letters if applicable from the REC. All correspondence with the REC will be retained in the Trial Master File/Investigator Site File.
Reports will be submitted to the REC in accordance with national requirements. It is the Chief Investigator's responsibility to produce the annual reports as required.

15.3 Authorisation of Participating Sites

The study protocol must be approved by the REC of each participating Center. As soon as the protocol has been approved by the REC, the Participating Center will notify the decision to the Chief Investigator. At this point the Center will be allowed to start patient recruitment.

15.4 Regulatory Compliance

The study will not commence until a Clinical Trial Authorisation (CTA) is obtained from the REC. The protocol conduct will comply with the Medicines for Human Use (Clinical Trials) Regulations 2004 and any relevant amendments.

15.5 Protocol Amendments

Protocol amendments must be reviewed and agreement received from the REC. The only circumstance in which an amendment may be initiated prior to REC approval is where the change is necessary to eliminate apparent, immediate risks to the patients (Urgent Safety Measures). In this case, accrual of new patients will be halted until the REC approval has been obtained.

15.6 Peer Review

This trial protocol has been reviewed by the IG-IBD Scientific Committee.

15.7 Declaration of Helsinki and Good Clinical Practice

The trial will be performed in accordance with the spirit and the letter of the declaration of Helsinki, the conditions and principles of Good Clinical Practice, the protocol and applicable local regulatory requirements and laws.

15.8 GCP Training

All trial staff holds evidence of appropriate GCP training.

16 Sponsorship, Financial and Insurance

The study has no financial support. Since it is not a therapeutic trial and the patients will not be subjected to additional diagnostic procedures besides those normally required in the follow-up of their disease, no insurance is needed.

17 Monitoring, Audit & Inspection

Should a monitoring visit or audit be requested, the investigator must make the trial documentation and source data available to the Monitor. All patient data must be handled and treated confidentially.
Remote monitoring will be conducted for all participating sites.

18 Protocol Compliance and Breaches of GCP

Prospective, planned deviations or waivers to the protocol are not allowed. In the event that eligibility criteria need to be changed/amended, then they MUST first be approved by REC via a substantial protocol amendment before they can be implemented. Protocol deviations, non-compliances, or breaches are departures from the approved protocol. They can happen at any time, but are not planned. They will be adequately documented on the relevant forms and reported to the Chief Investigator immediately.

Deviations from the protocol which are found to occur constantly again and again will not be accepted and will require immediate action and could potentially be classified as a serious breach.
Any potential/suspected serious breaches of GCP must be reported immediately to the Chief Investigator without any delay.

19 Publications policy

Ownership of the data arising from this trial resides with the trial team. On completion of the study the data will be analysed and tabulated and a Final Study Report prepared.
We expect that the study results will provide material for at least one publication. However, we do not exclude that data on the economic burden of anemia in IBD could be the subject of an additional paper. Authorship will be recognized to at least one representative of each participating Center that will contribute patients to the study and to the steering committee members. Contributions by other investigators will be recognized either by adding "for the RIDART I Study Investigators" at the end of the Authors' name list or by providing a complete list of Investigators in an Appendix.
Any funding or supporting body will be acknowledged within the publications, but they do not have review and publication rights of the data from the trial.
The patient can request trial results from their PI after the Final Study Report had been compiled.

20 References

Blanc B, Finch CA, Hallberg L, et al. Nutritional anaemias. Report of a WHO Scientific Group. WHO Tech Rep Ser 1968;405:1-40.

Bergamaschi G, Di Sabatino A, Albertini R et al. Prevalence and pathogenesis of anemia in inflammatory bowel disease. Influence of anti-tumor necrosis factor-alpha treatment. Haematologica 2010;95:199-205.

Bergamaschi G, Di Sabatino A, Albertini R, Costanzo F, Guerci M, Masotti M, Pasini A, Massari A, Campostrini N, Corbella M, Girelli D, Corazza GR. Serum hepcidin in inflammatory bowel diseases: biological and clinical significance. Inflamm Bowel Dis. 2013;19:2166-72.

Bozzini C, Campostrini N, Trombini P et al. Measurement of urinary hepcidin levels by SELDI-TOF-MS in HFE-hemochromatosis. Blood Cells Mol Dis. 2008;40:347–352.

Ciccocioppo R1, Klersy C, Russo ML, Valli M, Boccaccio V, Imbesi V, Ardizzone S, Porro GB, Corazza GR. Validation of the Italian translation of the Inflammatory Bowel Disease Questionnaire. Dig Liver Dis. 2011;43:535-41.

Dignass AU, Gasche C, Bettenworth Det al; European Crohn's and Colitis Organisation [ECCO]. European consensus on the diagnosis and management of iron deficiency and anaemia in inflammatory bowel diseases. J Crohns Colitis. 2015;9:211-22.

Filmann N, Rey J, Schneeweiss S, et al. Prevalence of anemia in inflammatory bowel diseases in european countries: a systematic review and individual patient data meta-analysis. Inflamm Bowel Dis. 2014;20(5):936–45.

Ganz T. Hepcidin and iron regulation, 10 years later. Blood. 2011;117:4425-4433.

Lee TW, Kolber MR, Fedorak RN, van Zanten SV. Iron replacement therapy in inflammatory bowel disease patients with iron deficiency anemia: A systematic review and meta-analysis. Journal of Crohn's and Colitis 2012;6:267–275.

Mecklenburg I, Reznik D, Fasler-Kan E, Drewe J, Beglinger C, Hruz P; Swiss IBD Cohort Study Group. Serum hepcidin concentrations correlate with ferritin in patients with inflammatory bowel disease. J Crohns Colitis. 2014;8:1392-7.

Patrick DL, Gagnon DD, Zagari MJ, Mathijs R, Sweetenham J; Epoetin Alfa Study Group. Assessing the clinical significance of health-related quality of life (HrQOL) improvements in anaemic cancer patients receiving epoetin alfa. Eur J Cancer. 2003;39:335-45.

Table 1. Study objectives and outcome measures

OBSERVATIONAL STUDY
Primary objective
Evaluation of the global prevalence of anemia in IBD
Secondary objectives
1. Evaluation of the pathogenesis of anemia in IBD 2. Evaluation of adherence to ECCO guidelines for the treatment of IDA in IBD 3. Influence of anemia on fatigue, quality of life, hospitalizations, additional outpatient visits, number of endoscopic examinations and further treatments
Primary outcome measure
Prevalence of anemia will be determined according to the WHO criteria for the diagnosis of anemia: Hb <13.0 g/dL for males and Hb <12 g/dL for females
Secondary outcome measure
1. Anemia differential diagnosis: the main objective is to differentiate IDA and AI; this will require that serum ferritin >100 µg/L and transferrin saturation ≤ 20% for AI, serum ferritin ≤100 µg/L and transferrin saturation ≤20% or serum ferritin ≤30 µg/L for isolated IDA and for the association of IDA and inflammation 2. To evaluate how many IBD patients with IDA actually receive any form of iron supplementation and which iron supplementation modalities are more commonly used 3. Quality of life and fatigue will be assessed by the IBDQ score (Ciccocioppo et al, 2011) and the visual analogue scale (VAS), respectively

Table 2. Precision that can be obtained in the evaluation of the prevalence of anemia in IBD, depending on the number of patients screened for anemia and on the prevalence of anemia

Hypothesis	Screened patients	Prevalence expected % (95% CI)	Precision %
1	5000	25 (23.8-26.2)	2.4
2	5000	40 (38.6-41.4)	2.8
3	8000	25 (24.1-26.0)	1.9
4	8000	40 (38.9-41.1)	2.2

CI: confidence interval