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**ANEMIA NAS DOENÇAS INFLAMATÓRIAS INTESTINAIS:  
PREVALÊNCIA E FATORES DE RISCO**

Juiz de Fora  
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Dissertação de Mestrado apresentada ao Curso de Mestrado em Saúde, Programa de Pós-Graduação em Saúde, área de concentração em Saúde Brasileira, da Universidade Federal de Juiz de Fora, como requisito parcial para obtenção do Título de Mestre em Saúde.

Orientador: Prof. Dr. Júlio Maria Fonseca Chebli  
Co-orientador: Prof. Dr. Abrahão Elias Hallack Neto

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*“A vida nem sempre segue a nossa vontade, mas ela é perfeita naquilo que tem que ser”.*  
*Francisco Cândido Xavier*

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## RESUMO

Anemia de difícil tratamento é uma manifestação clínica comumente observada nos pacientes portadores de doenças inflamatórias intestinais, sendo responsável por prejuízo significativo na qualidade de vida destes pacientes. O objetivo deste estudo foi avaliar, nos pacientes com doença inflamatória intestinal, a prevalência e os fatores de risco da anemia suas possíveis etiologias. Neste estudo de corte prospectivo observacional foram recrutados: 100 pacientes portadores de Doença de Crohn e 100 pacientes portadores de Retocolite ulcerativa, diagnosticados e acompanhados regularmente no Centro de Doenças Inflamatórias Intestinais do Hospital Universitário da Universidade Federal de Juiz de Fora, para avaliação hematológica, bioquímica e imunológica. Foram obtidas amostras de sangue (20 ml) e realizados os seguintes exames em todos os pacientes: hemograma completo, VGM, HGM, CHGM, plaquetas, ácido fólico, vitamina B12, reticulócitos, índice de saturação da transferrina, ferritina, ferro sérico, PCR e VHS. Foram adotados para o diagnóstico de anemia os mesmos critérios da WHO (World Health Organization). Foi considerado Anemia por Deficiência de Ferro quando houve diminuição dos níveis séricos de ferro ( $< 37 \mu\text{g/dl}$  para mulheres e  $< 59 \mu\text{g/dl}$  para homens), da ferritina ( $< 30 \mu\text{g/l}$  - na ausência de dados clínicos, laboratoriais ou endoscópicos de inflamação intestinal e  $< 100 \mu\text{g/l}$  - na presença de quaisquer destes dados), do índice de saturação da transferrina ( $< 16\%$ ). Foi considerado Anemia da Doença Crônica quando houve diminuição dos níveis séricos de ferro ( $< 37 \mu\text{g/dl}$  para mulheres e  $< 59 \mu\text{g/dl}$  para homens), aumento da ferritina ( $> 100 \mu\text{g/l}$ ) e diminuição do índice de saturação da transferrina ( $< 16\%$ ). - na presença de dados clínicos, laboratoriais ou endoscópicos de inflamação intestinal e Anemia Mista quando houve diminuição dos níveis séricos de ferro ( $< 37 \mu\text{g/dl}$  para mulheres e  $< 59 \mu\text{g/dl}$  para homens) e ferritina entre 30 e  $100 \mu\text{g/l}$ . As anemias foram classificadas em hiporregenerativas quando a contagem absoluta de reticulócitos estava abaixo de 50000 e normoproliferativas ou normorregenerativas quando a contagem absoluta de reticulócitos estava acima de  $100000/\text{mm}^3$ .

**Palavras-chave:** Doenças Inflamatórias Intestinais. Anemia. Doença de Crohn. Retocolite ulcerativa.

## ABSTRACT

Anemia difficult to treat is a clinical manifestation commonly seen in patients with inflammatory bowel disease, being responsible for significant impairment in quality of life of these patients. The aim of this study was to evaluate, in patients with inflammatory bowel disease, the prevalence and factors risk of anemia and possible etiologies of anemia in their possible occurrence. In this cross-sectional study of adult patients with inflammatory bowel disease (IBD) were recruited, of which: 100 patients with Crohn's disease and 100 patients with ulcerative colitis, diagnosed and regularly followed at the Center for Inflammatory Bowel Diseases, University Hospital, Federal University of Juiz de Fora, for haematological, biochemical and immunological evaluation. Blood samples (20 ml) were obtained and the following examinations were performed in all patients: CBC, MCV, MCH, CHCM, platelets, folic acid, vitamin B12, reticulocytes, transferrin saturation index, ferritin, serum iron, CRP and ESR. For the diagnosis of anemia the same criteria of WHO (World Health Organization) were adopted. Was considered Iron Deficiency Anemia when there was a decrease in serum iron levels ( $< 37$  mg / dl for women and  $< 59$  g / dl for men), ferritin ( $< 30\mu\text{g/l}$  - in the absence of clinical, laboratory data or endoscopic intestinal inflammation and  $< 100$  mg / l - in the presence of any of these data), the ratio of transferrin saturation ( $< 16\%$ ). Anemia was considered the Crohnic Disease Anemia when there was a decrease in serum iron levels ( $< 37$  mg / dl for women and  $< 59$  g / dl for men), elevated ferritin ( $> 100\mu\text{g} / \text{l}$ ) and decreased transferrin saturation index ( $< 16\%$ ). - in the presence of clinical, laboratory and endoscopic data of intestinal inflammation and Mix Anemia when there was a decrease in serum iron levels ( $< 37$  mg / dl for women and  $< 59$  g / dl for men) and ferritin between 30 and  $100\mu\text{g} / \text{l}$ . Anemia were classified into hiporregenerative when absolute reticulocyte count was below 50,000 and normoproliferative or normorregenerative when the absolute reticulocyte count was above 100,000.

**Keywords:** Inflammatory Bowel Diseases. Anemia. Crohn's disease. Ulcerative colitis.



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## LISTA DE ABREVIATURAS

ADC	- Anemia da doença crônica
CHGM	- Concentração de hemoglobina globular média
CNPq	- Conselho Nacional de Desenvolvimento Científico e Tecnológico
DC	- Doença de Crohn
DII	- Doença inflamatória intestinal
ERF	- Elemento responsivo ao ferro
DC	- Doença de Crohn
FAPEMIG	- Fundação de Amparo à Pesquisa do Estado de Minas Gerais
HGM	- Hemoglobina globular média
HU	- Hospital Universitário
IADC	- Índice de atividade de Doença de Crohn
IBD	- Inflammatory bowel disease
mRNA	- ácido ribonucleico mensageiro
OR	- Odds risk
PCR	- Proteína C reativa
PRF	- Proteína reguladora de ferro
RCUI	- Retocolite ulcerativa
RDW	- Red Cell Distribution Width
SPSS	- Statistical Package for the Social Sciences
UFJF	- Universidade Federal de Juiz de Fora
VGM	- Volume globular médio
VHS	- Velocidade de hemossedimentação
WHO	- World Health Organization

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## 1 INTRODUÇÃO

### 1.1 Etiopatogênese das Doenças Inflamatórias Intestinais (DII)

Embora já se reconheça que o processo inflamatório das DII seja decorrente de uma conjugação de fatores como os relacionados ao conteúdo do canal alimentar (antígenos bacterianos e/ou alimentares e aumento na permeabilidade intestinal a esses produtos) e da alteração na resposta imunológica da mucosa, determinada por alguns genes, ainda não há uma definição do verdadeiro agente causal das DII.

Sugere-se que uma alteração genética modifique a barreira epitelial intestinal, influenciando nas respostas imunológicas (inata e adaptativa) e na composição da microbiota intestinal, mas ainda não se conseguiu determinar uma causa específica.

### 1.2 Considerações gerais

As doenças inflamatórias intestinais (DII) constituem afecções multisistêmicas de origem idiopática que apresentam períodos de recorrências e remissões durante todo seu curso clínico (Lichtenstein, 2004). Enquanto a colite ulcerativa caracteriza-se por inflamação restrita à mucosa colônica, na Doença de Crohn (DC) observa-se inflamação transmural que pode afetar qualquer parte do trato gastrointestinal (Shanahan, 2001). Em muitos pacientes, estas afecções acarretam substancial custo pessoal, decorrente dos sintomas flutuantes imprevisíveis, do absenteísmo ao trabalho, da necessidade, muitas vezes, do uso de drogas de elevado custo, de cirurgias ou de cuidados multidisciplinares. Além disso, estas condições acarretam significativo impacto nos recursos públicos de cuidados de saúde (Friedman, 2004). Em vista do início frequente da doença em jovens, da morbidade, e dos possíveis efeitos adversos do tratamento medicamentoso, as doenças inflamatórias intestinais podem causar significativo impacto negativo na qualidade de vida destes pacientes. No Brasil, os primeiros estudos publicados sobre o comportamento das doenças inflamatórias intestinais, ocorreram em 1998, onde se demonstrou o crescimento da incidência da Doença de Crohn na segunda metade do século XX (Gaburri; Chebli, 1998). Posteriormente, Souza et al confirmaram estar havendo um aumento na

ocorrência das doenças inflamatórias intestinais em nosso meio, particularmente da Doença de Crohn.

### 1.2.1 Fatores envolvidos na patogênese das DII:

#### Fator ambiental

Nota-se que à medida que houve melhora nas condições de saúde, com menos infecções e doenças parasitárias, melhores condições sanitárias, acesso a vacinas e adoção de hábitos alimentares nas sociedades ocidentais, os pacientes foram menos expostos a infecções na infância, tornando o sistema imunológico menos preparado para lidar com as exposições antigênicas com o passar da vida. Talvez, explicando de modo parcial, o aumento das doenças inflamatórias intestinais

Loftus realizou uma extensa revisão em 2004, tentando correlacionar fatores ambientais, tais como dietéticos, fumo, contágio com vírus do sarampo, apendicectomia e uso de anticoncepcional oral, não obtendo qualquer sucesso, a não ser por uma correlação negativa com o tabagismo. (Loftus, 2004)

#### Fatores genéticos

Estudos genéticos identificaram que um diversificado leque de genes e mecanismos fisiopatológicos, incluindo o reconhecimento de microrganismos, a ativação de linfócitos, a sinalização de citocinas, e de defesa do epitélio intestinal estão ligados às DII, afetando a atividade da interleucina-23 e transcrição de fatores incluindo os fatores de transcrição relacionados ao NK2, locus 3 (NKX2-3), SMAD3, STAT3, ZMIZ1, e c-REL. Apesar da DC e da RCUI serem associadas com regiões genômicas que implicam produtos de genes envolvidos com leucócitos, há evidências de padrões de associação que são distintos entre DC e RCUI. Associações predominantes de DC incluem NOD2 e genes que regulam a autofagia. Já para a RCUI, o sinal de associação é predominante no cromossoma 6p21, na região do complexo principal de histocompatibilidade, perto de genes HLA de classe II. (CHO 2011)

Os genes que codificam os fatores que atuam na via da interleucina-23 têm sido associados com algumas doenças inflamatórias crônicas, em especial a psoríase e a espondilite anquilosante.

Também outras alterações mostram ligação entre DII e Doença Celíaca, indicando sobreposição significativa na fisiopatologia destas doenças.

O aumento da ocorrência de DII entre membros de uma mesma família, principalmente entre gêmeos monozigóticos fala a favor da importância genética para as DII, entretanto a não ocorrência em todos os gêmeos monozigóticos nos levam a acreditar que exista um gatilho não genético para o desenvolvimento de tais afecções (CHO, 2007).

#### Fatores microbianos e disfunção da mucosa

Atualmente se aceita que as DII estão associadas à perda de tolerância à microbiota intestinal normal, mas faltam estudos mais profundos sobre a composição do seu real conteúdo.

Também se sabe que nos doentes com DII ocorre uma facilitação à entrada de antígenos intra-luminais formados por bactérias ou produtos bacterianos através da mucosa intestinal, ou seja, um aumento da permeabilidade intestinal. (SARTOR, 2006).

#### 1.2.2 Imunidade Inata

Quando ocorre uma invasão bacteriana em pacientes portadores de DII, nota-se uma fraca resposta à invasão bacteriana, resultando em um menor estímulo à produção de citocinas pró-inflamatórias e a um clareamento bacteriano inadequado. Sabe-se que as células dendríticas intestinais ativadas nas DII produzem níveis elevados de citocinas IL-12 e IL-6. (Fiochi, 1998; Abraham, 2009; Sartor, 2006 e Podolsky, 2002).

### 1.2.3 Imunidade Adaptativa ou Específica

Quando a resposta inata não é capaz de eliminar determinados produtos bacterianos que entraram na mucosa, eles estimulam a imunidade adquirida, que é uma resposta tardia, dependente de memória imunológica.

A resposta tardia é derivada das células T (imunidade celular) e das células B (imunidade humoral).

A produção de anticorpos mediada pelas células B nas DII é aumentada tanto na mucosa quanto na corrente sanguínea (IgG, IgM e IgA).

Na DC, IL-12 e IL-23 estimulam a diferenciação das células T em células Th-1 e Th-17, com produção de INF-gama, IL-17 e IL-21.

A RCUI é uma condição associada a uma resposta Th-2 atípica, onde IL-12 e IL-23 estimulam a diferenciação das células T em Th-2, Th-17 e NK, com produção aumentada de IL-4, IL-5, IL-13 e IL-17.

Além das células T-helper, outro grupo celular é formado. As células T regulatórias (Treg) têm função de monitorar a resposta imunológica e prevenir sua ativação excessiva. Aparentemente, o número e a função das células Treg estão alterados nas DII.

O conhecimento da etiopatogenia das DII é muito importante. Infelizmente, ainda não é possível manipular os fatores genéticos e ambientais que estão associados. . (Fiochi, 1998; Abraham, 2009; Sartor, 2006 e Podolsky, 2002).

### 1.2.4 Anatomopatologia das DII

Na RCUI a lesão inicia no reto e se propaga, acometendo todo o cólon, mas normalmente não ultrapassa a válvula íleo-cecal. Esta lesão atinge somente a mucosa, não atingindo toda a parede do cólon, a não ser no caso do megacólon tóxico.

A lesão ativa demonstra congestão e edema de mucosa, focos de necrose do epitélio, abscessos crípticos e úlceras. O infiltrado de neutrófilos e linfoplasmocitário são proeminentes. Com resposta terapêutica favorável, a inflamação regride, as



úlceras desaparecem, mas ocorre hiperplasia das criptas levando a formação de pseudopólipos.

Quando ocorre remissão clínica, essas criptas podem sofrer atrofia com adelgaçamento da mucosa, encurtamento e distorção das criptas ( Barbieri, 2000);

A DC acomete todas as paredes do trato digestivo de maneira descontínua, podendo estender-se até mesentério e linfonodos .

Na DC, ocorre inflamação da mucosa com obstrução de linfáticos e edema que, quando localizados no intestino delgado, podem levar à má absorção de diversos nutrientes, como por exemplo ferro, dissacarídeos, proteína, folato e gorduras,. Quando o comprometimento ocorre mais a nível de íleo ocorrerá má absorção de sais biliares e vitamina B12. Além disto o organismo também perde proteína, eletrólitos, água e sangue pelas lesões ulceradas. No caso de doença estenosante ainda ocorre má absorção devido aos mecanismos luminares de supercrescimento bacteriano (Barbieri, 2000).

### 1.3 Anemia nas Doenças Inflamatórias Intestinais

#### 1.3.1 Importância e Prevalência das anemias nas Doenças Inflamatórias Intestinais

A anemia é um quadro clínico frequentemente observado nas doenças inflamatórias intestinais, causando grande prejuízo na qualidade de vida destes pacientes. Por ser uma complicação frequente que leva à hospitalização, torna-se de grande importância o seu estudo (Andrews,2004).

Em 1994, Gasche estudou 49 pacientes portadores de Doença de Crohn e encontrou, considerando anemia para os pacientes com índice de hemoglobina abaixo de 12 g/dl, uma prevalência de 32,6%. Em 1996, Schreiber estudou 342 pacientes com Retocolite Ulcerativa e, considerando como portadores de anemia aqueles pacientes que apresentassem hemoglobina abaixo de 10g/dl encontrou uma prevalência de anemia igual a 36,8%.

De acordo com uma revisão sobre a prevalência de anemia em pacientes com doença inflamatória intestinal, as taxas encontradas variaram, conforme a sub-população estudada, de 10,2% a 72,7% para pacientes com Doença de Crohn; de

8,8% a 66,6% para pacientes com Retocolite Ulcerativa e, em pacientes com doença inflamatória intestinal indeterminada, as taxas variaram de 17,5% a 73,5%. Gasche,(2000).

Outros estudos europeus relataram prevalência entre 17% a 20% (Bager, 2011 e Gisbert, et al., 2009), que difere da maior prevalência de anemia encontrada nos estudos de Bergamisch que foi em torno de 65 % e de Hoivik que encontrou prevalência de 48,8 % para Doença de Crohn e 20,2 % para os portadores de Retocolite Ulcerativa. (Bergamish et al, 2010 e Hoivik e al.2014).

### 1.3.2 Patogênese e Mecanismos das anemias nas Doenças Inflamatórias Intestinais

Dois tipos predominantes de anemia têm sido identificados nos pacientes portadores de doença inflamatória intestinal, a anemia por deficiência de ferro e a anemia da doença crônica. A patogênese da anemia na Doença de Crohn é multifatorial, desempenhando papel significativo: a inflamação crônica intestinal, as perdas sanguíneas evidentes ou ocultas pelo trato digestivo, deficiência de ácido fólico, hemólise, supressão da medula óssea por medicações, desnutrição, além da anorexia frequentemente observada. Estima-se que um terço dos pacientes portadores de doença inflamatória intestinal apresente hemoglobina inferior a 12 g/dl (Lichtenstein, 2004). Os dois padrões de anemia mais encontrados nas doenças inflamatórias intestinais são a anemia por deficiência de ferro e anemia da doença crônica (ADC), esta última também reconhecida como a maior causa de anemia em ambiente hospitalar (Gasche, 2004).

Do ponto de vista fisiopatológico, as anemias podem ser classificadas em hiporregenerativas, caracterizadas pela contagem absoluta de reticulócitos abaixo de  $50000/\text{mm}^3$ , sempre pela produção ineficiente de hemácias por carência de elementos (vit B12, ácido fólico e ferro), por falta de estimuladores (eritropoetina), por redução do tecido hematopoético normal, invasão da medula óssea (leucemias, linfomas, mielomas, metástases e fibrose) e anemia de doenças crônicas. As anemias regenerativas são tipicamente as anemias hemolíticas, mas podem ocorrer também no sangramento agudo, sendo caracterizada por contagem absoluta de reticulócitos acima de  $100000/\text{mm}^3$ .

O ferro é um micronutriente essencial, pois controla várias reações metabólicas. A quantidade insuficiente deste mineral leva à anemia, enquanto seu excesso leva ao aumento do estresse oxidativo nos tecidos corporais, gerando inflamações, morte celular e disfunções de sistemas orgânicos, podendo inclusive ser fator de risco para doenças tumorais (Oates, 2007).

Em média, um adulto armazena em seu corpo 2 a 4 g de ferro. A maioria deste está nas hemoglobinas dos eritrócitos sanguíneos, e uma deficiência sistêmica prolongada, resulta em diminuição na produção de hemoglobina e anemia. A manutenção do ferro para os precursores dos eritrócitos na matriz óssea e em outros tecidos é amplamente mantida, pela reciclagem de cerca de 20 mg de ferro a partir de eritrócitos senescentes. Apenas 1 a 2 mg diários de ferro precisam ser absorvidos da dieta, para repor as perdas ordinárias de ferro. A absorção do ferro ocorre no duodeno proximal e em seres humanos, ambos: ferro elementar e ferro heme são absorvidos pelos enterócitos duodenais (Nemeth; Ganz, 2006). Em circunstâncias normais a concentração de ferro no plasma e no tecido extracelular permanece relativamente estável, apesar da flutuação na oferta e demanda. Isto é atingido por uma delicada regulação no transporte celular, que envolve: absorção, estoque e reciclagem, mas a via desta regulação começou apenas recentemente a ser elucidada (Oates, 2007).

Na anemia por deficiência de ferro, a hipoferremia ocorre devido à inflamação e ulceração, as quais são os efeitos fisiológicos proeminentes nas doenças inflamatórias intestinais e podem resultar em franco sangramento intestinal. Além disto, a dor abdominal e a náusea, frequentemente, resultam em ingestão pobre e, a inflamação da mucosa do trato gastrointestinal pode levar a uma absorção inadequada de nutrientes. Contudo, o que realmente parece causar a anemia é a perda de ferro, que pode exceder a capacidade de absorção.

Em 1932, Locke observou que os quadros infecciosos associavam-se a hipoferremia, explicando parcialmente o achado de anemia em pacientes com infecções crônicas. Cartwright e Wintrobe demonstraram que a anemia associada à infecção era indistinguível da anemia da inflamação e estabeleceram que a hipoferremia resultava do sequestro de ferro reticuloendotelial e da interrupção da absorção intestinal de ferro. (Means, 2000).

Nos últimos anos, vários experimentos têm estabelecido que a hepcidina, um peptídeo liberado pelo fígado e regulador da homeostase do ferro seja a chave da hipoferrêmia na inflamação.

A Anemia da Doença Crônica tem como substrato fisiopatogênico, a inabilidade de aproveitar o ferro absorvido, havendo pouca utilização por parte do sistema hematopoiético, em detrimento do acúmulo no sistema reticuloendotelial. Na anemia da doença crônica foi recentemente descoberto que a hipoferrêmia resulta do sequestro de ferro reticuloendotelial e da interrupção da absorção intestinal de ferro, devido a um complexo processo envolvendo interleucinas inflamatórias (Gisbert, 2008).

Demonstrou-se que citocinas, tal como a interleucina-6 (IL-6) e mediadores da cinética do ferro como a hepcidina estão elevados em processos inflamatórios e em algumas anemias (Nemeth; Gabayan, 2004). A IL-6 é o principal estímulo para a liberação hepática de hepcidina. A hepcidina é uma proteína de 25 aminoácidos, rica em cisteína e que apresenta em sua cadeia molecular pontes de dissulfeto. Foi descoberta no ano de 2000, por Krause e colaboradores, sendo tida como um novo antimicrobiano endógeno. Nos anos seguintes, se demonstrou sua propriedade no controle da absorção duodenal de ferro, agindo como um mediador inibitório sobre a capacidade absorptiva do mesmo, além de levar a um acúmulo de ferro no sistema retículo endotelial em detrimento de sua utilização pelo sistema hematopoiético (Fleming, 2001). Estudos experimentais em cobaias revelaram um feed-back negativo entre a liberação de hepcidina e absorção duodenal de ferro (Raja; Duane, 1990). Outro achado interessante foi que eventos como a hipóxia, a inflamação mediada principalmente pela IL-6 e a anemia interferem na modulação endógena da hepcidina. Desta forma, a hipóxia e a anemia levam a uma diminuição, enquanto a inflamação potencializa a liberação de hepcidina (Nicolas; Viatte et al. 2002). A hepcidina hoje já é reconhecida como o principal hormônio regulador de ferro (Rossi, 2005).

Alguns autores especulam sobre uma possível interação entre a eritropoetina e a hepcidina. É admissível que um dos mecanismos que a eritropoetina atue no aumento da eritropoiese seja a partir de uma inibição da hepcidina, mas estudos ainda precisam ser conduzidos para provar tal hipótese (Deicher, Walter, 2004). Uma vez que a anemia observada nas doenças inflamatórias intestinais depende de

um conjunto complexo de interações celulares e fatores etiológicos, o tratamento da mesma pode ser desafiador e difícil (Wilson, Ofman, 2004).

### 1.3.3 Regulação celular e extracelular da concentração de ferro

O fluxo e a concentração de ferro são regulados em dois níveis: celular e sistêmico. A nível celular, a concentração de ferro é registrada por duas proteínas reguladoras PRF1 e 2 (proteína reguladora de ferro). Quando o ferro do citoplasma está baixo, as PRFs se ligam à sequência de elemento regulador (ERF – elemento responsivo ao ferro) no mRNAs das proteínas reguladoras de ferro. Dependendo da posição do ERF (5' ou 3' regiões não decodificadas), a ligação de PRF tem efeitos opostos na síntese de proteínas reguladoras do ferro: a ligação de PRFs ao 3'ERFs estabiliza o mRNA, resultando no aumento da síntese de proteínas, enquanto a ligação ao 5'ERFs previne a translação de mRNA, resultando num decréscimo da síntese proteica. Então, enquanto a concentração extracelular de ferro está em taxas normais, a homeostase de ferro celular é mantida pelo sistema PRF-ERF modificando a produção de proteínas envolvidas na obtenção, estoque e liberação de ferro, de acordo com as concentrações de ferro citoplasmático. (Nemeth, Ganz, 2004) A regulação sistêmica de ferro mantém uma concentração estável de ferro-transferrina no plasma e no fluido extracelular. Isto é ativado pela regulação do fluxo de ferro para o plasma: liberação de ferro da reciclagem de células vermelhas senescentes pelos macrófagos, reaproveitamento dos estoques de ferro dos hepatócitos, absorção do ferro da dieta pelos enterócitos duodenais e, durante o desenvolvimento fetal, a transferência de ferro da mãe para o feto através da placenta. Recentes estudos indicam que o ferro é liberado destes tecidos para o plasma através da membrana exportadora de ferro, ferroportina, a qual é posteriormente regulada pela hepcidina. A hepcidina causa a degradação da ferroportina, bloqueando assim o fluxo de ferro para o plasma. Os níveis plasmáticos de hepcidina são, por sua vez, regulados pelo ferro e anemia-hipóxia, assim completando a alça homeostática controlando a concentração sistêmica de ferro (Nemeth, Ganz, 2006).

A desregulação da hepcidina ou de seu receptor ferroportina, resulta num espectro de desordens do ferro. Por um lado, nas desordens inflamatórias e infecciosas, o excesso de hepcidina citocinas-induzidas contribuem para o desenvolvimento da anemia da inflamação, caracterizado por hipoferremia e anemia, a despeito de adequados estoques de ferro. Por outro lado, uma baixa produção de hepcidina, ocorrida por mutação no gene da hepcidina ou de seu regulador, parece ser a causa da maioria dos tipos de hemocromatoses hereditárias - caracterizada por uma absorção excessiva do ferro da dieta e deposição de ferro nos órgãos vitais. Os outros tipos de desordens do excesso de ferro hereditárias são causadas pela mutação na ferroportina que também desenvolve uma proteína não funcional (não exportando ferro, resultando na doença da ferroportina) ou não responsiva à hepcidina (exportando excessivamente ferro, resultando em hemocromatose mais típica)

A EPO (eritropoietina) é produzida pelo rim em condições de hipóxia ou deficiência de ferro, atuando, através da interação com o seu receptor (EPOR), como o principal agente anti-apoptótico nos eritroblastos. A injeção de EPO é um potente inibidor da síntese de hepcidina *in vivo*. O mecanismo, ou mecanismos, pelo qual a EPO atua como inibidor da hepcidina é ainda um assunto em investigação. Além do efeito indireto no aumento da eritropoiese, a EPO tem também um efeito direto de inibição da síntese de hepcidina pelos hepatócitos, através da regulação do fator de transcrição C/EBP $\alpha$ , mediada pelo EPOR. (Porto, 2012)

#### 1.3.4 Parâmetros do metabolismo do ferro

O diagnóstico de deficiência de ferro é tradicionalmente baseado em uma combinação de parâmetros, incluindo hematologia, e índices de metabolismo do ferro. A deficiência isolada de ferro pode ser caracterizada por baixos índices de ferro, ferritina e saturação da transferrina, mas um aumento na concentração de transferrina.

Contudo o diagnóstico de deficiência de ferro no contexto da doença inflamatória intestinal pode ser difícil, principalmente, quando o quadro de deficiência de ferro está associado à anemia da doença crônica. Nestas circunstâncias, muitas

das mensurações destes parâmetros podem ser irreais devido à inflamação (Nemeth; Ganz, 2006).

Na presença de inflamação crônica, a típica elevação dos níveis de transferrina da deficiência de ferro pode não ser encontrada, uma vez que pacientes com hipoalbuminemia tendem a apresentar também baixos níveis de transferrina. Da mesma maneira, os níveis de ferro sérico e capacidade de ligação do ferro são difíceis de interpretar na presença de inflamação (Weiss, 2005)

Finalmente, a ferritina sérica, o mais acessível parâmetro de estoque de ferro e o mais poderoso teste para deficiência de ferro pode ser normal ou às vezes elevado (em resposta à inflamação, uma vez que é um reagente de fase aguda) na presença de deficiência de ferro. Portanto, ao menos por enquanto, a ferritina geralmente, considerada o mais eficiente indicador de deficiência de ferro, pode não dar informações adequadas sobre estoque de ferro durante condições inflamatórias como a doença inflamatória intestinal. O aumento da concentração do receptor da transferrina distinguiria com segurança a anemia por doença crônica da anemia por deficiência de ferro, mas ainda não se tem este parâmetro disponível (Weiss, 2005).

Portanto, foi sugerido que o critério diagnóstico para deficiência de ferro deve ser adaptado ao nível de inflamação. Então, paciente sem bioquímica (PCR, etc.) ou clínica (diarréia e achados endoscópicos, etc) que evidenciem inflamação, o ponto de corte para definir um nível baixo de ferritina seria  $< 30 \mu\text{g/l}$ ; contudo, na presença de inflamação, o limite inferior deste parâmetro para ser considerado normal deve ser superior a  $100 \mu\text{g/l}$ . (Gasche, 2000).

## 2 OBJETIVOS

### GERAL

Avaliar a presença de anemia nos pacientes portadores de DII

### ESPECÍFICOS

- a) Avaliar a prevalência de anemia nos pacientes com DII
- b) Avaliar os fatores de risco para anemia nos pacientes portadores de DII
- c) Identificar as possíveis etiologias da anemia neste grupo de pacientes



### 3 JUSTIFICATIVA

É importante determinar a natureza e a gravidade da anemia nos pacientes portadores de doença inflamatória intestinal, para que a terapia possa ser definida da forma correta e ajustada de acordo com a necessidade do paciente. Além disto, devido ao fato da doença inflamatória intestinal ter impacto significativo no bem-estar físico e emocional dos pacientes, e por saber-se que a correção da anemia parece ter um impacto benéfico na qualidade de vida dos pacientes, tão importante quanto o controle da diarreia (Gisbert et al. 2009; Leifert, 2008 e Guagnozzi, 2014), estudar e tratar a anemia nos pacientes com doença inflamatória intestinal torna-se de fundamental importância.

A avaliação da prevalência, dos possíveis fatores de risco e das diversas etiologias da anemia nos pacientes portadores de doença inflamatória intestinal, poderá permitir que se consiga melhores resultados na busca da atenuação da morbi-mortalidade relacionada às doenças inflamatórias intestinais, bem como o oferecimento de melhor qualidade de vida para estes enfermos. Uma vez que sabe-se que o excesso de ferro reativa a inflamação e além disto que a capacidade de absorção de ferro é muito pequena, não se justificaria uma administração empírica deste mineral.

## 4 MATERIAL E MÉTODO

### 4.1 População de pacientes

Pacientes adultos com doença inflamatória intestinal, diagnosticados e acompanhados regularmente no Centro de Doenças Inflamatórias Intestinais do Hospital Universitário, da Universidade Federal de Juiz de Fora (UFJF). O diagnóstico de Retocolite Ulcerativa, assim como de Doença de Crohn foi baseado em critérios rotineiros clínicos, radiológicos, endoscópicos e histopatológicos.

Os critérios de inclusão foram: idade  $\geq 18$  anos e  $< 65$  anos e diagnóstico confirmado de doença inflamatória intestinal (Doença de Crohn ou Retocolite ulcerativa). Os pacientes foram excluídos quando quaisquer das seguintes condições estiveram presentes no “screening” inicial: hepatopatia pré-existente, insuficiência renal, doença pulmonar clinicamente significativa, infecção sistêmica, gravidez, história atual de qualquer tipo de malignidade (exceto cutânea), gastrectomia, tratamento de reposição com ferro, ácido fólico ou vitamina B12 nos últimos seis meses.

Foram considerados critérios clínicos sugestivos de inflamação no caso de pacientes portadores de Retocolite Ulcerativa: aumento da frequência de evacuações, diminuição da consistência das fezes, dor abdominal, tenesmo, urgência evacuatória, presença de muco ou sangue nas fezes, dor articular e febre. Foram considerados critérios laboratoriais para inflamação o aumento de PCR, VHS e leucocitose.

No caso dos pacientes portadores de Doença de Crohn foi considerado o IADC (Índice de Atividade da Doença de Crohn) como método de avaliação da inflamação conforme tabela 1 em anexo.

A doença foi considerada em remissão quando o IADC estava inferior a 150; leve a moderada quando o IADC entre 150 e 219; moderada a grave quando IADC entre 220 e 450, e grave ou fulminante quando se valores de IADC superiores a 450.

## 4.2 Desenho do Estudo e Acompanhamento Clínico-Laboratorial

Foi realizado um estudo de coorte transversal de pacientes ambulatoriais adultos com DII de um Centro de Doenças Inflamatórias Intestinais do Hospital Universitário da Universidade Federal de Juiz de Fora, Brasil.

Foram selecionados aleatoriamente 200 pacientes portadores de doença inflamatória intestinal, sendo 100 pacientes portadores de Retocolite Ulcerativa e 100 pacientes portadores de Doença de Crohn no período entre novembro de 2012 e janeiro de 2013.

Todo paciente foi informado de que para participar deste estudo, não teria nenhum gasto, nem receberia qualquer vantagem financeira. Os pacientes foram esclarecidos sobre o estudo em qualquer aspecto desejado e esteve livre para participar ou recusar-se a participar sabendo que esta recusa não acarretaria qualquer penalidade ou modificação na forma em que ele seria atendido. O paciente pôde retirar seu consentimento ou interromper sua participação a qualquer momento.

Os exames laboratoriais foram realizados no próprio Hospital Universitário, conforme já é rotina no acompanhamento dos pacientes portadores de doença inflamatória intestinal, não havendo risco para saúde diferente do que haveria num exame de sangue comum. Estes exames foram realizados com recursos próprios financiados pela FAPEMIG, obtidos pelo orientador.

O protocolo do estudo foi submetido para avaliação pelo Comitê de Ética em Pesquisa do HU/CAS e um termo de consentimento livre e esclarecido foi assinado por todos os pacientes que foram incluídos no estudo. Na visita inicial, todos os pacientes foram avaliados quanto aos critérios de elegibilidade e registrados seus dados demográficos (idade, sexo), peso corporal, história clínica (tabagismo; tipo, duração e extensão da doença inflamatória intestinal) e medicações de uso habitual, fenótipo da doença em caso de Doença de Crohn (fistulizante, fibroestenosante ou não-estenosante e não fistulizante), avaliada a realização de cirurgia prévia, de ressecção íleo-cecal, ressecção >100 cm de intestino delgado e ressecção colônica.

Na inclusão no estudo, foram obtidas amostras do sangue (20 ml) para avaliação hematológica, bioquímica e imunológica. Os seguintes exames

hematológicos e bioquímicos foram realizados em todos os pacientes: hemograma completo, VGM, HGM, CHGM, plaquetas, ácido fólico, vitamina B12, reticulócitos, índice de saturação da transferrina, ferritina, ferro sérico, PCR e VHS.

A coleta foi feita por um residente de bioquímica do Hospital Universitário de Juiz de Fora, assim como a realização dos exames, que ficou sob a supervisão dos professores da Residência de Bioquímica do Hospital Universitário.

Foram adotados como níveis anormais de hemoglobina, e, portanto, como anemia presente, quando os valores da hemoglobina estiveram inferiores a 13 g/dl no sexo masculino e 12g/dl no sexo feminino, similar aos valores recomendados para pacientes sem doença inflamatória intestinal, pela World Health Organization (WHO). Anemia foi considerada como grave quando os níveis da hemoglobina estivessem abaixo de 10g/dl em ambos os sexos (Gasche, 2007).

Anemia por deficiência de ferro foi definida quando houve diminuição dos níveis séricos de ferro ( $< 37 \mu\text{g/dl}$  para mulheres e  $< 59 \mu\text{g/dl}$  para homens), da ferritina ( $< 30 \mu\text{g/l}$  - na ausência de dados clínicos, laboratoriais ou endoscópicos de inflamação intestinal e  $< 100 \mu\text{g/l}$  - na presença de quaisquer destes dados), do índice de saturação da transferrina ( $< 16\%$ ) ( Gasche, 2007)

Anemia da Doença Crônica foi definida quando houve diminuição dos níveis séricos de ferro ( $< 37 \mu\text{g/dl}$  para mulheres e  $< 59 \mu\text{g/dl}$  para homens), aumento da ferritina ( $> 100 \mu\text{g/l}$ ) e diminuição do índice de saturação da transferrina ( $< 16\%$ ). - na presença de dados clínicos, laboratoriais ou endoscópicos de inflamação intestinal. (Gasche, 2007).

Anemia Mista foi definida quando houve diminuição dos níveis séricos de ferro ( $< 37 \mu\text{g/dl}$  para mulheres e  $< 59 \mu\text{g/dl}$  para homens) e ferritina entre 30 e  $100 \mu\text{g/l}$ . A Anemia por Deficiência de Vitamina B12 foi definida quando encontrado níveis séricos abaixo do normal desta vitamina ( $\text{Vit. B12} \leq 145 \text{pg/ml}$ ) nos pacientes portadores de anemia.

A Anemia por Deficiência de Ácido Fólico foi definida quando encontrado níveis séricos abaixo do normal desta vitamina (ácido fólico  $< 3 \text{ng/ml}$ ) nos pacientes portadores de anemia. As anemias foram classificadas em hiporregenerativas quando a contagem absoluta de reticulócitos estava abaixo de 50000 e

normoproliferativas ou normorregenerativas quando a contagem absoluta de reticulócitos estava acima de 100000.

Nos pacientes portadores de anemia, fizemos a correlação entre a presença de anemia e os fatores de risco como: fatores sócio-demográficos, peso corporal, história clínica (tabagismo; tipo, duração e extensão da doença inflamatória intestinal), se a doença encontrava-se em remissão ou em atividade, medicações de uso habitual, fenótipo da doença (em caso de Doença de Crohn), foi avaliada a realização de cirurgia prévia, de ressecção íleo-cecal, ressecção >100 cm de intestino delgado e ressecção colônica parcial.

Foram considerados os seguintes valores de referência:

Exame	Valor de referência
Eritrócitos	4,0 – 6,0 milhões/mm <sup>3</sup>
Hemoglobina	12-17 g/dl
Hematócrito	37-54 %
HGM	27-36 pg
VGM	80-98 fL
CHGM	30-36 %
RDW	11-15 %
Leucócitos	3600-11000 / mm <sup>3</sup>
Segmentados	45-66 %    2900 – 6600/ mm <sup>3</sup>
Linfócitos	20-35 %    1000 - 3500/ mm <sup>3</sup>
Eosinófilos	0-7 %        0 - 500/mm <sup>3</sup>
Bastões	0-5 %        0 - 500/mm <sup>3</sup>
Mielócitos	0 %            0/mm <sup>3</sup>
Pró-mielócitos	0 %            0/mm <sup>3</sup>
Basófilos	0-3 %        0 - 200/ mm <sup>3</sup>
Monócitos	2-10 %    100 -1000/mm <sup>3</sup>
Blastos	0 %            0/mm <sup>3</sup>
Plaquetas	140.000 – 500.000/ mm <sup>3</sup>
Ácido fólico	> 3 ng/ml    ou > 6,8 nmol/L
Vitamina B12	>145 pg/ml    ou > 107 pmol/L
Reticulócitos	0,5 – 2% do n. de eritrócitos
Índice de saturação da transferrina	20 – 50 %
Ferro sérico - homem	65-170 µg/dl
Ferro sérico – mulher	50-170 µg/dl
PCR	< 6mg/L
VHS – primeira hora	0-10 mm
VHS – segunda hora	0-20 mm

Fonte: WHO – World Health Organization

### 4.3 Análise estatística

A análise estatística foi realizada usando SPSS 16.0 (SPSS, Chicago, IL, EUA).

As variáveis contínuas são apresentadas como medianas e intervalos, e as variáveis categóricas são expressas em número e porcentagem de pacientes. A estatística descritiva de todas as variáveis relevantes para os grupos foram calculados.

Para análise dos dados, os pacientes foram divididos em dois grupos (com e sem anemia), de acordo com os seus níveis de hemoglobina. As comparações entre grupos, bem como possíveis relações entre a presença de anemia com variáveis sócio-demográficas e demais dados relatados foram analisados por meio de teste paramétricos t de Student, teste do qui-quadrado, ou não paramétricos o teste U de Mann - Whitney , quando apropriado . Análise logística univariada e multivariada foi realizada para identificar preditores independentes para a ocorrência de anemia em todo o grupo DII.

Os resultados são apresentados como chances de risco (OR) e intervalo de confiança de 95% (IC). Para efeitos de comparação, o nível de significância estatística foi estabelecido em  $P < 0,05$ .

## 5 RESULTADOS E DISCUSSÃO

Os resultados e a discussão são apresentados no formato do artigo científico a seguir. Este artigo foi submetido a revista indexada conforme norma do programa e o comprovante de submissão consta nos anexos.

**Title: Anemia in Brazilian inflammatory bowel disease outpatients: Prevalence, risk factors and etiology**

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**List of abbreviations**

ACD, anemia of chronic disease  
CD, Crohn's disease  
CDAI, Crohn's disease activity index  
CI, confidence intervals  
CRP, C-reactive protein  
Hb, hemoglobin  
IBD, inflammatory bowel disease  
IDA, iron deficiency anemia  
OR, odds risk  
TfS, transferrin saturation  
UC, ulcerative colitis



## ABSTRACT

**Goals:** To assess the prevalence, risk factors and etiology of anemia in Brazilian outpatients with inflammatory bowel disease (IBD).

**Background:** Anemia is common in IBD. However, epidemiological studies of non-western IBD populations are limited and may be confounded by demographic, socioeconomic and disease-related influences.

**Study:** In this cross-sectional study, 100 Crohn's disease (CD) patients and 100 ulcerative colitis (UC) subjects were assessed. Anemia workup included: complete blood count, ferritin, transferrin saturation, serum levels of folic acid and vitamin B12, and C-reactive protein (CRP) concentration.

**Results:** The overall prevalence of anemia on IBD was 21%. There was no significant difference in the prevalence of anemia between CD subjects (24%) and UC (18%). There was no correlation between the occurrence of anemia with demographics, IBD location or duration, behavior of CD and previous surgery. Moderate disease activity (OR: 3.48, 95% CI 1.95-9.64,  $P = .002$ ) as well as elevated CRP levels (OR: 1.8, 95% CI, 1.04-3.11,  $P = .02$ ) were independently associated with anemia. The most common etiologies of anemia found in both groups were iron deficiency anemia (IDA; 10% on CD and 6% on UC) followed by the anemia of chronic disease (ACD; 6% for both groups).

**Conclusions:** In Brazilian IBD outpatients, anemia is highly concurrent condition. Disease moderate activity as well as increased CRP were strongly associated with comorbid anemia. IDA and/or ACD were the most common etiologies. Periodic screening and appropriate management for anemia should be carried out routinely as part of quality of care improvement for IBD individuals.

**Key Words:** inflammatory bowel disease, Crohn's disease, ulcerative colitis, anemia, iron deficiency

## INTRODUCTION

Inflammatory bowel diseases (IBD) are idiopathic multisystemic disorders that present with periods of relapses and remissions throughout their clinical course.<sup>1</sup> While ulcerative colitis (UC) is characterized by inflammation limited to the colonic mucosa, in Crohn's disease (CD) is observed transmural inflammation that can affect any part of the gastrointestinal tract.<sup>2</sup> In many patients, these conditions cause substantial personal cost, due to the unpredictable fluctuating symptoms, absenteeism at work, use of high-cost drugs, surgeries or multidisciplinary care.

The severity of the intestinal manifestations (i.e. diarrhea, abdominal pain and bleeding) is usually an important guide for management of IBD. Occasionally, however, one or more extraintestinal manifestations can be the predominant clinical feature on patients with IBD. Notably, anemia is one of the most common extraintestinal manifestation (or complication) in IBD.<sup>3-5</sup> In this clinical setting, anemia can contribute to patients' poor quality of life, particularly because of its negative impact on the feeling of wellbeing, physical performance, mood, cognitive function, and capacity to perform social activities.<sup>6,7</sup> In addition, comorbid anemia in IBD individuals is a significant predictor of increased risk of hospitalization and even increased patient mortality.<sup>8-10</sup> Of note, anemia may occasionally both antecede the development of intestinal symptoms and be the key signal unmask IBD.<sup>3,6</sup>

The prevalence of anemia in IBD varies widely, with studies reporting a frequency between 8.8% and 74% depending on the assessed patient subpopulation.<sup>11,12</sup> Etiology of anemia in IBD is multifactorial. Nonetheless, iron deficiency and characteristics of chronic disease are the most prevalent in this clinical setting.<sup>11-13</sup> Periodic screenings for anemia as well as a systematic diagnostic approach are essential steps to the appropriate management of this condition on IBD. However, anemia is both underdiagnosed and undertreated in populations with IBD.<sup>14</sup> Furthermore, different geographic, demographic, socioeconomic, and disease-related characteristics may exist between South American and North American or European populations that may limit the generalization of the findings regarding the epidemiology of anemia on IBD individuals, as the data are mainly derived from North American or European studies.

Therefore the purpose of the present cross-sectional cohort study was to assess the prevalence, risk factors and etiology of anemia in a Brazilian population

with IBD. We hypothesized that anemia would be a concurrent condition in this patient population and correlated with inflammatory activity.

## **MATERIALS AND METHODS**

### **Study design**

We conducted a cross-sectional cohort study of adult outpatients with IBD from an IBD Center at the University Hospital of the Federal University of Juiz de Fora, Brazil, that were enrolled consecutively into this study for evaluation of the anemia between November 2012 and January 2013.

### **Participants**

Our goal was to recruit 200 IBD patients (100 with CD and 100 with UC), nearly 20% of the IBD cohort followed on our Center.

The diagnosis of CD or UC was confirmed by combinations of clinical, radiologic, endoscopic, and histopathological criteria generally accepted for CD or UC.<sup>2</sup> . The inclusion criteria were: age  $\geq 18$  and  $< 65$  years and a confirmed diagnosis of IBD (CD or UC). Patients were excluded if they were younger than 18 or older than 65 years or presented a severe IBD requiring hospitalization. Patients were also considered ineligible if they had a previous or current history of malignancies (except cutaneous), prior gastrectomy, systemic infections in the last 3 months, alcohol abusive use (daily alcohol consumption above 40 g), drug addiction, disabling chronic organ failure, or history of replacement therapy with iron, folic acid or vitamin B12 in the last six months. Pregnant women or nursing mothers were not selected.

### **Measurements and outcomes**

#### **Socio-demographic and disease-related characteristics**

On inclusion, the eligibility criteria were assessed and medical history recorded. Patient's relevant socio-demographic data included: age, gender, current smoking (more than one cigarette daily) and history of alcohol consumption.

The classification and extent of the patients' IBD were established using the Montreal classification.<sup>15</sup> CD activity was measured according to the Crohn's disease activity index (CDAI). Scores below 150 indicated remission and higher scores active disease (i.e. mild to moderate disease: CDAI between 150 and 219; moderate to severe disease: CDAI between 220 and 450). For UC patients we used the Truelove and Witt's criteria.<sup>16</sup> Thus, clinical remission was defined by  $\leq 2$  or 3 stools/day, without the presence of blood and/or pus in the stools, with no systemic symptoms; mild activity: up to 4 stools/day, with or without blood, no systemic involvement and increased inflammatory markers; moderate activity:  $>4$  stools per day with minimal systemic symptoms and increased inflammatory markers; severe activity:  $>6$  stools per day with blood and evidence of systemic involvement, such as fever, tachycardia, anemia and erythrocyte sedimentation above 30. In addition, disease duration and IBD-related surgical history were recorded as well.

### **Evaluation of anemia**

During the inclusion in the study, blood samples (20 ml) for hematological and clinical chemistry were obtained. Anemia workup included: complete blood count, ferritin, serum iron, transferrin saturation (TfS), serum levels of folic acid and vitamin B12, a reticulocyte count, erythrocyte sedimentation and quantitative C-reactive protein (CRP) concentration.

According to the definition of the World Health Organization, the cutoff point for anemia was hemoglobin (Hb) levels below 13 g/dL in males and below 12 g/dL in non-pregnant females.<sup>17</sup> Severe anemia was defined as Hb  $<10$  g/dL for both genders. CRP was considered as elevated when the CRP levels were  $>6$  mg/L. Patients were classified to have iron deficiency anemia (IDA) if there was a reduction in serum ferritin below 30  $\mu\text{g/L}$  or TfS was  $<16\%$  in the absence of clinical and/or biochemical (i.e. CDAI  $\leq 150$  and CRP below or equal to upper limit of normality) data suggestive of disease activity; in the presence of inflammation (e.g. CDAI  $>150$  and/or CRP above upper limit of normality) IDA was diagnosed by a serum ferritin  $<100$   $\mu\text{g/L}$  and/or TfS  $<16\%$ .<sup>6</sup>

In the presence of clinical or biochemical evidence of intestinal inflammation, the diagnostic criteria for anemia of chronic disease (ACD) were a serum ferritin  $>100$   $\mu\text{g/L}$  and TfS  $<16\%$ .<sup>6,9</sup> A combination of IDA and ACD was defined by finding of a serum ferritin level between 30 and 100  $\mu\text{g/L}$ .<sup>6,11</sup> Anemia due to vitamin B12

deficiency or anemia caused by folic acid deficiency was diagnosed when there was low serum levels of these vitamins in patients with anemia. If an absolute reticulocyte count was above 100,000 anemia was classified as hemolytic.

### **Statistical analysis**

The statistical analysis was performed using SPSS 16.0 (SPSS, Chicago, IL, USA). Continuous variables are presented as medians and ranges, and categorical variables are expressed as number and percentage of patients. Descriptive statistics of all relevant variables for the groups were calculated. For data analysis the patients were divided into two groups (subjects with and without anemia) according to their Hb levels. Comparisons between groups as well as possible relationships between the presence of anemia with socio-demographic and disease-related data were analyzed using parametric Student's t tests, nonparametric chi-squared tests, or the Mann-Whitney U test, as appropriate. Univariate and multivariate logistic analysis was performed to identify independent predictors for occurrence of anemia in the whole IBD group. The results are presented as odds risk (OR) and 95% confidence intervals (CI). For comparison, the level of statistical significance was set at  $P < .05$  and all reported P-values are two-tailed.

### **Ethics**

The study protocol was defined in accordance with the Declaration of Helsinki and was approved by the Human Use Investigation Committee of our institution. All patients signed a freely informed consent form before being admitted to the study. The confidentiality patient identity and records were maintained.

## **RESULTS**

### **Baseline patient characteristics**

A total of 221 adult IBD outpatients were screened for the study. Of these, 18 individuals (8.1%) were not enrolled because they presented some of the exclusion criteria: severe IBD requiring hospitalization (n=4), colon cancer (n=1), active infectious (n=2), alcohol abusive use (n=2), previous gastrectomy (n=1), end-stage renal disease (n=1), pregnancy (n=2) and recent therapy with iron (n=5). Three patients refused to participate after reading the informed consent.

Thus 200 patients (90.5%) of the eligible population (75 males, 125 females, median age 42 years (range 19-60) were included. The demographic and clinical characteristics of the IBD cohort at baseline are shown in Table 1.

### **Assessment of anemia in patients with IBD**

The overall prevalence of anemia was 21%. The prevalence was similar among patients with CD (24%) and UC subjects (18 %;  $P = .25$ ) (Table 1). No patient presented severe anemia. There was no correlation between the occurrence of anemia with demographics, IBD location or duration, behavior of CD and previous surgery for IBD. Conversely, IBD patients with moderate disease activity (CDAI between 220 and 450 for CD or  $>4$  stools per day and increased inflammatory markers for UC) had significantly higher prevalence of anemia (66.7%) than patients in remission (17.8%) or with mildly active IBD (18.9%) ( $P < .001$  for both comparisons). This finding was more notable for UC individuals with moderate disease activity ( $P = .001$ ) than in the CD patients ( $P = .024$ ) presenting moderate activity (Table 1). In addition, CRP levels were significantly higher in both diagnostic groups with anemia than in the population without anemia ( $P < .001$  for CD and  $P = .009$  for UC). Interestingly, smokers had a lower prevalence of anemia ( $P = .047$ ). As shown by univariate logistic regression analysis, the risk of anemia was 4-fold higher for moderate disease activity and 2-fold higher for no smoking (Table 2). Increase in CRP by each 1 mg/L increased this risk by 90%. In multivariate model, independent variables associated with anemia were moderate disease activity and CRP. Moderate disease increased 3.5-fold the risk of anemia, whereas each 1 mg/L increase in CRP increased this probability by 80%. However, in multivariate analysis no smoking was not associated with an increased OR for anemia.

The most common etiologies of anemia found in both patients with CD and UC were IDA (10% on CD and 6% on UC subjects) followed by the ACD (6% in both groups) and B12 vitamin deficiency (5% in both groups). A total of 4% of the IBD cohort presented concomitant IDA and ACD and only one patient showed folate deficiency associated to IDA (Table 3).

## DISCUSSION

This is the first study to highlight the magnitude of comorbid anemia in Brazilian patients with IBD. It clearly shows that current anemia was strongly associated with both moderate disease activity and higher CRP levels. Furthermore, IDA and/or CDA were the most common etiologies of the anemia on the IBD setting.

The prevalence of anemia in our patients with IBD was 21%. This finding is in accordance with a overall prevalence of anemia (24%, 95% CI, 18-31) found on a recent meta-analysis evaluating 2192 IBD patients in European countries.<sup>18</sup> Other studies reported similar prevalence between 17% to 20%<sup>19,20</sup> which differs from the higher prevalence of anemia found on the studies of Bergamisch and colleagues<sup>4</sup> (65%) and Hoivik et al<sup>13</sup> (48.8% on CD and 20.2% on UC). This probably is due to the fact that our study was conducted in patients who were already in IBD treatment while the latter two studies evaluated patients before starting treatment. In current study the prevalence of comorbid anemia was similar among patients with CD and UC subjects. Disparately, other studies reported anemia occurring more frequently in patients with CD than in those with UC.<sup>4,11</sup> It is possible that selection bias on inclusion in our study (i.e. more patients with UC presented disease activity than CD patients) can justify this difference.

No patient in current study had severe anemia. It, however, included only outpatients and, individuals with severe IBD requiring hospitalization and more probable of presenting severe anemia were excluded. Furthermore, our study population is followed in an IBD center where patients are regularly monitored and treated to the target of tight control of intestinal inflammation. Notoriously, the degree of anemia in IBD patients correlates with underlying disease activity.<sup>18,21</sup> It is' another good reason to opt for expeditious, aggressive restraint of intestinal inflammation.

Similar to the finding in other studies<sup>6, 7,12,19</sup> we have revealed that the two most commonly found patterns of anemia in IBD were IDA and that of chronic disease. Although the distinction between IDA and the ACD is important for IBD individuals, both conditions commonly overlap. It is well known that continuous or recurrent intestinal blood loss, reduced iron absorption from the inflamed bowel and systemic iron sequestration drive a negative iron balance in IBD.<sup>1,3,11</sup> Conversely a main mechanism driving ACD is the interleukins-6 release from the inflamed intestine that can trigger increased hepcidin hepatic synthesis, which potentially

decreased duodenal absorption of iron and retained iron within cells of the reticular-endothelial system.<sup>22</sup> In addition, IBD individuals can have difficulty for utilizing iron appropriately because it has inappropriately low levels of erythropoietin for their severity of anemia.<sup>21</sup> Systemic inflammatory cytokines increase (eg, interleukins-6, TNF-alpha, among others) have been shown to decrease mRNA expression of erythropoietin.<sup>23</sup> It is interestingly to highlight that treatment with anti-tumour necrosis factor-alpha agents has been shown to improve iron deficiency by improving erythropoiesis, implicating a role for TNF-alpha in development of ACD and/or IDA on IBD patients.<sup>13</sup>

Our findings demonstrate a strong correlation between anemia and disease activity. Moderate disease activity was an independent factor that increased on 3.5-fold the risk of anemia. In particular, patients with moderate activity have a higher prevalence of anemia than those on remission or presenting mild activity. This finding was more evident for UC subjects with moderate disease activity than in the CD. It is possible to speculate that UC patients during flares can present higher bleeding rate than CD patients, which can entail more IDA. Other previous studies found that IBD patients with active disease status are more likely to have anemia than those being in remission.<sup>1,11,18</sup> Indeed, it should be emphasized that more inflammatory activity probably results in more blood loss, increased release of hepcidin and decreased iron absorption from the intestine. Additionally, our observation that elevated CRP is positively associated with anemia is consistent with that of Hoivik et al.<sup>4</sup> It is worth remembering that CRP is an acute phase protein that represents a nonspecific serum marker of inflammation. Active IBD, especially CD is associated with a CRP response in 40-80% of patients.<sup>24</sup> Thus, intestinal inflammation plays an integral role in the development of anemia on IBD individuals. Particularly on the setting of active disease and/or an increased CRP levels the suspicion of underlying anemia should be raised, which should be looked for carefully. However, it is important to point that about 18% (Table 1) of the patients with IBD had anemia despite having no active disease. Therefore, regardless of whether the patient has IBD on remission, screening for anemia may be still worthwhile.

Interestingly, in agreement with Hoivik et al<sup>4</sup>, we have revealed a negative correlation between smoking and anemia by univariate analysis. It has been shown that smokers can develop secondary polycythaemia caused by exposure to carbon monoxide by smoking.<sup>25</sup> Nonetheless, in our multivariate model smoking was not



associated with a reduced odds for anemia. Hence, the possible protective role of the cigarette against the development of the anemia on IBD remains open to debate.

Ultimately, we believe that an attention about the prevalence, risk factors and different etiologies of anemia in patients with IBD could allow that personalized, targeted therapies for patients be correctly adjusted according to the patient's need, thus potentially offering an opportunity for improvement of health-related quality of life, working ability, and patient satisfaction.

A limitation of the study is the fact that the occurrence of the anemia on IBD should ideally be assessed in a longitudinal rather than a cross-sectional study design. Furthermore, the burden of anemia found in our IBD patients may not completely reflect the true prevalence of anemia in the general IBD population. In the present study, the patients with IBD were at an IBD center of a university hospital, which makes them more likely to be a more ill or troubled group of IBD patients than those who would be seen in a community setting. It is generally accepted that patients with more severe disease are more likely to experience anemia.<sup>21</sup> This might bias the results toward a higher prevalence than in the IBD population in general. Thus our results should be interpreted in the appropriate context. Ultimately, future research through longitudinal studies is needed to examine which is the impact of the aggressive management of IBD on both the incidence and severity of the anemia in IBD patients.

## **CONCLUSIONS**

In Brazilian IBD outpatients, anemia is highly concurrent condition, deserving special attention. IDA and ACD are the most common types of anemia in this clinical setting. Our data underline that anemia in IBD is largely correlated with inflammatory activity. So adequate treatment should target both the proper correction of Hb levels and aggressive management of active IBD with close monitoring, which could afford the opportunity for giving a better patient's quality of life. The high prevalence of anemia in IBD patients together with the availability of effective treatment for this condition indicates that it is timely to recommend that periodic screening and appropriate management for anemia should be carried out routinely as part of quality of care improvement for all patients with IBD.

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TABLE 1. Demographic and clinical characteristics of the inflammatory bowel disease patients with and without anemia

		Crohn's disease		Ulcerative colitis		Total	
		Anemia	No anemia	Anemia	No Anemia	Anemia	No anemia
<b>Total n (%)</b>		24 (24)	76 (76)	18 (18)	82 (82)	42 (21)	158 (79)
<b>Gender n (%)</b>	<b>Female</b>	11 (17.2)	53 (82.8)	11 (18)	50 (82)	22 (17.6)	103 (82.4)
	<b>Male</b>	13 (36.1)	23 (63.9)	07 (17.9)	32 (82.1)	20 (26.7)	55 (73.3)
<b>Age year</b>		38.9	42.2	41.1	45.6	41.1	43.9
<b>(median/range)</b>		(18-64)	(19-63)	(20-64)	(19-65)	(18-64)	(19-65)
<b>Disease activity* n (%)</b>	<b>In remission</b>	17 (19.5)	70 (80.5)	7 (14.6)	41 (85.4)	24 (17.8)	111 (82.2)
	<b>Mild</b>	4 (50)	4 (50)	6 (13.3)	39 (86.7)	10 (18.9)	43 (81.1)
	<b>Moderate</b>	3 (60)	2 (40)	5 (71.4)	2 (28.6)	8 (66.7)	4 (33.3)
<b>CD Location n (%)</b>	<b>Heal</b>	8 (34.8)	15 (65.2)				
	<b>Colonic</b>	7 (20.6)	27 (79.4)				
	<b>Ileocolonic</b>	9 (20.9)	34 (79.1)				
<b>UC Location n (%)</b>	<b>Ulcerative proctitis</b>			8 (15.1)	45 (84.9)		
	<b>Left sided UC</b>			2 (10)	18 (90)		
	<b>Extensive UC</b>			8 (29.6)	19 (70.4)		
<b>Behavior of CD n (%)†</b>	<b>B1</b>	8 (21.6)	29 (78.4)				
	<b>B2</b>	7 (25)	21 (75)				
	<b>B3</b>	9 (25.7)	26 (74.3)				
<b>Disease duration (year) (median/range)</b>		6.4 (1.4-12.8)	8 (0.8-11.6)	7.4 ± 7.3 (2.7-10.8)	7.7 ± 5.2 (1-11)	6.8 ± 7.0 (1.4-12.8)	7.8 ± 6.2 (0.8-11.6)
<b>Previous surgery n (%)</b>	<b>Yes</b>	9 (23.1)	30 (76.9)	1 (50)	1 (50)	10 (24.4)	31 (75.6)
	<b>No</b>	15 (24.6)	46 (65.4)	17 (17.3)	81 (82.7)	32 (20.1)	127 (79.9)
<b>Smoking** n (%)</b>	<b>Yes</b>	2 (11.1)	16 (88.9)	1 (5.9%)	16 (94.1)	3 (8.6%)	32 (91.4)
	<b>No</b>	22 (26.8)	60 (73.2)	17 (20.5)	66 (79.5)	39 (23.6)	126 (76.4)

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<b>CRP ***</b>						
<b>(mg/L)</b>	12.9	4.6	9.9	4.9	11	3.5
<b>(median/range)</b>	(1- 80)	(1-45)	(1-60)	(1-30)	(1- 80)	(1-45)

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CD, Crohn's disease; UC, ulcerative colitis; CRP, C-reactive protein

\* For moderate disease activity there was a statistically significant difference with  $P = .024$  (CD),  $P = .001$  (UC) and  $P < .001$  (for total population)

\*\* For smoking there was a statistically significant difference with  $P = .047$  (for total population)

\*\*\* For CRP there was a statistically significant difference with  $P < 0.001$  (CD) and  $P = .009$  (UC)

# For all other characteristics, there was no significant difference

† B1 - non-stricturing, non-penetrating; B2 - stricturing; B3 - penetrating

TABLE 2. Univariate and multivariate logistic regression analysis for occurrence of anemia in the whole inflammatory bowel disease group

	OR	- 95% CI	+ 95% CI	P
<b>Univariate logistic regression analysis for anemia risk</b>				
Gender	0.957	0.912	1.242	.63
Age	1.263	0.674	3.487	.59
Inactive disease	1.438	0.497	2.764	.75
Mild disease activity	2.614	1.345	6.243	.09
Moderate disease activity	4.101	2.503	10.542	< .001
Disease duration	0.989	0.646	1.728	.21
Previous surgery	1.345	0.587	3.843	.57
No smoking	1.912	1.088	4.531	.04
CRP *	1.905	1.047	3.354	.001
<b>Multivariate logistic regression analysis for anemia risk</b>				
No smoking	0.912	0.878	2.531	.14
Moderate disease activity	3.487	1.954	9.643	.002
CRP	1.803	1.048	3.113	.02

OR, odds risk; CI, confidence intervals; CRP, C-reactive protein

TABLE 3. Etiology of the anemia on patients with inflammatory bowel disease

	ACD (%)	B <sub>12</sub> deficiency (%)	IDA (%)	<i>ACD+IDA</i> (%)
Crohn's disease (n=100)	6	5	10*	3
Ulcerative colitis (n=100)	6	5	6	1
Total (n=200)	12	10	16	4

ACD, anemia of chronic disease; IDA, Iron deficiency anemia

\* One Crohn's disease patient presented concomitant IDA and folate deficiency

## 6 CONCLUSÕES

A prevalência de anemia em DII foi semelhante à da literatura, 21% dos casos avaliados.

Anemia por deficiência de ferro e Anemia da doença crônica foram os tipos mais comuns de anemia nos pacientes portadores de DII e associaram-se com atividade moderada da doença;

Atividade moderada da doença e PCR elevada associaram-se independentemente com o risco de anemia

Anemia é frequente na Doença Inflamatória Intestinal, merecendo atenção e cuidados específicos.



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## **ANEXOS**

## ANEXO 1 Índice de Atividade da Doença de Crohn (IADC)

Para chegar-se ao IADC deve-se multiplicar o valor da coluna 1 pelo da coluna 2, anotando o resultado na coluna Subtotal. Finalmente deve-se somar todos subtotais para encontrar o valor total do IADC

Variável	Fator Multiplicador	Subtotal
Média do número de evacuações líquidas ou pastosas por dia nos últimos 7 dias.	X 2	
Dor abdominal, em média nos últimos 7 dias (0-sem dor, 1- dor leve, 2- dor moderada, 3- dor acentuada)	X 5	
Sensação de bem-estar, média dos últimos 7 dias (0- bom, 1- um pouco abaixo da média, 3- ruim, 4- muito ruim, 5- terrível)	X 7	
Número de complicações 1 - artrite ou artralgia 2 - irite ou uveíte 3 - eritema nodoso ou pioderma gangrenoso ou estomatite aftóide 4 - fissura anal ou fístula ou abscesso perirretal 5 - febre acima de 37,8° C	X 20	
Massa abdominal (0-não, 2- questionável, 5- definida) x 10	X 10	
Hematócrito (homens: 47 menos Ht; mulheres: 42 menos Ht em %)	X6	
Percentual acima ou abaixo do peso corporal habitual (1 menos [peso/peso habitual] x 100 (o resultado deve ser somado ou diminuído ao restante de acordo com o sinal)	X1	

## ANEXO 2 - Questionários da Pesquisa

**PACIENTE PORTADOR DE DOENÇA DE CROHN****Data:** \_\_\_\_\_

1-Nome:\_\_\_\_\_ 2-RG Hospitalar: \_\_\_\_\_

3-Idade: \_\_\_\_\_ 4- Sexo: \_\_\_\_\_ 5- Peso: \_\_\_\_\_

6- Tabagismo atual:

 Sim  Não

7-Duração da Doença de Crohn: \_\_\_\_\_

8- Extensão da Doença de Crohn:

 TGI superior  Ileocolônica  Ileíte extensa  Colônica

9- História familiar de doença inflamatória intestinal

 Sim  Não

10- Drogas utilizadas no tratamento atual da doença:

\_\_\_\_\_

11- Situação da doença no momento: Índice de Atividade da Doença de Crohn

\_\_\_\_\_

 Em remissão  Em atividade leve  Em atividade moderada

12- Fenótipo da Doença de Crohn:

 Fistulizante  Fibroestenósante  Não fistulizante e não estenosante

13- Cirurgia prévia:

 Ressecção ileocolônica  Ressecção >100 cm de intestino delgado Ressecção colônica parcial  Ressecção colônica total



14- Resultados dos exames:

Data dos resultados: \_\_\_\_\_

<b>Exame</b>	<b>Resultados</b>
Eritrócitos	
Hemoglobina	
Hematócrito	
HGM	
VGM	
CHGM	
RDW	
Leucócitos	
Segmentados	
Linfócitos	
Eosinófilos	
Bastões	
Mielócitos	
Pró-mielócitos	
Basófilos	
Monócitos	
Blastos	
Plaquetas	
Ácido fólico	
Vitamina B12	
Reticulócitos	
Índice de saturação da transferrina	
Ferro sérico	
Ferritina	
PCR	
VHS – primeira hora	
VHS – segunda hora	

**PACIENTE PORTADOR DE RETOCOLITE ULCERATIVA****Data:** \_\_\_\_\_

1-Nome: \_\_\_\_\_ 2-RG Hospitalar: \_\_\_\_\_

3-Idade: \_\_\_\_\_ 4- Sexo: \_\_\_\_\_ 5- Peso: \_\_\_\_\_

6- Tabagismo atual:

 Sim  Não

7-Duração da RCUI: \_\_\_\_\_

8- Extensão da Doença:

 Colite distal/proctite  Colite esquerda  Pancolite

9- História familiar de doença inflamatória intestinal

 Sim  Não

10- Drogas utilizadas no tratamento atual da doença:

\_\_\_\_\_

11- Doença no momento:

 Em remissão  Em atividade leve  Em atividade moderada

12- Ressecção colônica:

 Sim  Não

13- Resultados dos exames:

Data dos resultados: \_\_\_\_\_

<b>Exame</b>	<b>Resultados</b>
Eritrócitos	
Hemoglobina	
Hematócrito	
HGM	
VGM	
CHGM	
RDW	
Leucócitos	
Segmentados	
Linfócitos	
Eosinófilos	
Bastões	
Mielócitos	
Pró-mielócitos	
Basófilos	
Monócitos	
Blastos	
Plaquetas	
Ácido fólico	
Vitamina B12	
Reticulócitos	
Índice de saturação da transferrina	
Ferro sérico	
Ferritina	
PCR	
VHS – primeira hora	
VHS – segunda hora	

## ANEXO 3 - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO



**MINISTÉRIO DA EDUCAÇÃO  
UNIVERSIDADE FEDERAL DE JUIZ DE FORA  
HOSPITAL UNIVERSITÁRIO  
COMITÊ DE ÉTICA EM PESQUISA - CEP-HU CAS/UFJF  
JUIZ DE FORA - MG – BRASIL**

**SERVIÇO DE GASTROENTEROLOGIA – AMBULATÓRIO DE DOENÇA INFLAMATÓRIA INTESTINAL**

Pesquisador Responsável: Júlio Maria Fonseca Chebli

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**TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO**

O Sr. (a) está sendo convidado (a) como voluntário (a) a participar da pesquisa “Anemia nas doenças inflamatórias intestinais: prevalência e fatores de risco”. Neste estudo pretendemos realizar exames de sangue para verificarmos se o Sr. (a) é portador de anemia. O motivo que nos leva a estudar é para verificarmos a incidência da anemia nos pacientes que têm doença inflamatória intestinal e qual a sua provável origem. Para este estudo adotaremos os seguintes procedimentos: faremos a coleta de seu sangue, aqui mesmo no Hospital Universitário, conforme já é rotina no seu acompanhamento para doença inflamatória intestinal e faremos as análises necessárias, não há risco para sua saúde diferente do que há no fazer um exame de sangue comum. Para participar deste estudo você não terá nenhum custo, nem receberá qualquer vantagem financeira.

Você será esclarecido (a) sobre o estudo em qualquer aspecto que desejar e estará livre para participar ou recusar-se a participar. Poderá retirar seu consentimento ou interromper a participação a qualquer momento. A sua participação é voluntária e a recusa em participar não acarretará qualquer penalidade ou modificação na forma em que é atendido pelo pesquisador

O pesquisador irá tratar a sua identidade com padrões profissionais de sigilo.

Os resultados da pesquisa estarão à sua disposição quando finalizada. Seu nome ou o material que indique sua participação não será liberado sem a sua permissão.

O (A) Sr (a) não será identificado em nenhuma publicação que possa resultar deste estudo.

Este termo de consentimento encontra-se impresso em duas vias, sendo que uma cópia será arquivada pelo pesquisador responsável, no CAS do Hospital Universitário e a outra será fornecida a você.

Não haverá risco, se por ventura houver será ressarcido pelo pesquisador responsável.

Eu, \_\_\_\_\_, portador do documento de Identidade \_\_\_\_\_ fui informado (a) dos objetivos do estudo “Anemia nas doenças inflamatórias intestinais: prevalência e fatores de risco”, de maneira clara

e detalhada e esclareci minhas dúvidas. Sei que a qualquer momento poderei solicitar novas informações e modificar minha decisão de participar se assim o desejar.

Declaro que concordo em participar desse estudo. Recebi uma cópia deste termo de consentimento livre e esclarecido e me foi dada à oportunidade de ler e esclarecer as minhas dúvidas.

Juiz de Fora, \_\_\_\_\_ de \_\_\_\_\_ de \_\_\_\_\_ .

Nome

Assinatura participante

Data

Nome

Assinatura pesquisador

Data

Nome

Assinatura testemunha

Data

Em caso de dúvidas com respeito aos aspectos éticos deste estudo, você poderá consultar o CEP- Comitê de Ética em Pesquisa do HU/CAS da UFJF

Rua Catulo Breviglieri, s/n

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## APÉNDICE

## Medicine

### Anemia in Brazilian inflammatory bowel disease outpatients: Prevalence, risk factors and tiology

--Manuscript Draft--

<b>Manuscript Number:</b> MD-D-14-00149	MD-D-14-00149
<b>Article Type:</b>	OA: Observational Study (STROBE Compliant)
<b>Section/Category:</b>	Gastroenterology and hepatology
<b>Keywords:</b>	inflammatory bowel disease, Crohn's disease, ulcerative colitis, anemia, iron deficiency
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<b>Manuscript Region of Origin:</b>	BRAZIL
<b>Abstract:</b>	<p><b>Goals:</b> To assess the prevalence, risk factors and etiology of anemia in Brazilian outpatients with inflammatory bowel disease (IBD).</p> <p><b>Background:</b> Anemia is common in IBD. However, epidemiological studies of non-western IBD populations are limited and may be confounded by demographic, socioeconomic and disease-related influences.</p> <p><b>Study:</b> In this cross-sectional study, 100 Crohn's disease (CD) patients and 100ulcerative colitis (UC) subjects were assessed. Anemia workup included: complete blood count, ferritin, transferrin saturation, serum levels of folic acid and vitamin B12,and C-reactive protein (CRP) concentration.</p> <p><b>Results:</b> The overall prevalence of anemia on IBD was 21%. There was no significant difference in the prevalence of anemia between CD subjects (24%) and UC (18%).There was no correlation between the occurrence of anemia with demographics, IBD location or duration, behavior of CD and previous surgery. Moderate disease activity (OR: 3.48, 95% CI 1.95-9.64, P = .002) as well as elevated CRP levels (OR: 1.8, 95% CI, 1.04-3.11, P = .02) were independently associated with anemia. The most common etiologies of anemia found in both groups were iron deficiency anemia (IDA; 10% on CD and 6% on UC) followed by the anemia of chronic disease (ACD; 6% for both groups).</p> <p><b>Conclusions:</b> In Brazilian IBD outpatients, anemia is highly concurrent condition. Disease moderate activity as well as increased CRP were strongly associated with comorbid anemia. IDA and/or ACD were the most common etiologies. Periodic screening and appropriate management for anemia should be carried out routinely as part of quality of care improvement for IBD individuals.</p>

**Anemia in Brazilian inflammatory bowel disease outpatients: Prevalence, risk factors and etiology**

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Conflicts of Interest: The authors have no conflicts of interest to disclose.

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**List of abbreviations**

ACD, anemia of chronic disease

CD, Crohn's disease

CDAI, Crohn's disease activity index

CI, confidence intervals CRP, C-reactive protein Hb, hemoglobin

IBD, inflammatory bowel disease

IDA, iron deficiency anemia

OR, odds risk

TfS, transferrin saturation

UC, ulcerative colitis

## ABSTRACT

**Goals:** To assess the prevalence, risk factors and etiology of anemia in Brazilian outpatients with inflammatory bowel disease (IBD).

**Background:** Anemia is common in IBD. However, epidemiological studies of non-western IBD populations are limited and may be confounded by demographic, socioeconomic and disease-related influences.

**Study:** In this cross-sectional study, 100 Crohn's disease (CD) patients and 100 ulcerative colitis (UC) subjects were assessed. Anemia workup included: complete blood count, ferritin, transferrin saturation, serum levels of folic acid and vitamin B<sub>12</sub>, and C-reactive protein (CRP) concentration.

**Results:** The overall prevalence of anemia on IBD was 21%. There was no significant difference in the prevalence of anemia between CD subjects (24%) and UC (18%). There was no correlation between the occurrence of anemia with demographics, IBD location or duration, behavior of CD and previous surgery. Moderate disease activity (OR: 3.48, 95% CI 1.95-9.64,  $P = .002$ ) as well as elevated CRP levels (OR: 1.8, 95% CI, 1.04-3.11,  $P = .02$ ) were independently associated with anemia. The most common etiologies of anemia found in both groups were iron deficiency anemia (IDA; 10% on CD and 6% on UC) followed by the anemia of chronic disease (ACD; 6% for both groups).

**Conclusions:** In Brazilian IBD outpatients, anemia is highly concurrent condition. Disease moderate activity as well as increased CRP were strongly associated with comorbid anemia. IDA and/or ACD were the most common etiologies. Periodic screening and appropriate management for anemia should be carried out routinely as part of quality of care improvement for IBD individuals.

**Key Words:** inflammatory bowel disease, Crohn's disease, ulcerative colitis, anemia, iron deficiency

## INTRODUCTION

Inflammatory bowel diseases (IBD) are idiopathic multisystemic disorders that present with periods of relapses and remissions throughout their clinical course.<sup>1</sup> While ulcerative colitis (UC) is characterized by inflammation limited to the colonic mucosa, in Crohn's disease (CD) is observed transmural inflammation that can affect any part of the gastrointestinal tract.<sup>2</sup> In many patients, these conditions cause substantial personal cost, due to the unpredictable fluctuating symptoms, absenteeism at work, use of high-cost drugs, surgeries or multidisciplinary care.

The severity of the intestinal manifestations (i.e. diarrhea, abdominal pain and bleeding) is usually an important guide for management of IBD. Occasionally, however, one or more extraintestinal manifestations can be the predominant clinical feature on patients with IBD. Notably, anemia is one of the most common extraintestinal manifestation (or complication) in IBD.<sup>3-5</sup> In this clinical setting, anemia can contribute to patients' poor quality of life, particularly because of its negative impact on the feeling of wellbeing, physical performance, mood, cognitive function, and capacity to perform social activities.<sup>6,7</sup> In addition, comorbid anemia in IBD individuals is a significant predictor of increased risk of hospitalization and even increased patient mortality.<sup>8-10</sup> Of note, anemia may occasionally both antecede the development of intestinal symptoms and be the key signal unmask IBD.<sup>3,6</sup>

The prevalence of anemia in IBD varies widely, with studies reporting a frequency between 8.8% and 74% depending on the assessed patient subpopulation.<sup>11,12</sup> Etiology of anemia in IBD is multifactorial. Nonetheless, iron deficiency and characteristics of chronic disease are the most prevalent in this clinical setting.<sup>11-13</sup>

Periodic screenings for anemia as well as a systematic diagnostic approach are essential steps to the appropriate management of this condition on IBD. However, anemia is both underdiagnosed and undertreated in populations with IBD.<sup>14</sup> Furthermore, different geographic, demographic, socioeconomic, and disease-related characteristics may exist between South American and North American or European populations that may limit the generalization of the findings regarding the epidemiology of anemia on IBD individuals, as the data are mainly derived from North American or European studies.

Therefore the purpose of the present cross-sectional cohort study was to assess the prevalence, risk factors and etiology of anemia in a Brazilian population with IBD. We hypothesized that anemia would be a concurrent condition in this patient population and correlated with inflammatory activity.

## **MATERIALS AND METHODS**

### **Study design**

We conducted a cross-sectional cohort study of adult outpatients with IBD from an IBD Center at the University Hospital of the Federal University of Juiz de Fora, Brazil, that were enrolled consecutively into this study for evaluation of the anemia. between November 2012 and January 2013.

### **Participants**

Our goal was to recruit 200 IBD patients (100 with CD and 100 with UC), nearly 20% of the IBD cohort followed on our Center.

The diagnosis of CD or UC was confirmed by combinations of clinical, radiologic, endoscopic, and histopathological criteria generally accepted for CD or UC.<sup>2</sup> The inclusion criteria were: age  $\geq 18$  and  $< 65$  years and a confirmed diagnosis of IBD (CD or UC). Patients were excluded if they were younger than 18 or older than 65 years or presented a severe IBD requiring hospitalization. Patients were also considered ineligible if they had a previous or current history of malignancies (except cutaneous), prior gastrectomy, systemic infections in the

last 3 months, alcohol abusive use (daily alcohol consumption above 40 g), drug addiction, disabling chronic organ failure, or history of replacement therapy with iron, folic acid or vitamin B<sub>12</sub> in the last six months. Pregnant women or nursing mothers were not selected.

### **Measurements and outcomes**

#### **Socio-demographic and disease-related characteristics**

On inclusion, the eligibility criteria were assessed and medical history recorded. Patient's relevant socio-demographic data included: age, gender, current smoking (more than one cigarette daily) and history of alcohol consumption.

The classification and extent of the patients' IBD were established using the Montreal classification.<sup>15</sup> CD activity was measured according to the Crohn's disease activity index (CDAI). Scores below 150 indicated remission and higher scores active disease (i.e. mild to moderate disease: CDAI between 150 and 219; moderate to severe disease: CDAI between 220 and 450). For UC

patients we used the Truelove and Witt's criteria.<sup>16</sup> Thus, clinical remission was defined by  $\leq 2$  or 3 stools/day, without the presence of blood and/or pus in the stools, with no systemic symptoms; mild activity: up to 4 stools/day, with or without blood, no systemic involvement and increased inflammatory markers; moderate activity:  $>4$  stools per day with minimal systemic symptoms and increased inflammatory markers; severe activity:  $>6$  stools per day with blood and evidence of systemic involvement, such as fever, tachycardia, anemia and erythrocyte sedimentation above 30. In addition, disease duration and IBD-related surgical history were recorded as well.

### **Evaluation of anemia**

During the inclusion in the study, blood samples (20 ml) for hematological and clinical chemistry were obtained. Anemia workup included: complete blood count, ferritin, serum iron, transferrin saturation (TfS), serum levels of folic acid and vitamin B<sub>12</sub>, a reticulocyte count, erythrocyte sedimentation and quantitative C-reactive protein (CRP) concentration.

According to the definition of the World Health Organization, the cutoff point for anemia was hemoglobin (Hb) levels below 13 g/dL in males and below 12 g/dL in non-pregnant females.<sup>17</sup> Severe anemia was defined as Hb  $<10$  g/dL for both genders. CRP was considered as elevated when the CRP levels were  $>6$  mg/L. Patients were classified to have iron deficiency anemia (IDA) if there was a reduction in serum ferritin below 30  $\mu\text{g/L}$  or TfS was  $<16\%$  in the absence of clinical and/or biochemical (i.e. CDAI  $\leq 150$  and CRP below or equal to upper limit of normality) data suggestive of disease activity; in the presence of inflammation (e.g. CDAI  $>150$  and/or CRP above upper limit of normality) IDA was diagnosed by a serum ferritin  $<100$   $\mu\text{g/L}$  and/or TfS  $<16\%$ .<sup>6</sup>

In the presence of clinical or biochemical evidence of intestinal inflammation, the diagnostic criteria for anemia of chronic disease (ACD) were a serum ferritin  $>100$   $\mu\text{g/L}$  and TfS  $<16\%$ .<sup>6,9</sup> A combination of IDA and ACD was defined by finding of a serum ferritin level between 30 and 100  $\mu\text{g/L}$ .<sup>6,11</sup> Anemia due to vitamin B<sub>12</sub> deficiency or anemia caused by folic acid deficiency was diagnosed when there was low serum levels of these vitamins in patients with anemia. If an absolute reticulocyte count was above 100,000 anemia was classified as hemolytic.

### **Statistical analysis**

The statistical analysis was performed using SPSS 16.0 (SPSS, Chicago, IL, USA). Continuous variables are presented as medians and ranges, and categorical variables are expressed as number and percentage of patients. Descriptive statistics of all relevant variables for the groups were calculated. For data analysis the patients were divided into two groups (subjects with and

without anemia) according to their Hb levels. Comparisons between groups as well as possible relationships between the presence of anemia with socio-demographic and disease-related data were analyzed using parametric Student's *t* tests, nonparametric chi-squared tests, or the Mann-Whitney *U* test, as appropriate. Univariate and multivariate logistic analysis was performed to identify independent predictors for occurrence of anemia in the whole IBD group. The results are presented as odds risk (OR) and 95% confidence intervals (CI). For comparison, the level of statistical significance was set at  $P < .05$  and all reported *P*-values are two-tailed.

### **Ethics**

The study protocol was defined in accordance with the Declaration of Helsinki and was approved by the Human Use Investigation Committee of our institution. All patients signed a freely informed consent form before being admitted to the study. The confidentiality patient identity and records were maintained.

## **RESULTS**

### **Baseline patient characteristics**

A total of 221 adult IBD outpatients were screened for the study. Of these, 18 individuals (8.1%) were not enrolled because they presented some of the exclusion criteria: severe IBD requiring hospitalization (n=4), colon cancer (n=1), active infectious (n=2), alcohol abusive use (n=2), previous gastrectomy (n=1), end-stage renal disease (n=1), pregnancy (n=2) and recent therapy with iron (n=5). Three patients refused to participate after reading the informed consent. Thus 200 patients (90.5%) of the eligible population (75 males, 125 females, median age 42 years (range 19-60) were included. The demographic and clinical characteristics of the IBD cohort at baseline are shown in Table 1.

### **Assessment of anemia in patients with IBD**

The overall prevalence of anemia was 21%. The prevalence was similar among patients with CD (24%) and UC subjects (18 %;  $P = .25$ ) (Table 1). No patient presented severe anemia. There was no correlation between the occurrence of anemia with demographics, IBD location or duration, behavior of CD and previous surgery for IBD. Conversely, IBD patients with moderate disease

activity (CDAI between 220 and 450 for CD or >4 stools per day and increased inflammatory markers for UC) had significantly higher prevalence of anemia (66.7%) than patients in remission (17.8%) or with mildly active IBD (18.9%) ( $P < .001$  for both comparisons). This finding was more notable for UC individuals with moderate disease activity ( $P = .001$ ) than in the CD patients ( $P = .024$ ) presenting moderate activity (Table 1). In addition, CRP levels were significantly higher in both diagnostic groups with anemia than in the population without anemia ( $P < .001$  for CD and  $P = .009$  for UC). Interestingly, smokers had a lower prevalence of anemia ( $P = .047$ ).

As shown by univariate logistic regression analysis, the risk of anemia was 4-fold higher for moderate disease activity and 2-fold higher for no smoking (Table 2). Increase in CRP by each 1 mg/L increased this risk by 90%. In multivariate model, independent variables associated with anemia were moderate disease activity and CRP. Moderate disease increased 3.5-fold the risk of anemia, whereas each 1 mg/L increase in CRP increased this probability by 80%. However, in multivariate analysis no smoking was not associated with an increased OR for anemia.

The most common etiologies of anemia found in both patients with CD and UC were IDA (10% on CD and 6% on UC subjects) followed by the ACD (6% in both groups) and B<sub>12</sub> vitamin deficiency (5% in both groups). A total of 4% of the IBD cohort presented concomitant IDA and ACD and only one patient showed folate deficiency associated to IDA (Table 3).

## DISCUSSION

This is the first study to highlight the magnitude of comorbid anemia in Brazilian patients with IBD. It clearly shows that current anemia was strongly associated with both moderate disease activity and higher CRP levels. Furthermore, IDA and/or CDA were the most common etiologies of the anemia on the IBD setting.

The prevalence of anemia in our patients with IBD was 21%. This finding is in accordance with a overall prevalence of anemia (24%, 95% CI, 18-31) found on a recent meta-analysis evaluating 2192 IBD patients in European countries.<sup>18</sup> Other studies reported similar prevalence between 17% to 20%<sup>19,20</sup> which differs from the higher prevalence of anemia found on the studies of Bergamisch and colleagues<sup>4</sup> (65%) and Hoivik et al<sup>13</sup> (48.8% on CD and 20.2% on UC). This probably is due to the fact that our study was conducted in patients who were already in IBD treatment while the latter two studies evaluated patients before starting treatment. In current study the prevalence of comorbid anemia was similar among patients with CD and UC subjects. Disparately, other studies reported anemia occurring more frequently in patients with CD than in those with UC.<sup>4,11</sup> It is possible that selection bias on inclusion in our study (i.e. more patients with UC presented disease activity than CD patients) can justify this difference.

No patient in current study had severe anemia. It, however, included only outpatients and, individuals with severe IBD requiring hospitalization and more probable of presenting severe anemia were excluded. Furthermore, our study population is followed in an IBD center where patients are regularly monitored and treated to the target of tight control of intestinal inflammation. Notoriously, the degree of anemia in IBD patients correlates with underlying disease activity.<sup>18,21</sup> It is' another good reason to opt for expeditious, aggressive restraint of intestinal inflammation.

Similar to the finding in other studies<sup>6,7,12,19</sup> we have revealed that the two most commonly found patterns of anemia in IBD were IDA and that of chronic disease. Although the distinction between IDA and the ACD is important for IBD individuals, both conditions commonly overlap. It is well known that continuous or recurrent intestinal blood loss, reduced iron absorption from the inflamed bowel and systemic iron sequestration drive a negative iron balance in IBD.<sup>1,3,11</sup> Conversely a main mechanism driving ACD is the interleukins-6 release from the inflamed intestine that can trigger increased hepcidin hepatic synthesis, which potentially decreased duodenal absorption of iron and retained iron within cells of the reticular- endothelial system.<sup>22</sup> In addition, IBD individuals can have difficulty for utilizing iron appropriately because it' has inappropriately low levels of erythropoietin for their severity of anemia.<sup>21</sup> Systemic inflammatory cytokines increase (eg, interleukins-6, TNF-alpha, among others) have been shown to decrease



mRNA expression of erythropoietin.<sup>23</sup> It is interestingly to highlight that treatment with anti-tumour necrosis factor-alpha agents has been shown to improve iron deficiency by improving erythropoiesis, implicating a role for TNF-alpha in development of ACD and/or IDA on IBD patients.<sup>13</sup>

Our findings demonstrate a strong correlation between anemia and disease activity. Moderate disease activity was an independent factor that increased on 3.5-fold the risk of anemia. In particular, patients with moderate activity have a higher prevalence of anemia than those on remission or presenting mild activity. This finding was more evident for UC subjects with moderate disease activity than in the CD. It is possible to speculate that UC patients during flares can present higher bleeding rate than CD patients, which can entail more IDA. Other previous studies found that IBD patients with active disease status are more likely to have anemia than those being in remission.<sup>1,11,18</sup> Indeed, it should be emphasized that more inflammatory activity probably results in more blood loss, increased release of hepcidin and decreased iron absorption from the intestine. Additionally, our observation that elevated CRP is positively associated with anemia is consistent with that of Hoivik et al.<sup>4</sup> It is worth remembering that CRP is an acute phase protein that represents a nonspecific serum marker of inflammation. Active IBD, especially CD is associated with a CRP response in 40-80% of patients.<sup>24</sup> Thus, intestinal inflammation plays an integral role in the development of anemia on IBD individuals. Particularly on the setting of active disease and/or an increased CRP levels the suspicion of underlying anemia should be raised, which should be looked for carefully. However, it is important to point that about 18% (Table 1) of the patients with IBD had anemia despite having no active disease. Therefore, regardless of whether the patient has IBD on remission, screening for anemia may be still worthwhile.

Interestingly, in agreement with Hoivik et al.<sup>4</sup>, we have revealed a negative correlation between smoking and anemia by univariate analysis. It has been shown that smokers can develop secondary polycythaemia caused by exposure to carbon monoxide by smoking.<sup>25</sup> Nonetheless, in our multivariate model smoking was not associated with a reduced odds for anemia. Hence, the possible protective role of the cigarette against the development of the anemia on IBD remains open to debate.

Ultimately, we believe that an attention about the prevalence, risk factors and different etiologies of anemia in patients with IBD could allow that personalized, targeted therapies for patients be correctly adjusted according to the patient's need, thus potentially offering an opportunity for improvement of health-related quality of life, working ability, and patient satisfaction.

A limitation of the study is the fact that the occurrence of the anemia on IBD should ideally be assessed in a longitudinal rather than a cross-sectional study design. Furthermore, the burden of anemia found in our IBD patients may not completely reflect the true prevalence of anemia in the general IBD population. In the present study, the patients with IBD were at an IBD center of a university hospital, which makes them more likely to be a more ill or troubled group of IBD patients

than those who would be seen in a community setting. It is generally accepted that patients with more severe disease are more likely to experience anemia.<sup>21</sup> This might bias the results toward a higher prevalence than in the IBD population in general. Thus our results should be interpreted in the appropriate context. Ultimately, future research through longitudinal studies is needed to examine which is the impact of the aggressive management of IBD on both the incidence and severity of the anemia in IBD patients.

## CONCLUSIONS

In Brazilian IBD outpatients, anemia is highly concurrent condition, deserving special attention. IDA and ACD are the most common types of anemia in this clinical setting. Our data underline that anemia in IBD is largely correlated with inflammatory activity. So adequate treatment should target both the proper correction of Hb levels and aggressive management of active IBD with close monitoring, which could afford the opportunity for giving a better patient's quality of life. The high prevalence of anemia in IBD patients together with the availability of effective treatment for this condition indicates that it is timely to recommend that periodic screening and appropriate management for anemia should be carried out routinely as part of quality of care improvement for all patients with IBD.

## FUNDING

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TABLE 1. Demographic and clinical characteristics of the inflammatory bowel disease patients with and without anemia

		Crohn's disease		Ulcerative colitis		Total	
		Anemia	No anemia	Anemia	No Anemia	Anemia	No anemia
<b>Total n (%)</b>		24 (24)	76 (76)	18 (18)	82 (82)	42 (21)	158 (79)
<b>Gender n (%)</b>	<b>Female</b>	11 (17.2)	53 (82.8)	11 (18)	50 (82)	22 (17.6)	103 (82.4)
	<b>Male</b>	13 (36.1)	23 (63.9)	07 (17.9)	32 (82.1)	20 (26.7)	55 (73.3)
<b>Age year (median/range)</b>		38.9 (18-64)	42.2 (19-63)	41.1 (20-64)	45.6 (19-65)	41.1 (18-64)	43.9 (19-65)
<b>Disease activity* n (%)</b>	<b>In remission</b>	17 (19.5)	70 (80.5)	7 (14.6)	41 (85.4)	24 (17.8)	111 (82.2)
	<b>Mild</b>	4 (50)	4 (50)	6 (13.3)	39 (86.7)	10 (18.9)	43 (81.1)
	<b>Moderate</b>	3 (60)	2 (40)	5 (71.4)	2 (28.6)	8 (66.7)	4 (33.3)
<b>CD Location n (%)</b>	<b>Ileal</b>	8 (34.8)	15 (65.2)				
	<b>Colonic</b>	7 (20.6)	27 (79.4)				
	<b>Ileocolonic</b>	9 (20.9)	34 (79.1)				
<b>UC Location n (%)</b>	<b>Ulcerative proctitis</b>			8 (15.1)	45 (84.9)		
	<b>Left sided UC</b>			2 (10)	18 (90)		
	<b>Extensive UC</b>			8 (29.6)	19 (70.4)		
<b>Behavior of CD n (%)†</b>	<b>B1</b>	8 (21.6)	29 (78.4)				
	<b>B2</b>	7 (25)	21 (75)				
	<b>B3</b>	9 (25.7)	26 (74.3)				
<b>Disease duration (year) (median/range)</b>		6.4 (1.4-12.8)	8 (0.8-11.6)	7.4 ± 7.3 (2.7-10.8)	7.7 ± 5.2 (1-11)	6.8 ± 7.0 (1.4-12.8)	7.8 ± 6.2 (0.8-11.6)
<b>Previous surgery n (%)</b>	<b>Yes</b>	9 (23.1)	30 (76.9)	1 (50)	1 (50)	10 (24.4)	31 (75.6)
	<b>No</b>	15 (24.6)	46 (65.4)	17 (17.3)	81 (82.7)	32 (20.1)	127 (79.9)
<b>Smoking** n (%)</b>	<b>Yes</b>	2 (11.1)	16 (88.9)	1 (5.9%)	16 (94.1)	3 (8.6%)	32 (91.4)
	<b>No</b>	22 (26.8)	60 (73.2)	17 (20.5)	66 (79.5)	39 (23.6)	126 (76.4)

<b>CRP ***</b>						
<b>(mg/L)</b>	12.9	4.6	9.9	4.9	11	3.5
<b>(median/range)</b>	(1- 80)	(1-45)	(1-60)	(1-30)	(1- 80)	(1-45)

CD, Crohn's disease; UC, ulcerative colitis; CRP, C-reactive protein

\* For moderate disease activity there was a statistically significant difference with  $P = .024$  (CD),  $P = .001$  (UC) and  $P < .001$  (for total population)

\*\* For smoking there was a statistically significant difference with  $P = .047$  (for total population)

\*\*\* For CRP there was a statistically significant difference with  $P < 0.001$  (CD) and  $P = .009$  (UC)

# For all other characteristics, there was no significant difference

† B1 - non-stricturing, non-penetrating; B2 – stricturing; B3 - penetrating

TABLE 2. Univariate and multivariate logistic regression analysis for occurrence of anemia in the whole inflammatory bowel disease group

	OR	- 95% CI	+ 95% CI	P
<b>Univariate logistic regression analysis for anemia risk</b>				
Gender	0.957	0.912	1.242	.63
Age	1.263	0.674	3.487	.59
Inactive disease	1.438	0.497	2.764	.75
Mild disease activity	2.614	1.345	6.243	.09
Moderate disease activity	4.101	2.503	10.542	< .001
Disease duration	0.989	0.646	1.728	.21
Previous surgery	1.345	0.587	3.843	.57
No smoking	1.912	1.088	4.531	.04
CRP *	1.905	1.047	3.354	.001
<b>Multivariate logistic regression analysis for anemia risk</b>				
No smoking	0.912	0.878	2.531	.14
Moderate disease activity	3.487	1.954	9.643	.002
CRP	1.803	1.048	3.113	.02

OR, odds risk; CI, confidence intervals; CRP, C-reactive protein

TABLE 3. Etiology of the anemia on patients with inflammatory bowel disease

	ACD (%)	B <sub>12</sub> deficiency (%)	IDA (%)	<i>ACD+IDA</i> (%)
Crohn's disease (n=100)	6	5	10*	3
Ulcerative colitis (n=100)	6	5	6	1
Total (n=200)	12	10	16	4

ACD, anemia of chronic disease; IDA, Iron deficiency anemia

\* One Crohn's disease patient presented concomitant IDA and folate deficiency



## STROBE Statement

Checklist of items that should be included in reports of observational studies

Section/Topic	Item N°	Recommendation	Reported on Page N°
<b>Title and abstract</b>	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of that was done and what was found	3
<b>Introduction</b>			
Background/rationale	2	Explain the scientific background and rationale for investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4,5
<b>Methods</b>			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	
Participants	6	(a) <i>Cohort study</i> -Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> -Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional</i> -Give the eligibility criteria, and the sources and methods of selection of participants	5
		(b) <i>Cohort study</i> -For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> -For matched studies, give matching criteria and the number of controls per case	-
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effects modifiers. Give diagnostic criteria, if applicable	5,6,7
Data source/measurement	8*	For each variable of interest, give source of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5,6,7
Bias	9	Describe any efforts to address potential sources of bias	-
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5,6,7
		(a) Describe all statistical methods, including those used to control for confounding	7
Statistical methods	12	(b) Describe any methods used to examine subgroups and interactions	7
		(c) Explain how missing data were	-
		(d) <i>Cohort study</i> -If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> -If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> -If applicable, describe analytical methods taking account of sampling strategy	-
		(e) Describe any sensitivity analyses	-
			-

Section/Topic	Item N°	Recommendation	Reported on Page N°
<b>Results</b>			
Participants	13*	(a) Report numbers of individuals at each stage of study-eg numbers potentially eligible, examined for eligibility, confirmed, included in the study, completing follow-up, and analysed	7,8
		(b) Give reasons for non-participation at each stage	7,8
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	-
		(c) <i>Cohort study</i> -Summarise follow-up time (eg, average and total amount)	-
Outcome	15*	<i>Cohort study</i> -Report numbers of outcome events or summary measures over time	-
		<i>Case-control study</i> -Report numbers in each exposure category, or summary measures of exposure	-
		<i>Cross-sectional study</i> -Report number of outcome events or summary measures	8
Main results	16*	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8
		(b) Report category boundaries when continuous variables were categorized	-
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	8
Other analyses	17	Report other analyses done-eg analyses of subgroups and interactions, and sensitivity analyses	-
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9,10,11
Generalisability	21	Discuss the generalizability (external validity) of the study results	9,10,11
<b>Other Information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with article (freely available on the the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annls.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).