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Titolo della Tesi

The role of Nutritional Status on Disease Activity in Patients with Inflammatory Bowel Diseases

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SUMMARY

ABSTRACT

Background: Nutritional deficiency, including malnutrition, undernutrition, and overnutrition—are common throughout the disease course. These may result from inadequate intake, altered nutrient requirements and metabolism, malabsorption, excessive gastrointestinal losses, and medication effects. Whether low serum micronutrient levels are a cause or consequence of active disease remains unclear, as does the therapeutic potential of supplementation. Nevertheless, the available evidence consistently shows that active disease is linked to low serum micronutrient concentrations.
The objective of this study was to evaluate whether targeted nutritional assessment and correction of identified nutritional deficiencies can modify the risk of disease relapse and activity in patients with IBD.

Material and methods: This multicenter, prospective, randomized, open-label study enrolled all patients with a confirmed diagnosis of IBD (either in remission or with mild disease activity) who presented with at least one nutritional deficiency at baseline (T0), including low levels of vitamin D, vitamin B12, folic acid, serum iron, ferritin, albumin, or hemoglobin levels. Eligible patients were randomized in a 1:1:1 ratio to a nutritional supplementation group, a control group, or a healthy control group without any nutritional deficiencies.
The efficacy of nutritional interventions, delivered according to a predefined protocol, was assessed at the end of follow-up (T1, 54 weeks). Clinical outcomes included disease activity, evaluated using the Partial Mayo Score and Harvey–Bradshaw Index, as well as therapeutic modifications (e.g., treatment optimization, switching of advanced therapies, or corticosteroid initiation). Quality of life was measured at baseline and at week 54 using the SF-36 and IBD-DI questionnaires. Moreover, the relationship between nutritional status (including micronutrient deficiencies) and clinical as well as laboratoristics disease activity was evaluated at the baseline in patient without any supplementation therapies.

Results: At the end of the enrollment period, 120 IBD patients (46 CD; 74 UC) were included. Overall, 27/120 (22%) patients presented clinical activity at the enrollment period. Patients with clinical active disease had significantly lower hemoglobin, iron, ferritin, and vitamin D levels compared to inactive patients ($p = 0.007$, $p < 0.001$, $p = 0.005$, and $p = 0.003$, respectively). No significant differences were observed for folate, vitamin B12, or albumin.
A total of 21/120 patients (17.5%) experienced a clinical relapse during follow-up or had active disease at T1. No significant differences were observed between the intervention and control groups ($p = 0.94$; 95% CI 0.33–2.79). However, when patients were stratified according to the presence or absence of nutritional deficiencies at baseline, those without deficiencies had a significantly lower risk of clinical relapse ($p = 0.047$; 95% CI 0.07–0.98). Notably, only patients in the intervention group demonstrated a significant improvement in quality of life, as measured by the SF-36 score, from baseline to the end of follow-up (T0: 118 vs. T1: 142; $p = 0.007$).

Conclusion: The results of this study indicate that, when standard-of-care therapy is maintained, nutritional supplementation alone does not reduce the risk of clinical relapse. However, supplementation was associated with improved quality of life at the end of follow-up. Conversely, patients without nutritional deficiencies at baseline had a lower risk of relapse, suggesting that optimal nutritional status is associated with deeper remission.

1. INTRODUCTION

Inflammatory bowel diseases (IBD) are chronic, immune-mediated disorders that affect the gastrointestinal (GI) tract. The two major forms are Crohn's disease (CD) and ulcerative colitis (UC). CD can involve any region of the GI tract and is characterized by transmural inflammation, whereas UC is typically confined to the rectum and colon, affecting primarily the mucosal and submucosal layers [1,2].

The etiology of IBD remains incompletely understood. However, substantial evidence supports the hypothesis that disease onset results from a complex interplay among genetic susceptibility, immune dysregulation, and environmental factors that influence the gut microbiome[3–5]. Among these, diet represents a key environmental contributor. The global rise in IBD incidence has been linked to Western lifestyle and dietary patterns, particularly a high intake of protein and red meat. Such diets promote the production of bacterial metabolites—including ammonia, indoles, phenols, and sulfides—while reducing short-chain fatty acids (SCFAs), all of which may contribute to IBD pathogenesis [6,7].

Accordingly, dietary modifications have been investigated as potential therapeutic strategies. For instance, in pediatric IBD, exclusive enteral nutrition has proven effective in inducing clinical remission, regardless of the specific formula used [8–10].

Nutritional abnormalities—including malnutrition, undernutrition, and overnutrition—are common throughout the disease course [11]. These may result from inadequate intake, altered nutrient requirements and metabolism, malabsorption, excessive gastrointestinal losses, and medication effects [12]. At diagnosis, up to 60% of patients with CD and 35% of those with UC are underweight; however, this proportion has declined in recent years, paralleling the global rise in obesity [13,14]. Currently, 20–40% of adult IBD patients are overweight (body mass index [BMI] 25–30 kg/m²), and an additional 15–40% are obese (BMI > 30 kg/m²) [15].

Obesity in IBD is associated with poorer outcomes, including reduced response to therapy—particularly anti-TNF agents—higher rates of hospitalization, and lower rates of endoscopic remission [16–19]. In overweight IBD patients (BMI ≥ 25 kg/m²), sarcopenia is an independent predictor of surgical intervention ($p = 0.002$) [20]. Moreover, nutritional deficiencies and low serum micronutrient levels negatively affect both induction and maintenance of remission, as well as patients' quality of life [21,22]. Consequently, nutritional assessment is essential in the management of IBD, and current guidelines recommend regular screening for nutritional status, micronutrient deficiencies, and bone mineral density [22,23].

Despite this, data remain limited regarding how malnutrition—particularly micronutrients deficiency and undernutrition—affects disease progression and therapeutic response in IBD. Furthermore, it is unclear whether micronutrient or vitamin supplementation (e.g., vitamin D) exerts therapeutic effects or merely reflects disease activity [24,25].

1.1 Nutritional status and micronutrients in Inflammatory bowel disease

In the increasingly ambitious management of inflammatory bowel disease (IBD), the treat-to-target strategy has evolved to encompass progressively stricter therapeutic goals—ranging from mucosal and transmural healing to histological remission and, ultimately, deep disease remission [26,27]. Within this context, nutritional status must also be regarded as a key therapeutic consideration.

Several nutritional factors may serve as potential targets to achieve better symptom control, deeper remission, and improved overall quality of life in IBD patients. However, evidence regarding the influence of nutritional status on remission induction and surgical risk remains limited [26]. Certain clinical outcomes—such as the risk of surgery, postoperative recurrence (POR), and clinical relapse—require data from randomized controlled trials (RCTs) or long-term interventional studies, which are still largely lacking. Furthermore, most available evidence arises from observational studies characterized by heterogeneous inclusion criteria and outcome measures.

Among the most extensively studied targets, albumin, hemoglobin, iron, ferritin, vitamin B12, folic acid, and vitamin D show the strongest correlations with disease activity, at least in cross-sectional analyses.

1.1.1 Albuminemia

Chronic mucosal inflammation leads to both malabsorption and intestinal protein loss, resulting in hypoalbuminemia.

In IBD, hypoalbuminemia correlates with higher relapse rates, secondary loss of response to biological therapies and increased clearance of biological drugs, such as infliximab and golimumab [28–31].

In CD, higher serum albumin levels are linked to greater clinical remission following infliximab dose escalation [32], whereas hypoalbuminemia is associated with poorer outcomes, increased relapse risk, and discontinuation of anti-TNF therapy when albumin levels fall below 3.5 g/dL ($p = 0.0274$) [33–35]. In general albumin concentrations are directly proportional to adalimumab levels and inversely proportional to anti-adalimumab antibody titters as shown in a cross-sectional study [36]. In UC, hypoalbuminemia is a negative prognostic factor, associated with a more severe disease course [37], greater corticosteroid use, poorer endoscopic response or remission, and primary nonresponse to infliximab [38,39].

Moreover, among patients with acute severe ulcerative colitis, hypoalbuminemia is associated with a higher likelihood of colectomy [38,40,41]. Albumin levels > 3.5 g/dL are an independent predictor of colectomy-free survival (OR 3.03; 95% CI 1.12–8.22; $p = 0.029$) [42], whereas levels < 3 g/dL (HR 2.67; 95% CI 1.20–5.92) are linked to an increased colectomy risk. Admission albumin < 2.45 g/dL independently predicts colectomy (OR 6.10; 95% CI 1.83–20.30) [43,44].

In CD, low serum albumin levels are associated with an increased risk of postoperative—particularly septic—complications, with ORs ranging from 1.21 to 2.23 [45–49]. Serum albumin consistently appears as a key marker of disease course, therapeutic response, surgical outcomes,

and postoperative complications in both CD and UC. However, current evidence does not establish a causal relationship between albumin levels and disease courses.

1.2 Anemia and Micronutrients

Chronic inflammation and intestinal damage contribute to nutrient malabsorption, leading to deficiencies in vitamins and micronutrients essential for overall health [11]. Whether low serum micronutrient levels are a cause or consequence of active disease remains unclear, as does the therapeutic potential of supplementation. Nevertheless, the available evidence consistently shows that active disease is linked to low serum micronutrient concentrations.

Micronutrients most frequently affected in IBD include iron, vitamin D, vitamin B12, folic acid, and vitamins A, E, C, K, B1, and B6. These deficiencies may result from IBD itself or from coexisting autoimmune conditions, such as autoimmune atrophic gastritis (impairing iron and vitamin B12 absorption) and celiac disease (causing iron and folate malabsorption) [50].

1.2.1 Hemoglobin levels

Anemia is a frequent complication of IBD. According to the World Health Organization (WHO), it is defined as hemoglobin (Hb) < 13 g/dL in men and < 12 g/dL in non-pregnant women [51]. The main causes of anemia in IBD include iron deficiency, folate or vitamin B12 deficiency, and chronic disease anemia due to inflammation, or mixed etiologies. Identifying the underlying cause is essential for appropriate treatment [52,53].

Pure iron deficiency anemia is defined by ferritin < 30 µg/L with normal CRP, anemia of chronic disease by ferritin > 100 µg/L with elevated CRP, and mixed anemia by ferritin < 100 µg/L with elevated CRP [54].

Anemia negatively affects quality of life, cognitive function, work capacity, hospitalization rates, and healthcare costs [55].

A retrospective study in CD demonstrated that anemia predicts loss of response to anti-TNF therapy [198]. Anemic CD patients had approximately twice the odds of severe hospitalization compared to non-anemic individuals (OR 1.49; 95% CI 1.37–1.61) [56].
In UC, anemia serves as a marker of disease severity (e.g., Truelove and Witts index) [57,58], a predictor of relapse in 5-ASA–treated patients, and a risk factor for hospital readmission [59,60]. Two prospective studies further confirmed that anemia correlates with disease extension, treatment escalation, and adverse outcomes (hospitalization and surgery) in both CD and UC, suggesting its value in stratifying disease severity [59,61].
Seven retrospective studies examining CD patients undergoing bowel resection found that preoperative anemia is associated with increased postoperative morbidity and mortality [62,63], higher risks of sepsis [49], surgical site infection [64], postoperative complications [176], and prolonged ileus [65].

1.2.2 Iron

Iron deficiency (ID) is one of the most common global disorders, affecting approximately 50% of IBD patients [53,66,67], with a prevalence ranging from 26.5% to 62.5%, making it the most frequent micronutrient deficiency in this population [53,68–70].
Diagnostic thresholds vary with inflammation severity: during remission, ID is defined by ferritin < 30 µg/L and transferrin saturation (TSAT) < 16%, whereas during active disease (CRP > 5 mg/L and/or fecal calprotectin > 150 mg/kg), ID is defined in case of ferritin < 100 µg/L. Iron plays vital roles in oxygen transport, mitochondrial function, gene regulation, and DNA synthesis. Deficiency manifests as fatigue, sleep disturbance, restlessness, impaired cognition and immunity, and reduced physical performance—all of which markedly impair quality of life [71]. Female sex and severe disease activity increase risk, owing to menstrual losses and bloody diarrhea, respectively.

Iron deficiency, even in the absence of anemia, adversely affects health-related quality of life (HRQoL) in IBD, making its early detection and correction clinically important [71–73]. Several studies evaluating intravenous iron therapy in IBD patients without anemia demonstrated significant improvements in ferritin, serum iron, TSAT, symptoms, and HRQoL, irrespective of disease activity [74–76].

No standardized recommendations exist for treating iron deficiency without anemia (IDWA). Oral supplementation is poorly tolerated in up to 20% of patients and may exacerbate symptoms [68,77–79]. Intravenous iron is generally more effective, better tolerated, and associated with fewer adverse effects than oral formulations, but its higher cost limits its use to cases of overt iron-deficiency anemia [80,81].

1.2.3 Vitamin B12 and Folic Acid

Vitamin B12 and folate deficiencies are frequent in IBD. Folate deficiency arises from multiple factors: inadequate intake, malabsorption, increased demand due to inflammation, resection, enteric fistulas, and use of drugs such as sulfasalazine and methotrexate [82,83]. A recent meta-analysis showed that serum folate levels are significantly lower in IBD patients than in healthy controls, particularly in UC [84].

On the other hand, vitamin B12 deficiency is more prevalent in CD (33%) than in UC (16%) [85]. Prior intestinal surgery is an independent risk factor for low vitamin B12 levels in CD [86], and ileal resections > 20 cm markedly increase risk [87], consistent with the distal ileum being the main absorption site.

1.2.4 Vitamin D

Growing evidence supports an immunomodulatory role of vitamin D in IBD [25,88]. Multiple observational and cross-sectional studies, summarized in three meta-analyses, demonstrate lower

serum vitamin D levels in IBD patients than in healthy controls [183,223,224]. Latitude does not appear to influence this association ($p = 0.34$) [223].

All meta-analyses report an inverse relationship between serum vitamin D and disease activity in both UC and CD [183,184]. Low vitamin D levels are associated with increased odds of active disease, mucosal inflammation, and clinical relapse (OR 1.53; 95% CI 1.32–1.77; OR 1.30; 95% CI 1.06–1.60; OR 1.23; 95% CI 1.03–1.47, respectively) [184]. Causality remains uncertain—whether vitamin D deficiency contributes to or results from disease activity.

A meta-analysis of seven RCTs found that vitamin D supplementation reduced the risk of clinical relapse (RR 0.64; 95% CI 0.46–0.89) among 458 IBD patients, particularly in CD in remission (OR 0.47; 95% CI 0.27–0.82) [89]. However, pooled continuous outcomes (e.g., disease activity indices) showed nonsignificant effects.

Other meta-analyses reported reductions in CRP and modest improvements in disease indices [90–93]. Optimal efficacy appears with intermediate doses (10,000–15,000 IU/day) [89]. Observational studies and two RCTs also indicate that low vitamin D levels correlate with poorer response to biologics [24,94–96].

A retrospective study showed that vitamin D < 20 ng/mL was associated with increased risks of surgery (OR 1.76; 95% CI 1.24–2.51) and hospitalization compared with levels ≥ 30 ng/mL in both CD and UC [97]. Vitamin D levels > 30 ng/mL were linked to lower risk of endoscopic recurrence after intestinal resection [98], although supplementation alone did not prevent recurrence in CD [99].

The vast majority of available data comes from observational studies. Consequently, the only robust conclusion that can be drawn is the presence of a significant association between disease activity and nutritional status or micronutrient deficiencies. Whether low micronutrient levels are a cause or a consequence of active disease—and whether supplementation offers therapeutic benefit—remains uncertain.

2. AIMS OF THE STUDY

The objective of this study is to evaluate the efficacy of targeted nutritional assessment and correction of identified nutritional deficiencies in patients with IBD in modifying the risk of disease relapse and activity.

Primary outcomes

Efficacy of nutritional interventions (according to the following protocol) at the end of follow up (54 weeks) evaluated as:

- Clinical activity
- Quality of life

Secondary outcomes

- Relapse rate in patients with baseline clinical deficiencies compared with those without deficiencies.
- The relationship between nutritional status (including micronutrient deficiencies) and clinical as well as laboratoristics disease activity.
- Diagnostic accuracy of micronutrient deficiencies in classifying patients as having active or inactive disease at baseline.

3. METHODS

This is a prospective, randomized, open-label study that will include all patients diagnosed with IBD, irrespective of disease activity (either in remission or with mild disease activity), who are referred to the Gastroenterology, Hepatology and Nutrition, and Internal Medicine and Nephrology Units of the "San Salvatore" Hospital in L'Aquila — Department of Clinical Medicine, Public Health, Life and Environmental Sciences (MeSVA), University of L'Aquila — as well as the Gastroenterology Unit of the Galliera Hospital (Genoa), Gastreoenterlogy Unit of the Ospedale degli Infermi (Rimini).

The following baseline characteristics were collected for all enrolled patients: sex, age, disease type, Montreal classification, disease duration, current therapies, and disease activity status, assessed using clinical scores (Partial Mayo Score [pMS] for ulcerative colitis and Harvey–Bradshaw Index for Crohn's disease [HBI]) and laboratory parameters (C-reactive protein [CRP] and fecal calprotectin).

At baseline, all patients underwent to administration of the Malnutrition Inflammation Risk Tool (MIRT) score for nutritional assessment, along with blood sampling for measurement of circulating hemoglobin, albumin, iron, ferritin, vitamin D, vitamin B12, and folic acid levels.

Patients with IBD and suboptimal nutritional status were randomized to receive:

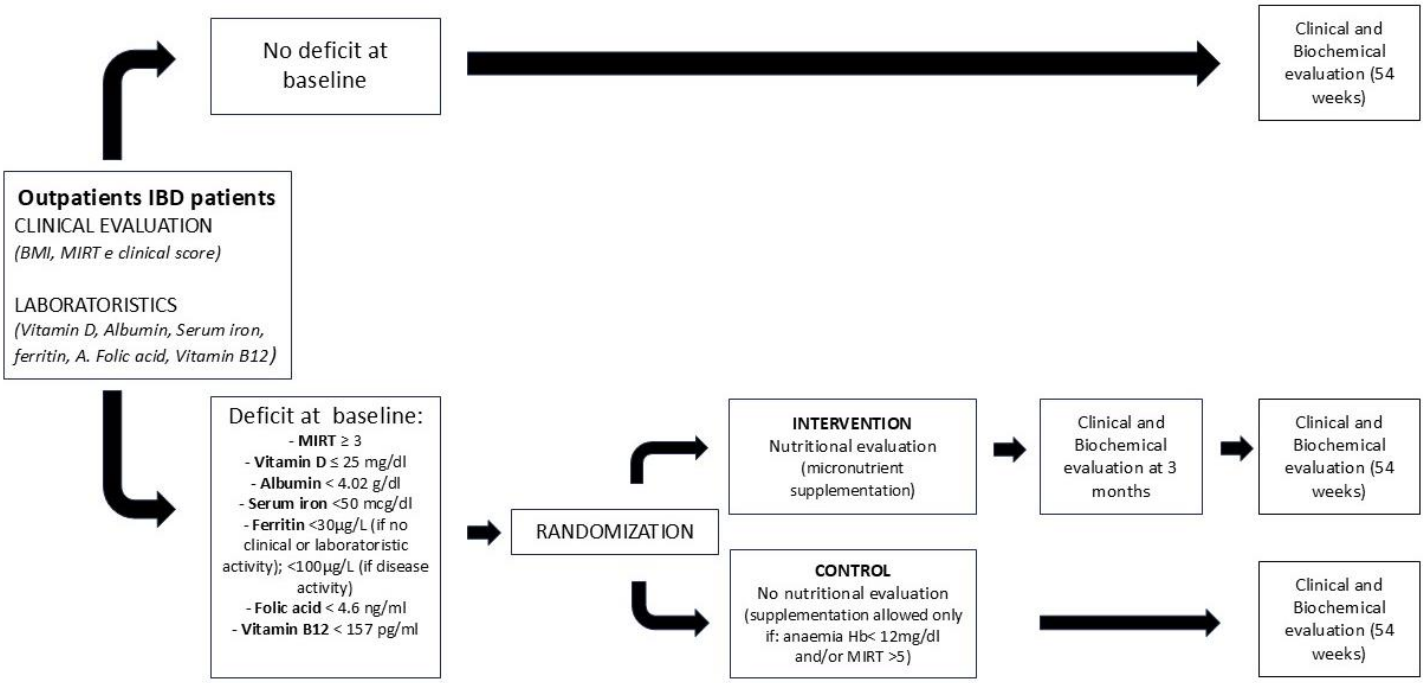
(1) targeted nutritional evaluation with correction of all laboratory deficiencies (intervention group)

(2) no additional nutritional assessment (control group).

(3) Patients without nutritional deficiencies were managed according to standard clinical practice at each enrollment site.

The flow chart outlining the study protocol is shown in Figure 1

Figure 1: Study protocol flow-chart



Inclusion Criteria

- Endoscopic and histological diagnosis of IBD
- Presence of at least one nutritional deficiency as defined in **Table 1**
- Blood tests performed within four weeks of clinical assessment, without changes in therapy during this interval

Exclusion Criteria

- Ongoing supplementation with folic acid, vitamin D, vitamin B12, iron, and/or other nutritional supplements
- Moderate or severe disease activity evaluated with HBI o pMS
- Anemic patients (Hb < 12 g/dL) with known alpha or beta thalassemia trait

Table 1: Definition of deficiencies

Parameter	Definition of Deficiency
MIRT	MIRT ≥ 3
Vitamin D	≤ 25 mg/dL
Albumin	< 4.02 g/dL
Iron	< 50 µg/dL
Ferritin	< 30 µg/L (no clinical/lab activity); < 100 µg/L (with clinical or lab activity)
Folic Acid	< 4.6 ng/mL
Vitamin B12	< 157 pg/mL

Recruitment Period

Eligible patients meeting inclusion and exclusion criteria were enrolled over one year period, from January 1, 2024, to October 30, 2024.

3.1 Randomization

All eligible patients were randomized into either the intervention or control arm using dedicated software managed by the study center of Galliera Hospital (Genoa). The randomization outcome will be communicated simultaneously to the respective investigators.

3.2 Study Procedures and Expected Outcomes

All patients diagnosed with IBD will undergo nutritional assessment using the MIRT (Appendix table 1) and blood tests to determine levels of hemoglobin, albumin, iron, ferritin, vitamin D, vitamin B12, and folate.

Patients will be allocated in a 1:1:1 ratio to the following groups:

1) **Intervention:**

- Active supplementation arm in nutritional deficiency patients

2) **Control:**

- Nutritional deficiency without nutritional supplementations

Patients with malnutrition (MIRT > 5) or anemia (Hb < 12 g/dL) will receive supplementation at the discretion of the treating physician, regardless of study arm.

In cases of micronutrient deficiency without anemia or hypoalbuminemia and with vitamin D > 20 ng/mL, patients in the control group will not receive supplementation.

3) Healthy Control:

- Patients without nutritional deficiencies were included outside of randomization and were managed according to standard clinical practice.

3.3 Disease Activity

The patients included were categorized as dichotomous groups as active and inactive disease at the baseline as follows:

Clinical activity:

- UC: Partial Mayo clinical score (PMS) ≥ 2
- CD: Harvey-Bradshaw Index (HBI) ≥ 5

Laboratory activity:

- Active disease based on calprotectin levels ≥ 150 mcg/kg

3.4 Intervention Procedures (Supplementation Protocol)

Patients in the intervention arm received nutritional interventions according to the following protocol:

- **Folic acid:** 1 tablet daily for 60 days if serum folate < 4.6 ng/mL [100]
- **Cholecalciferol:** 25,000 IU/2.5 mL oral solution every 15 days for 3 months if vitamin D < 25 ng/mL
- **Oral iron:** 1 tablet daily for 60 days if iron < 50 µg/dL, ferritin < 30 µg/L (in the absence of active disease), and iron-binding capacity < 25%

- **Intravenous iron:** Two doses of ferric carboxymaltose administered one week apart in patients with Hb < 12 g/dL (women) or < 13.4 g/dL (men), iron < 50 µg/dL, ferritin < 30 µg/L, and iron-binding capacity < 25%
- **Nutritional counseling:** Referral to a dedicated dietitian if albumin < 4.02 g/dL and/or MIRT ≥ 3
- **Vitamin B12 supplementation:**
    - Intramuscular cyanocobalamin 1000 µg/mL once weekly for 4 weeks if vitamin B12 < 157 pg/mL
    - Oral cyanocobalamin 50 µg/mL daily (Long-Life B12) for 4 weeks if vitamin B12 between 200–400 pg/mL [101,102]

3.4 Efficacy of Nutritional Correction on disease activity

All patients with IBD and suboptimal nutritional status who were randomized to receive either targeted nutritional assessment (by a gastroenterologist or dietitian) or no additional intervention were followed for the entire study period. Disease activity, assessed through clinical evaluation, activity scores, and inflammatory markers, was monitored throughout the study according to the scheduled follow-up visits.

Specifically, follow-up visits will occur at 3 months for the intervention group and at 12 months for all study arms, in line with good clinical practice. Additional visits required for chronic disease management will be permitted and will not result in study withdrawal.

Clinical relapse was defined as the recurrence of symptoms necessitating a change in therapy or the introduction of corticosteroids or a Partial Mayo Score (PMS) ≥ 5 or Harvey-Bradshaw index (HBI) ≥7

3.5 Relationship Between Disease Activity and Serum Nutrient Concentrations

In all enrolled patients, correlations were assessed at baseline between disease activity—evaluated clinically (history, physical examination, and clinical activity scores) and biochemically (CRP and fecal calprotectin)—and nutritional status.

3.6 Statistical analysis

All statistical analyses were performed using appropriate parametric and non-parametric methods, depending on data distribution. Continuous variables were assessed for normality using the Shapiro–Wilk test and are presented as mean ± standard deviation or median with range, as appropriate. Categorical variables were reported as frequencies and percentages.

Categorical variables were compared using the χ^2 test or Fisher's exact test when expected cell counts were <5. Group comparisons of continuous variables were conducted using analysis of variance (ANOVA) with a Kruskal-Wallis test planned for normally distributed data.

An unpaired two-sample Welch t-test was performed to compare the micronutrients mean levels among active and inactive patients at the baseline.

The micronutrients that demonstrated differences between the active and inactive disease groups were included in subsequent analyses.

Pearson correlation analysis was performed to explore the relationship between disease activity and nutritional status. Additionally, receiver operating characteristics (ROC) analysis were performed to determine for identifying patients with active diseases. This was performed twice, for clinical and laboratory evidence of disease activity, respectively.

A multivariate analysis was performed, including the micronutrients that differed between the active and inactive groups, and adjusting for CRP levels.

MS Excel v.2016 (Microsoft Corporation, USA, 2016) and Stata v.17 (College Station, TX: StataCorp LLC, 2021) were used for calculations and graph creation. Significance level was set at p = 0.05 for all inferential analysis.

3.7 Sample size

A cumulative 1-year CD relapse frequency of 50% has been reported [103].

We powered the study at 70% and alpha at 5%. To detect a relative risk reduction of relapse of 50% or ⁄ and an absolute risk of 30% in the intervention group, we needed 31 in each treatment arm. We planned to enroll 40 patients in each treatment-arm as we took into account an approximate drop-out of 10%

4. RESULTS

4.1 Patients' characteristics

At the end of the enrollment period, 120 IBD patients (46 CD; 74 UC) were included.

Overall, 27/120 (22%) patients presented clinical activity, 40/120 (33%) showed high value of calprotectin. The baseline characteristics are reported in Table 2.

Table 2: Baseline characteristics among active/inactive groups.

	Clinical Active Disease (n = 27)	**Clinical Inactive Disease (n = 93)**
Disease		
CD	10	35
UC	17	58
Sex gender		
Male	13	55
Female	14	38
Therapy		
Advanced therapies	13	50
Conventional therapies	14	43
Disease duration (months)	Median: 88.7 (Range: 6–300)	Median: 96.0 (Range: 6–588)
BMI		
CD	23.35 (4.41)	24.29 (2.56)
UC	24.5 (3.23)	24.63 (2.90)
Localization		
CD		
Ileal	5	19
Ileo-colic	5	14

Colic	0	2
UC		
Ulcerative proctitis	7	19
Left side colitis	6	26
Extensive colitis	4	13

4.2 Primary Outcome

A total of 21/120 patients (17.5%) experienced a clinical relapse during follow-up or had active disease at T1 (9 cases across the intervention and 9 control groups; 3 in the healthy control group). No significant difference was observed between the intervention and control groups (p = 0.94; 95% CI 0.33–2.79). However, when patients were stratified according to the presence or absence of nutritional deficiencies at baseline, those without deficiencies had a significantly lower risk of clinical relapse (p = 0.047; 95% CI 0.07–0.98; FigureS 2 and 3). Notably, only patients in the intervention group demonstrated a significant improvement in quality of life, as measured by the SF-36 score, from baseline to the end of follow-up (T0: 118 vs. T1: 142; p = 0.007; Table 3). Conversely, no significant reduction in IBD-DI scores was observed in either the intervention or control groups (Figures 4 and 5).

Table 3: Mean quality of life evaluated with SF-36 and IBD-DI scores among intervention and control groups.

	SF-36			**IBD-DI**		
	T0	**T1**	Delta (*p*)	**T0**	**T1**	Delta (*p*)
Intervention	118	142	Δ + 24 (0.007)	56	53	Δ - 3 (0.29)
Control	122	109	Δ - 13 (0.2)	58	53	Δ - 5 (0.26)

4.3 Micronutrients Serum Level and Disease Activity

The serum level of Hb, iron, ferritin and vitamin D was different among the active and inactive group (p: 0.007; p: 0.001; p: 0.005; p: 0.003) while no difference was found among the other micronutrients evaluated (folic acid, vitamin B12) and albumin. The results were reported in Table

4. Figure 6 showed the box plot of the mean serum level of Hb, iron, ferritin and vitamin D among the active and inactive groups. Mean serum levels among the active and inactive groups are reported as Supplementary Table S2 in the appendix section.

Table 4: Biochemical mean levels and differences among active and inactive groups, high and low calprotectin level groups, and active and inactive groups.

Item	**Mean Difference Among Clinical Active/Inactive Groups (Standard Error)**	***p*-Value (95% CI)**	**Mean (SD)**
Hemoglobin	Δ + 1.02 mg/dL (0.39)	$p = 0.007$ (13.45–14.26)	Active: 13.05 (1.42) Inactive: 14.07 (2.13)
Iron	Δ + 33.03 µg/dL (8.82)	$p < 0.001$ (79.46–97.13)	Active: 61.88 (31.73) Inactive: 94.90 (43.55)
Ferritin	Δ + 44.59 ng/dL (16.90)	$p = 0.005$ (70.25–107.68)	Active: 53.87 (56.40) Inactive: 98.46 (96.87)
Vitamin D	Δ + 6.84 mg/dL (2.35)	$p = 0.003$ (2.2.79–27.68)	Active: 19.67 (7.61) Inactive: 26.51 (11.59)
Vitamin B12	Δ + 107.3 pg/dL (102.90)	$p = 0.16$ (−336.72–122.12)	Active: 370.00 (136.62) Inactive: 477.30 (318.67)
Folic Acid	Δ − 1.90 ng/dL (2.98)	$p = 0.267$ (−8.30–4.49)	Active: 8.43 (10.40) Inactive: 6.53 (5.39)
Albumin	Δ + 0.12 g/dL (0.13)	$p = 0.203$ (−0.16–0.39)	Active: 4.12 (4.05) Inactive: 4.23 (3.90)
Calprotectin	Δ − 917.44 µg/gr (285.27)	$p = 0.002$ (186.10–474.20)	Active: 1062.25 (1266.58) Inactive: 144.81 (303.93)
Item	**Mean difference among Low/High Calprotectin groups (Standard Error)**	***p* value (95% CI)**	**Mean (SD)**
Hemoglobin	Δ + 0.79 mg/dL (0.31)	$p = 0.006$ (0.18–1.42)	High: 13.49 (1.60) Low: 14.29 (1.41)
Iron	Δ + 17.04 µg/dL (9.18)	$p = 0.033$ (−1.21–35.29)	High: 77.87 (49.56) Low: 94.91 (37.96)
Ferritin	Δ + 66.32 ng/dL (18.76)	$p < 0.001$ (29.03–103.60)	High: 50.83 (40.98) Low: 117.14 (104.54)
Vitamin D	Δ + 5.51 mg/dL (2.49)	$p = 0.015$ (0.56–10.47)	High: 21.48 (8.72) Low: 26.99 (11.83)
Vitamin B12	Δ + 72.66 pg/dL (52.63)	$p = 0.08$ (−33.09–178.41)	High: 352.09 (123.65) Low: 424.75 (225.85)
Folic Acid	Δ + 0.42 ng/dL (1.77)	$p = 0.407$ (−3.12–3.96)	High: 6.79 (7.76) Low: 7.21 (6.05)
Albumin	Δ + 0.01 g/dL (0.17)	$p = 0.466$ (−0.33–0.36)	High: 4.19 (0.35) Low: 4.21 (0.81)
Item	**Mean difference among Active/Inactive groups † (Standard Error)**	***p* value (95% CI)**	**Mean (SD)**
Hemoglobin	Δ + 0.48 mg/dL (0.20)	$p = 0.106$ (−0.28–1.23)	Healthy: 14.04 (2.29) Diseased: 13.56 (1.55)
Iron	Δ+ 14.78 µg/dL (9.43)	$p = 0.061$ (−4.06–33.61)	Healthy: 94.23 (38.37) Diseased: 79.45 (48.77)
Ferritin	Δ + 48.66 ng/mL (16.06)	$p = 0.002$ (16.74–80.58)	Healthy: 108.61 (105.09) Diseased: 59.95 (50.66)
Vitamin D	Δ + 5.47 mg/dL (2.25)	$p = 0.009$ (0.98–9.95)	Healthy: 27.24 (11.80) Diseased: 21.77 (9.09)

Vitamin B12	Δ − 5.14 pg/dL (52.83)	$p = 0.462$ (−111.91–101.64)	Healthy: 387.82 (144.51) Diseased: 392.96 (226.11)
Folic Acid	Δ + 0.79 ng/mL (1.72)	$p = 0.322$ (−2.64–4.24)	Healthy: 7.29 (6.22) Diseased: 6.49 (7.25)
Albumin	Δ − 0.01 g/dL (.15)	$p = 0.469$ (−0.31–0.28)	Healthy: 4.20 (1.81) Diseased: 4.22 (0.33)
Calprotectin	Δ − 734.12 µg/gr (156.87)	$p < 0.001$ (−1051.39–416.85)	Healthy: 41.15 (39.15) Diseased: 775.28 (991.61)

4.4. Clinical Activity: Logistic Regressions and ROC Analysis

Hb and the micronutrients that demonstrated differences between the active and inactive disease groups were included in subsequent analyses.

Hb levels showed a suboptimal correlation with clinical disease activity in IBD patients (OR 0.80; 95% CI 0.62–1.03). The ROC analysis yielded an Area Under the Curve (AUC) of 0.70 (p = 0.06), suggesting limited discriminatory power. Iron and vitamin D levels demonstrated the highest accuracy in the ROC analysis, with AUCs of 0.76 (p < 0.001) and 0.68 (p = 0.013), respectively. Iron levels were associated with an OR of 0.97 (95% CI: 0.95–0.98), while vitamin D levels had an OR of 0.92 (95% CI: 0.86–0.99), indicating a significant inverse association with disease activity (Table 5; Figure 7).

4.5 Calprotectin: Logistic Regressions and ROC Analysis

Vitamin D and Ferritin showed the better performance at the ROC analysis considering the disease activity assessed as dichotomic value based on calprotectin levels.

However, their AUC were sub-optimal (AUC 0.68; p < 0.001; AUC 0.66; p = 0.19) with an OR of 0.98 (95% CI 0.97–0.99) and 0.94 (95% CI 0.89–0.99), respectively (Table 5; Figure 8).

Table 5: Normal levels of micronutrients and relative Odds Ratio and Area Under the Curve in active group (both clinical and calprotectin activity).

	Clinical Activity		Calprotectin	
Micronutrients and Hemoglobin	**Odds Ratio (95% CI)**	**AUC**	**Odds Ratio (95% CI)**	**AUC**
Hemoglobin	0.80 (95% CI: 0.62–1.03)	0.70 ($p = 0.06$)	0.69 (95% CI: 0.51–0.93)	0.64; $p = 0.01$
Iron	0.97 (95% CI: 0.95–0.98)	0.76 ($p < 0.001$)	0.98 (95% CI: 0.97–1.00)	0.68; $p = 0.05$
Ferritin	0.98 (95% CI: 0.97–1.00)	0.68 ($p = 0.018$)	0.94 (95% CI: 0.89–0.99)	0.68; $p = 0.19$
Vitamin D	0.92 (95% CI: 0.86–0.99)	0.69 ($p = 0.013$)	0.98 (95% CI: 0.97–0.99)	0.66; $p < 0.001$

4.6 Multivariate Analysis of Micronutrients and Disease Activity

In the multivariate model, which included micronutrients and adjusted for inflammatory status using serum CRP values, the association between micronutrient levels and disease activity was weak and not statistically significant in the multivariate analysis (Table 6).

Table 6: Multivariate model incorporating micronutrients and disease activity, defined by both calprotectin levels and clinical activity.

Micronutrients and Hemoglobin	**Odds Ratio (95% CI)**	***p* Value**
Hemoglobin	0.75 (0.50–1.12)	0.170
Iron	0.99 (0.98–1.00)	0.655
Ferritin	0.99 (0.92–1.02)	0.082
Vitamin D	0.97 (0.93–1.02)	0.270
CRP	1.07 (0.93–1.24)	0.314

5. DISCUSSION

An increasing body of evidence has highlighted a strong correlation between nutritional status and disease activity in IBD [104]. As a result, the assessment of nutritional status in these patients is considered relevant, and current guidelines recommend routine screening for nutritional status, micronutrient deficiencies, and bone mineral density [22,105104].

Deficiencies in nutrients and low serum levels of micronutrients have been associated with poorer outcomes, including reduced quality of life and impaired induction and maintenance of remission [17]. However, existing data are often conflicting and not sufficiently robust to draw definitive conclusions regarding the impact of micronutrient levels on disease course .

Moreover, evidence on how micronutrient deficiencies influence disease progression and therapeutic response remains limited [21]. It also remains unclear whether supplementation of specific micronutrients or vitamins, such as vitamin D, acts as a therapeutic intervention or simply reflects disease activity and nutritional depletion secondary to inflammation [26,106].

Conversely, the use of micronutrients and standard biochemical parameters (e.g., leukocyte and platelet counts, hemoglobin levels) as biomarkers of IBD activity has been investigated, but results remain inconsistent [24,107–109].

The primary outcome of this study showed that, under standard-of-care therapy, nutritional supplementation alone did not reduce the risk of clinical relapse ($p = 0.94$; 95% CI 0.33–2.79). However, supplementation was associated with a significant improvement in quality of life exclusively in the intervention group. The mean SF-36 score increased from 118 at baseline (T0) to 142 at follow-up (T1) ($p = 0.007$).

In contrast, when the study population was stratified by baseline nutritional status, patients without deficiencies demonstrated a significantly lower risk of clinical relapse compared with those with deficiencies ($p = 0.047$; 95% CI 0.07–0.98; Figures 2 and 3).

The subsequent analysis was conducted exploring the correlation among nutritional deficiency and disease activity at the baseline. All the conducted analysis showed a suboptimal correlation between the evaluated micronutrients and disease activity. It could be possible that the outpatient setting contributed to the selection of a patient population with less severe disease and better nutritional status (only patients with mild or inactive disease were included as per protocol). These rigorous inclusion criteria could reduce the observed differences between active and inactive groups. Nonetheless, this inclusion criteria allows us to select a more homogeneous population among the deficiency and the healthy control group that likely presented less severe disease. Thus, this clinical context closely reflects the unselected patient population encountered in routine clinical practice. Additionally, the relatively small sample size limited the ability to perform sub-group analyses, which may have provided further insights. As reported in other studies, deficiencies in certain micronutrients appear to be more pronounced in patients with CD than in those with UC. This finding is more robust for vitamin D and vitamin B12, especially in CD patients who have undergone ileal resection [89,106]. However, the limited sample size in the present study does not allow for sub-analyses to robustly verify this observation.

Conversely, the stringent inclusion criteria, which excluded patients receiving any form of nutritional supplementation, enhance the robustness and reproducibility of our results.

Notably, serum vitamin D levels demonstrated the highest accuracy in discriminating disease activity, both clinical and biochemical, in ROC analyses—with AUCs of 0.68 ($p = 0.013$) and 0.68 ($p < 0.001$), respectively. Furthermore, vitamin D showed a significant inverse association with active disease, with odds ratios of 0.92 (95% CI: 0.86–0.99) for clinical activity and 0.98 (95% CI: 0.97–0.99) for biochemical activity. The data concerning vitamin D appears to be the most robust among the evaluated micronutrients and is consistent with previously published findings in the literature [110].

Previous evidence has suggested a relationship between vitamin D supplementation and disease course in patients with IBD [89]. However, these findings remain conflicting, as highlighted in a recent meta-analysis [106].

Regarding serum hemoglobin, mean levels were higher in the inactive group; however, the correlation with disease activity was less pronounced (OR 0.80; 95% CI: 0.62–1.03).

Although ferritin is an acute-phase reactant and its serum levels may be elevated in IBD patients with active inflammation, its association with disease activity appears more reliable in patients with low-grade systemic inflammation, as evidenced by the good correlation observed in the population included in this study. Although differences between the active and inactive groups were consistent for all micronutrients analyzed, some minor discrepancies emerged (e.g., ferritin was associated with calprotectin but not with clinical activity).

This discrepancy can be speculatively explained by the weak correlation between clinical activity and endoscopic inflammatory activity. Micronutrient deficiencies are more prevalent in hospitalized patients, as well as in those with active IBD and long-standing CD. According to current guidelines, monitoring of micronutrient levels should be considered in people with IBD, and supplementation is recommended in cases of deficiency [111].

These data showed no benefit of a treat-to-target nutritional strategy in reducing the risk of clinical relapse in an unselected outpatient cohort of patients with IBD. These findings suggest that, although nutritional deficiencies (e.g., vitamin D) correlate well with disease activity, targeted supplementation as an adjunctive therapeutic strategy does not appear to reduce the risk of relapse. Moreover, the limited magnitude of these differences does not support the use of serum micronutrient levels as reliable biomarkers for identifying disease activity.

In conclusion, a nutritional treat-to-target strategy does not appear to influence the clinical course in an unselected outpatient IBD population. However, it does seem to improve overall quality of life. On the other hand, hemoglobin, iron, ferritin, and vitamin D levels were associated with disease activity status. However, despite these correlations, their accuracy in distinguishing between active and inactive disease was suboptimal, limiting their utility as reliable biomarkers. In contrast, folic acid, vitamin B12, and albumin levels demonstrated poor concordance with disease activity, further reducing their potential clinical relevance in this context.

6. FIGURES

Figure 1: Study protocol flow-chart

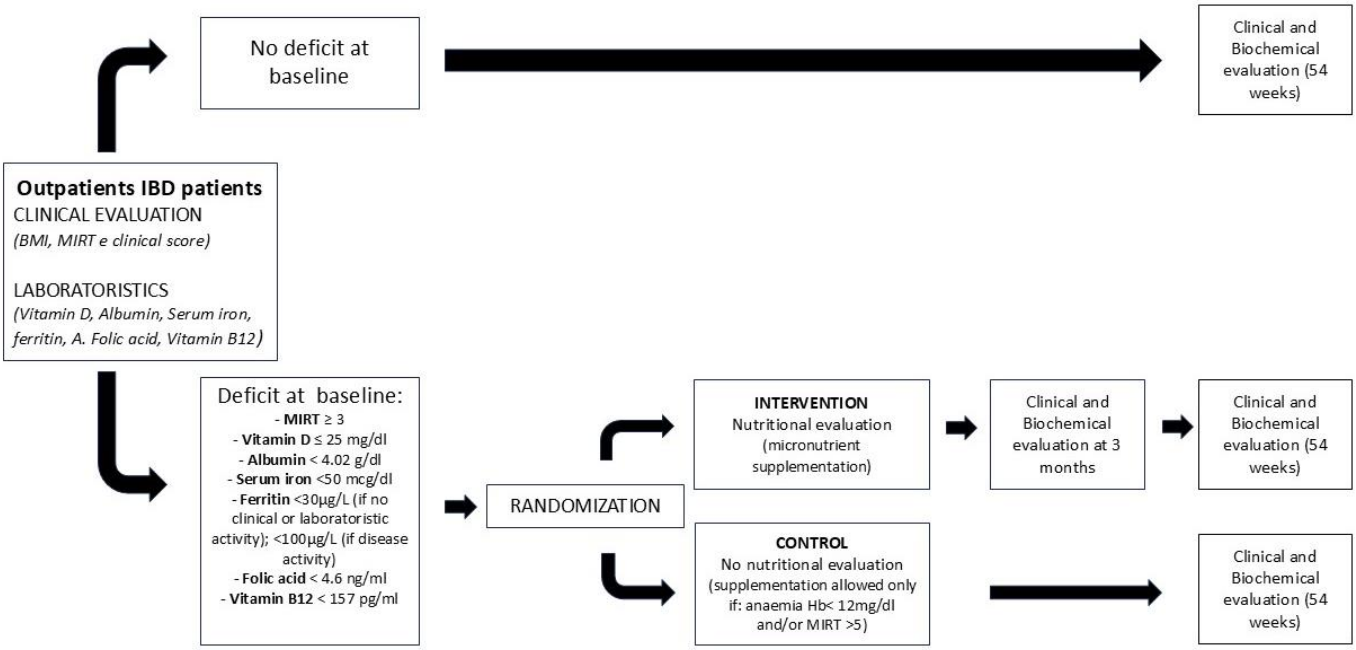


Figure 2: Risk of clinical relapse at the end of study period among the three groups

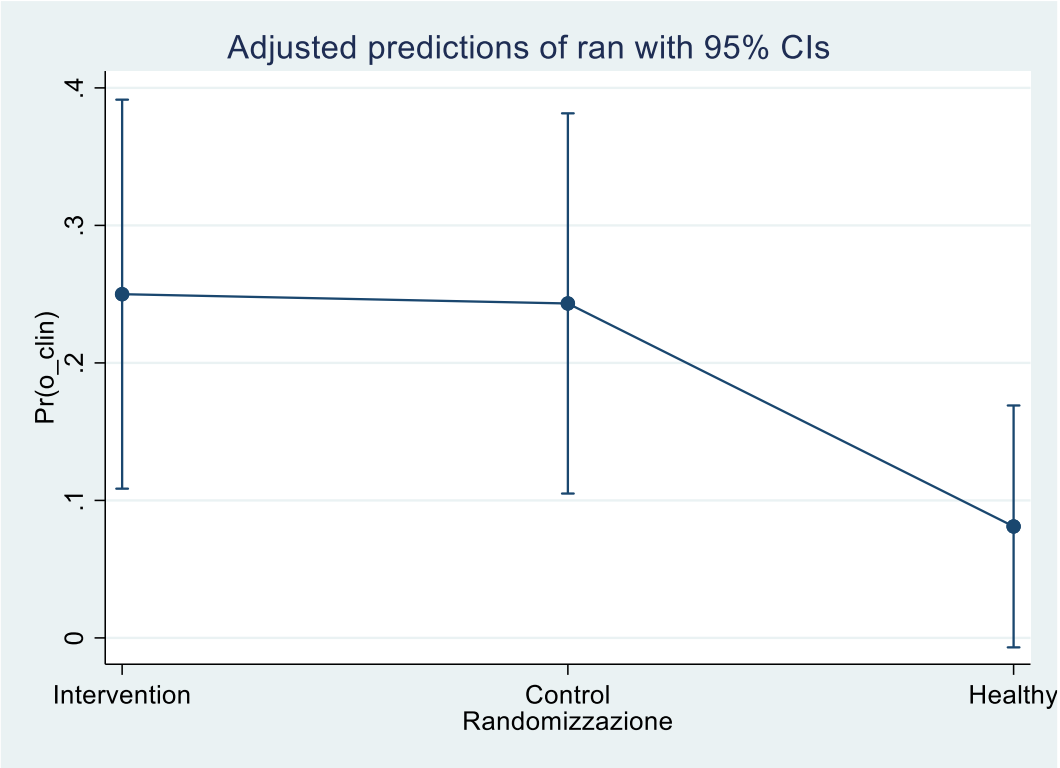


Figure 3: Clinical relapses at the end of follow-up stratified by baseline nutritional deficiency.

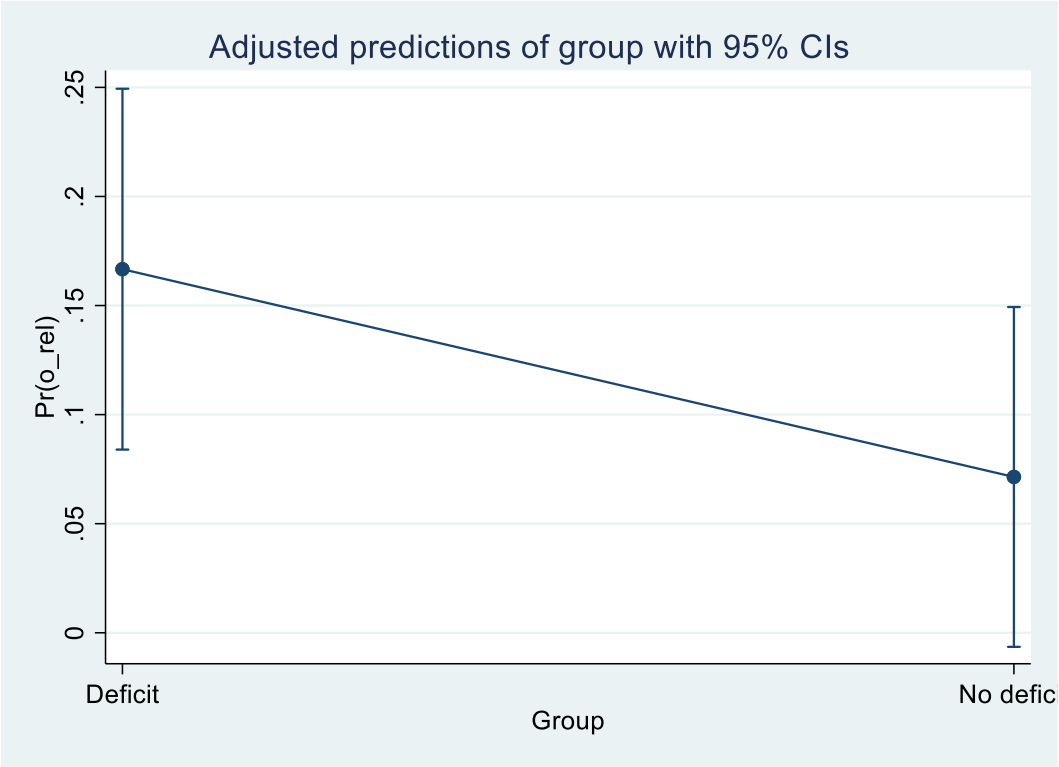


Figure 4: QoL evaluated with SF-36 score at T0 and T1 among intervention and control groups.

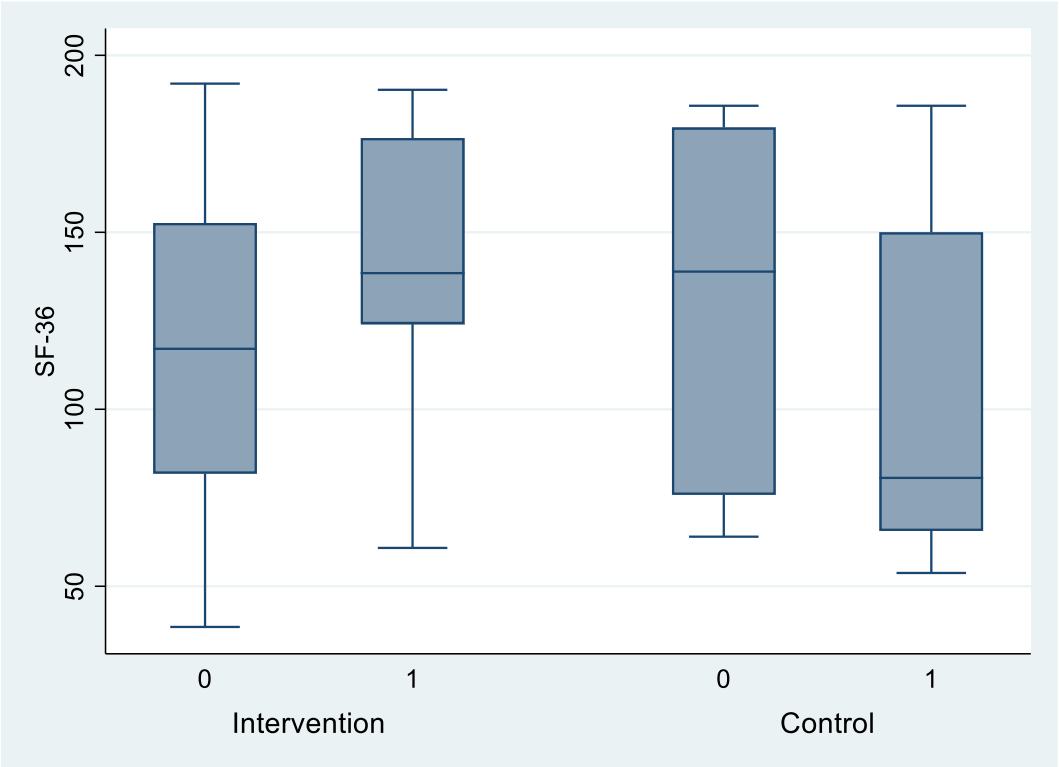


Figure 5: QoL evaluated with IBD-DI score at T0 and T1 among intervention and control groups.

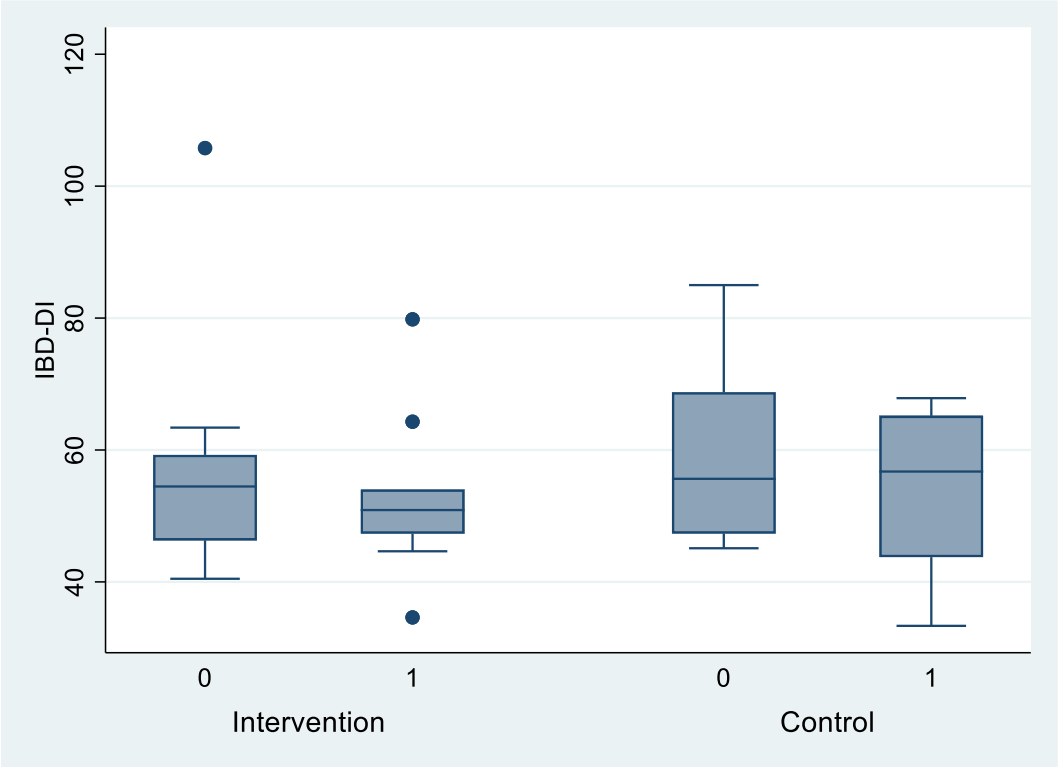


Figure 6: The boxplot shows the mean serum level of ferritin, iron, vitamin D and Hemoglobin among active and inactive groups

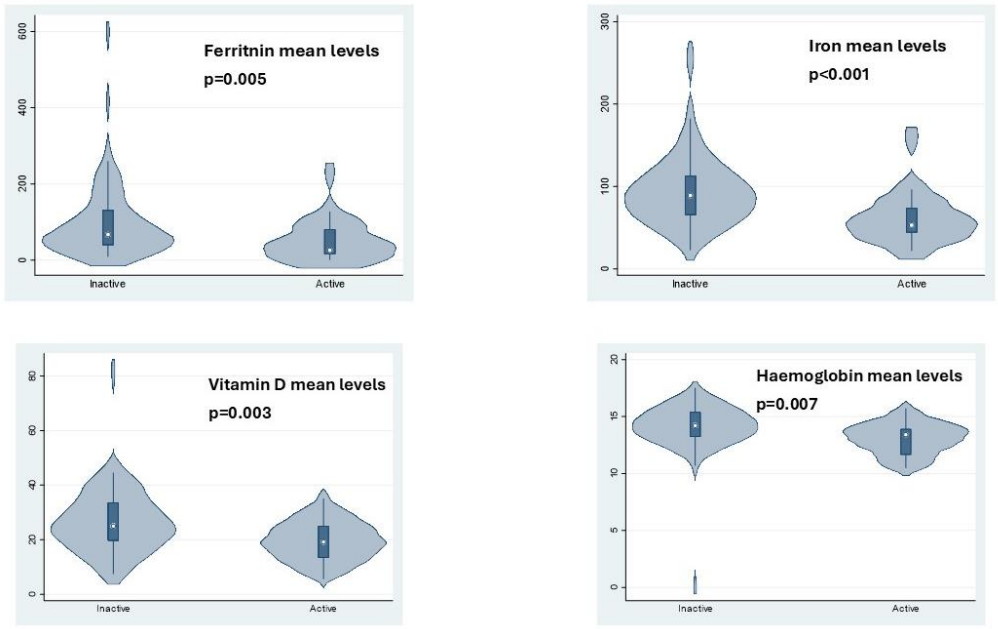


Figure 7: The receiver operating characteristic (ROC) curve shown accuracy of micronutrients and hemo-globin to identify patients with active diseases based on clinical activity. AUC: Area Under the Curve.

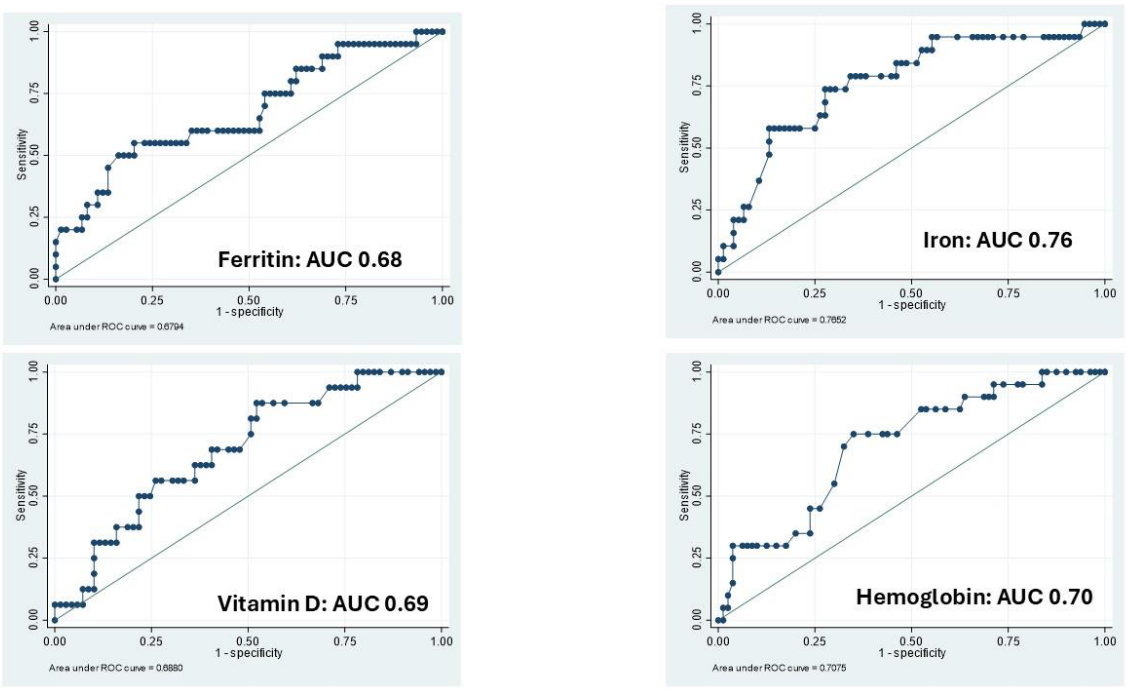
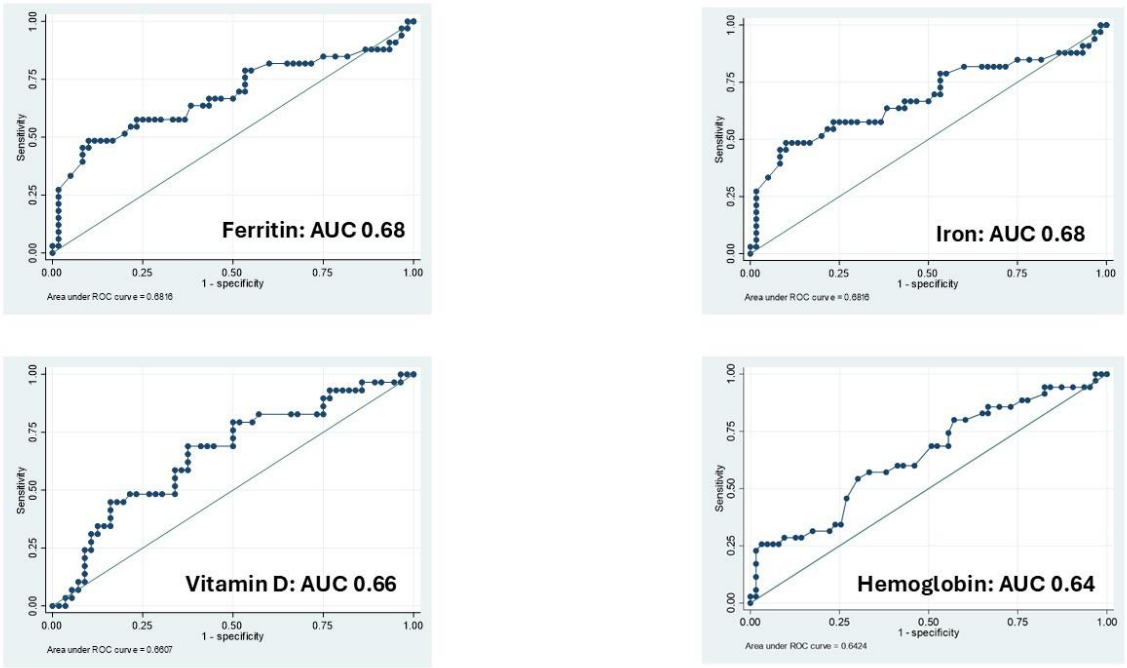


Figure 8: The receiver operating characteristic (ROC) curve shown accuracy of micronutrients and hemoglobin to identify patients with active diseases based on calprotectin value. AUC: Area Under the Curve.



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APPENDIX

Table S1: Malnutrition Inflammation Risk tool (MIRT)

		Points		Points		Points
BMI (kg/m^2)	>20	0	18·5–20·0	1	<18·5	2
Weight loss 3 months (%)	<5	0	5–<10	2	≥10	3
CRP (mg/l)	<5	0	5–50	2	≥50	3

* Score: 0–8 points.

Table S1: Mean serum level (±SD) among active and inactive groups

	Inactive group (±SD)	**Active group (±SD)**
Hemoglobin	14.06 (2.13)	13.05 (1.4)
Iron	94.90 (43.54)	61.87 (31.72)
Ferritin	98.45 (96.87)	53.87 (56.40)
Vitamin D	26.51 (11.59)	19.66 (7.60)
Vitamin B12	370 (136.61)	477.3 (318.66)
Folic acid	6.52 (5.38)	8.42 (10.40)
Albumin	4.23 (0.69)	4.11 (0.35)

Table S2: normal reference values and corresponding units of measurement

	Normal values
Hemoglobin (gr/dl)	Male ≥ 13; Female ≥ 12
Iron (µg/dl)	50-170
Ferritin ng/ml	4-210
Vitamin D – 25(OH)D (mg/dl)	<25
Vitamin B12 (pg/ml)	157-1059
Folic acid (ng/ml)	4.6-18.7
Albumin g/dl	4.02-4.76