Exploring the concept of deep remission in Crohn's disease: correlation between transmural healing and biomarkers

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Abstract

Background: While mucosal healing (MH) and transmural healing (TH) predict relevant clinical outcomes in Crohn's disease (CD), little is known about the real significance and clinical impact of deep remission (DR).

Objectives: To better explore the concept of DR, toward a direct correlation between MH, TH, and biomarkers.

Design: Real-world observational longitudinal study to evaluate the rate of clinical remission (CR), MH and TH, and the fecal calprotectin (FC)/C-reactive protein (CRP) levels in all consecutive CD patients on biologics.

Methods: A receiver operating characteristic (ROC) curve was constructed to define the best FC and CRP cut-offs associated with MH and TH. Finally, patients achieving CR, MH, and TH, in association with the target FC/CRP values, were considered in DR.

Results: Among 118 CD patients, CR, MH, and TH were achieved in 62.7, 44.1, and 32.2%, respectively. After 2 years, the mean FC levels decreased from $494 \pm 15.4 \mu g/g$ to $260 \pm 354.9 \mu g/g$ ($p < 0.01$). Using the ROC curve analysis, an FC cut-off value of 94 µg/g was associated with both MH [sensitivity: 94.2%, specificity: 84.8%, positive predictive value (PPV): 83.05%, negative predictive value (NPV): 94.92%, area under the curve (AUC): 0.95] and TH (sensitivity: 92.1%, specificity: 70%, PPV: 64.4%, NPV: 94.9%, AUC: 0.88). CRP < 5 mg/L was associated with both MH (sensitivity: 96.1%, specificity: 62.1%, PPV: 66.7%, NPV: 95.35%, AUC: 0.85) and TH (sensitivity: 97.4%, specificity: 52.5%, PPV: 52%, NPV: 95.35%, AUC: 0.78). When considering CD patients with concomitant CR, MH, and TH associated with an FC < 94 µg/g and CRP < 5 mg/L, this association was found identified in 33 patients (27.9%).

Conclusion: An FC < 94 µg/g and a normal CRP are associated with CR, MH, and TH and could be included in the definition of DR in association. So by definition, DR could be achieved in approximately 30% of CD patients during maintenance treatment with biologics.

Keywords: biologics, Crohn's disease, deep remission, fecal calprotectin, mucosal healing, transmural healing

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Introduction

Crohn's disease (CD) is a transmural, chronic, and life-threatening inflammatory disease, which often leads to surgery due to complications such as strictures, fistulae, and abscesses.[1–3] To prevent such complications and the subsequent irreversible bowel damage, the cornerstone of the treatment is the disease remission, which should ideally comprise clinical, biochemical, mucosal, and transmural healing (TH), although the ultimate therapeutic target in CD is still a matter of debate.[4]

Practically, Abundant evidence has previously shown that mucosal healing (MH), defined as the endoscopic healing of any inflamed tract of the mucosal layer, is a pivotal outcome in CD able to predict lower request for steroids, hospitalization, and surgery in the years following treatment.[5–9]

More recently, some studies have 'explored the deep', demonstrating that TH, defined as the normalization of the bowel wall thickness (BWT) of all inflamed segments involved in CD,[3,10–16] is associated with improved long-term outcomes in CD by reducing clinical relapses, hospitalization, and surgery rates, even better than MH.

Thanks to this new evidence, in the last few years, the concept of deep remission (DR) has changed from clinical and endoscopic remission (MH)[17] to combined clinical, endoscopic (MH), and TH.[4]

The current definition of DR does not take into account the role of biochemical markers, such as C-reactive protein (CRP) and fecal calprotectin (FC), which have demonstrated a prognostic significance. In particular, FC is able to predict long-term clinical outcomes when measured 12 weeks after the onset of medical treatment.[18] The STRIDE-II systematic review supported using an FC cut-off value of 150 mg/g to identify MH.[4] Although the correlation between FC and endoscopic findings has been well-explored, very few studies report the relationship between FC and TH.

Similarly, CRP, which is widely used in clinical practice but is outperformed by FC, is characterized by high specificity but low sensitivity[19,20] and has been associated with response to treatment and risk of relapse.[21]

Due to the importance of the treat-to-target strategy, further studies are urgently needed to evaluate the new endpoints (i.e. TH, DR) that take into account the entire complexity of the disease.

The aim of the present study was to better explore the concept of DR in CD, toward the correlation between MH, TH, and biochemical markers, especially FC.

Materials and methods

Study population and study design
From 2018 to 2021, we carried out a real-world observational, longitudinal, prospective study to evaluate the rate of clinical remission (CR), MH, and TH in all consecutive adult patients with CD undergoing maintenance treatment with biologics (infliximab, adalimumab, vedolizumab, and ustekinumab) for 2 years at our tertiary Inflammatory Bowel Disease (IBD) Unit of 'Federico II' University Medical School in Naples, Italy. For each included subject, we investigated the CRP and FC levels associated with CR, MH, and TH in order to better assess the concept of DR.

The CD diagnosis was made in accordance with current European Crohn's and Colitis Organization (ECCO) guidelines.[22] The indications for the use of biologics in CD patients had followed ECCO and Italian guidelines.[22,23]

All patients underwent clinical assessment, laboratory exams, including CRP and FC evaluation, at our centralized laboratory, and bowel sonography (BS) and endoscopy before and after a 2-year treatment period with biologics, as routinely performed at our center. All these evaluations were performed within 14 days. Based on the combined clinical, endoscopic, and sonographic findings, all patients that participated in the study were divided into three groups: (a) the TH group, which included patients who had achieved TH plus MH; (b) the MH group, which comprised patients who showed MH only; and (c) the CR group, which included patients with CR, but who displayed pathological BS and endoscopy.

We excluded all subjects without FC and/or CRP evaluation, who were treated with biologics for less than 2 years and who refused endoscopic examination. Furthermore, we excluded all subjects who underwent biologic treatment for prophylaxis of post-operative recurrence.

Stool samples were collected at the patients' homes, refrigerated at 2°C–8°C, then sent to our centralized laboratory to be processed and analyzed according to the manufacturer's instructions (Calprest; Eurospital Spa, Trieste, Italy). FC levels were expressed as µg/g of feces. An FC

value >120 μg/g was considered 'positive' or indicative of active inflammation; a CRP value <5 mg/L was considered 'negative', while a result higher than 5 mg/L was considered 'positive'.

The reporting of this study conforms to the STROBE statement.[24]

Clinical remission

In accordance with ECCO statements,[22] steroid-free CR was identified in the presence of Harvey–Bradshaw index <5 in patients who did not need treatment with systemic steroids or budesonide.

Mucosal healing

Ileocolonoscopy was used to assess MH. It was performed with a conventional colonoscope (Olympus Exera CV-190) by two expert operators (NI and AR) who were blinded to the outcome of the other diagnostic procedures, after standard bowel cleansing with a split dose of 4-L solution of polyethylene glycol. The endoscopic activity of CD and occurrence of MH both at the start of the study and during the follow-up were assessed using the Simple Endoscopic Score for Crohn's Disease (SES-CD).[9,25,26] MH was identified in the presence of endoscopic remission in the entire bowel by a per-patient evaluation (SES-CD ≤ 2).[9] An esophagogastroduodenoscopy was performed in case of involvement of the proximal gastrointestinal tract by CD.

Transmural healing

BS was used to evaluate TH. It was performed at the start of the study and every 3 months during the follow-up period after overnight fasting using a Logiq S7 ultrasound system with linear and convex probes (5–9 MHz). Neither special preparation nor contrast and/or paralytic agents were administered. The ultrasound procedure was performed by two experienced gastroenterologists (FC and AT) who were blinded to the outcomes of other diagnostic procedures. Each patient underwent a systematic scanning of the abdomen.

BWT was considered normal for values ≤3 mm.[27] Positive BS was defined as the occurrence of concentric and regular increased BWT > 3 mm.[28] The BS definition of complicated disease was defined in accordance with the literature.[28] In particular, strictures were diagnosed in the presence of thickened (>4 mm) intestinal wall, narrowed intestinal lumen, and fluid-distended or echogenic content-filled loops just above the thickened intestinal tract. Furthermore, enterocutaneous, enteroenteric, and enteromesenteric fistulas were scored when hypoechoic duct-like structures with fluid or air content were seen between skin and intestinal loops, between two intestinal loops, or between intestinal loop and mesentery, respectively.[28] TH was identified and recorded with BS by a global per-patient evaluation. In accordance with existing evidence, a decrease in BWT to values ≤3 mm was considered diagnostic of TH.[28,29] Moreover, sonography was able to identify antral or duodenal inflamed or healed segments in all enrolled cases.

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS software v.15.0, Chicago, IL, United States) for Windows and the StatsDirect Statistical Software (v.3.0). The descriptive statistics used included determination of mean values and standard deviations (SDs), or medians and interquartile ranges, of the continuous variables, and of percentages and proportions of the categorical variables.

Statistical analysis was performed using chi-square, the Mann–Whitney *U*-test, Student's *t*-test, or analysis of variance (ANOVA), as appropriate.

A receiver operating characteristic (ROC) curve was constructed to define the best FC and CRP cut-off values associated with MH and TH. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and the area under the curve (AUC), with the respective 95% confidence interval (95% CI), were calculated. Finally, CD patients who achieved CR, MH, or TH, in association with the target FC and CRP values, were considered to have achieved DR. In addition, the agreement (k) between MH/FC and TH/FC, and between FC and CRP, was calculated. A *p*-value < 0.05 was considered significant.

Results

The study included 118 patients with CD (males 65.3%; mean age 36.1 ± 15.1) who enrolled in our Unit and completed a 2-year treatment phase. Seventy-nine of them (66.9%) were treated with anti-tumor necrosis factor (TNF) alpha (infliximab or adalimumab), 18 subjects (15.3%) with vedolizumab, and 15 (12.7%) with ustekinumab. Six subjects (5.1%) received a combination treatment with anti-TNF alpha plus azathioprine. Furthermore, 18 out of 85 patients (21.2%) treated with anti-TNF alpha agents and 6 out of 18 subjects (33.3%) treated with vedolizumab, were escalated during the first 6 months of therapy. Their baseline features of all patients are shown in Table 1.

All patients who participated in the study underwent clinical exam, laboratory investigation for CRP and FC, and sonographic and endoscopic evaluation both before starting the use of biologics and 2 weeks to completion of the 2-year treatment course with biological drugs. These assessments revealed a CR rate of 62.7% (74 subjects out of 118); the prevalence of MH was 44.1% (52 out of 118 subjects), whereas TH was found in 38 patients (32.2%). All patients with TH also showed MH on endoscopic evaluation.

At baseline, the mean FC level of the entire population was $494 \pm 515.4 \,\mu g/g$, whereas after 2 years the FC level decreased to $260 \pm 354.9 \,\mu g/g$ ($p < 0.01$). More specifically, the FC level decreased from $307.5 \pm 248.2 \,\mu g/g$ to $45.4 \pm 31.3 \,\mu g/g$ in the TH group ($p < 0.01$), from $384.5 \pm 355.8 \,\mu g/g$ to $44.9 \pm 27.8 \,\mu g/g$ in the MH group ($p < 0.01$), and from $523.4 \pm 591.7 \,\mu g/g$ to $105.9 \pm 156.9 \,\mu g/g$ in the CR group ($p < 0.01$) (Table 2). No differences were seen in FC levels between the TH and MH groups 2 years from the onset of treatment with biologics, but subjects with CR displayed higher FC levels than those with MH or TH ($p < 0.01$) (Table 3).

Moreover, patients with colonic or ileocolonic CD showed higher but insignificant FC levels than isolated ileal CD patients both at baseline ($530.4 \pm 561.3 \,\mu g/g$ *versus* $402.7 \pm 364.1 \,\mu g/g$, $p = 0.3$) and during follow-up ($274.3 \pm 384.5 \,\mu g/g$ *versus* $217.1 \pm 251.7 \,\mu g/g$, $p = 0.5$), without differences in the rate of CR, MH, and TH according to the disease location.

Similarly, the CRP level decreased from $11.7 \pm 14.8 \,mg/L$ at baseline in the entire study group to $10.1 \pm 18.7 \,mg/L$ after 2 years, although this decrease was not significant ($p = 0.6$) (Table 2). However, the CRP level significantly decreased from $11.1 \pm 15.6 \,mg/L$ to $2.5 \pm 1.7 \,mg/L$ in the TH group ($p < 0.01$), from $11.3 \pm 14.04 \,mg/L$ to $2.4 \pm 1.6 \,mg/L$ in the MH group ($p < 0.01$), and from $13.2 \pm 17.8 \,mg/L$ to $5 \pm 13.3 \,mg/L$ in the CR group ($p < 0.01$) (Table 3). Table 4 summarizes the differences between FC and CRP levels in patients that reached or did not reach any outcome (TH, MH, or CR).

The ROC curve analysis showed that an FC cut-off value of $94 \,\mu g/g$ was associated with both MH (sensitivity: 94.2%, 95% CI: 84.1–98.8%, specificity: 84.8%, 95% CI: 73.9–92.5%, PPV: 83.05%, 95% CI: 73.4–89.7%, NPV: 94.92%, 95% CI: 86.1–98.2%, AUC: 0.95) (Figure 1(a)) and TH (sensitivity: 92.1%, 95% CI: 78.6–98.3%, specificity: 70%, 95% CI: 58.7–79.7%, PPV: 64.4%, 95% CI: 55.4–72.5%, NPV: 94.9%, 95% CI: 86.2–98.2%, AUC: 0.88) (Figure 1(b)). The MH/FC and TH/FC k of agreement was 0.81 ($p < 0.01$) and 0.58 ($p < 0.01$), respectively.

Moreover, a CRP cut-off value of $5 \,mg/L$ (also named 'negative' CRP) was associated with both MH (sensitivity: 96.1%, 95% CI: 86.8–99.5%, specificity: 62.1%, 95% CI: 49.3–73.8%, PPV: 66.7%, 95% CI: 59.4–73.2%, NPV: 95.35%, 95% CI: 83.87–98.78%, AUC: 0.85) (Figure 2(a)) and TH (sensitivity: 97.4%, 95% CI: 86.2–99.9%, specificity: 52.5%, 95% CI: 41–63.8%, PPV: 52%, 95% CI: 45.8–58.1%, NPV: 95.35%, 95% CI: 83.9–98.7%, AUC: 0.78) (Figure 2(b)). The MH/CRP and TH/CRP k of agreement was 0.63 ($p < 0.01$) and 0.54 ($p < 0.01$), respectively.

The FC/CRP k of agreement was 0.82 ($p < 0.01$) for MH and 0.74 ($p < 0.01$) for TH.

When considering CD patients with concomitant CR, MH, and TH associated with an FC level $< 94 \,\mu g/g$ and negative CRP ($< 5 \,mg/L$), this association was identified in 33 patients (27.9%) (Figure 3).

No significant differences were seen in terms of effectiveness of a specific treatment and the rates of concomitant clinical, biochemical remission, MH, and TH (Table 5).

Table 1. Baseline features of study population.

	Crohn's disease (118)
Male gender, *n* (%)	77 (65.3)
Age at enrollment (years), mean ± SD	36.1 ± 15.1
Disease duration (months), mean ± SD	123.4 ± 104.5
Age at diagnosis, *n* (%)	
A1	32 (27.1)
A2	67 (56.8)
A3	19 (16.1)
Disease location, *n* (%)	
L1	23 (19.5)
L2	8 (6.8)
L3	84 (71.2)
L4	3 (2.5)
Disease behavior, *n* (%)	
B1	46 (39)
B2	52 (44.1)
B3	20 (16.9)
Perianal disease, *n* (%)	30 (25.4)
Smoking habits, *n* (%)	
Yes	36 (30.5)
Not	59 (50)
Ex	23 (19.5)
Familial history, *n* (%)	23 (19.5)
Need for steroids at diagnosis, *n* (%)	60 (50.8)
Previous surgery, *n* (%)	58 (49.2)
Previous thiopurines exposure, *n* (%)	47 (39.8)
Extra-intestinal manifestations, *n* (%)	48 (40.7)
Biologic, *n* (%)	
IFX	10 (8.5)

(Continued)

Table 1. (Continued)

	Crohn's disease (118)
ADA	75 (63.6)
VDZ	18 (15.3)
UST	15 (12.7)
Combination therapy, *n* (%)	6 (5.1)

Combination therapy: the concomitant association of anti-TNF alpha and immunomodulators such as azathioprine or 6-mercaptopurine.
ADA, adalimumab; SD, standard deviation; IFX, Infliximab; n, number; TNF, tumor necrosis factor; UST, ustekinumab; VDZ, vedolizumab.

Discussion

The treatment strategies for CD have evolved in the last few years, moving from a clinical-based management to a treat-to-target approach, which includes objective monitoring and tight disease control.[4] Recent evidence shows that a decision-making strategy based on accurate and tight evaluation with even more stringent endpoints (i.e., TH) is associated with better long-term outcomes, reducing hospitalization, the rate of surgery, progressive bowel damage, and disease burden and disability.[10,11,15] From this perspective, biomarkers such as CRP and FC have emerged as non-invasive useful tools for monitoring disease activity. In particular, FC is able to predict long-term clinical outcomes when measured 12 weeks after the onset of medical treatment[18]; the STRIDE-II systematic review supported using an FC cut-off value of 150 µg/g to identify MH.[4] Despite this evidence, the current definition of DR does not take into account the role of biochemical markers, such as CRP and FC.[30] In effect, current evidences, although encouraging, are too limited to support the correlation between radiological findings and biomarkers.[30]

In the present study, with the aim to establish a direct correlation between MH and TH with biochemical markers (FC and CRP), we found that an FC cut-off value of 94 µg/g was associated with both MH (sensitivity: 94.2%, specificity: 84.8%, PPV: 83.05%, NPV: 94.92%) and TH (sensitivity: 92.1%, specificity: 70%, PPV: 64.4%, NPV: 94.9%). For CRP, a cut-off of 5 mg/L was

Table 2. Clinical, laboratory, endoscopic, and sonographic findings at baseline and after 2 years.

	Baseline	After 2 years	*p*
Clinical			
HBI, mean ± SD	6.69 ± 1.32	5.19 ± 2.11	<**0.001**
Laboratory			
CRP levels (mg/L), mean ± SD	11.75 ± 14.84	10.11 ± 18.78	0.63
FC levels (µg/g), mean ± SD	493.83 ± 515.45	260.15 ± 354.93	<**0.001**
Endoscopy			
SES-CD, mean ± SD	9.65 ± 4.84	5.45 ± 5.27	<**0.001**
Bowel sonography			
BWT at BS (mm), mean ± SD	6.57 ± 1.66	4.81 ± 2.39	<**0.001**
Disease extension at BS (cm), mean ± SD	15.47 ± 7.79	9.4 ± 10.1	<**0.001**

Significant differences have been highlighted in bold.
BS, bowel sonography; BWT, bowel wall thickness; CRP, C-reactive protein; HBI, Harvey-Bradshaw index; FC, fecal calprotectin; SD, standard deviation; SES-CD, Simple Endoscopic Score for Crohn's Disease.

Table 3. Fecal calprotectin and CRP levels according with TH, MH, and CR.

	Baseline FC	After 2 years FC	*p*
TH (*n* = 38)	307.53 ± 248.23	45.45 ± 31.26	<**0.001**
MH (*n* = 52)	384.48 ± 355.86	44.92 ± 27.78	<**0.001**
CR (*n* = 74)	523.4 ± 591.76	105.9 ± 156.89	<**0.01**
	Baseline CRP	**After 2 years CRP**	*p*
TH (*n* = 38)	11.1 ± 15.58	2.5 ± 1.76	<**0.001**
MH (*n* = 52)	11.3 ± 14.04	2.4 ± 1.66	<**0.001**
CR (*n* = 74)	13.2 ± 17.85	5 ± 13.32	<**0.01**

Significant differences have been highlighted in bold.
CR, clinical remission; CRP, C-reactive protein, results are expressed as mg/L; FC, fecal calprotectin, results are expressed as µg/g; MH, mucosal healing; TH, transmural healing.

associated with both MH (sensitivity: 96.1%, specificity: 62.1%, PPV: 66.7%, NPV: 95.35%) and TH (sensitivity: 97.4%, specificity: 52.5%, PPV: 52%, NPV: 95.35%). When considering CD patients with concomitant CR, MH, and TH associated with an FC level <94 µg/g and negative CRP (<5 mg/L), this association was found

in 33 patients and by this definition DR was achieved in 27.9% of treated CD population.

FC is considered an excellent biomarker associated with both clinical and endoscopic disease activity.[31] In fact, most data commonly reveal that FC is significantly reduced in IBD subjects with

Table 4. Differences in FC and CRP levels after 2 years of treatment according with any achieved outcome.

	TH yes (38/118)	TH not (80/118)	*p*
FC (μg/g), mean ± SD	45.45 ± 31.26	391.68 ± 362.12	<**0.001**
CRP (mg/L), mean ± SD	2.5 ± 1.76	21.91 ± 13.7	**0.002**
	MH yes (52/118)	**MH not (66/118)**	*p*
FC (μg/g), mean ± SD	44.92 ± 27.78	429.73 ± 399.8	<**0.001**
CRP (mg/L), mean ± SD	2.4 ± 1.66	23.41 ± 16.2	<**0.001**
	CR yes (74/118)	**CR not (44/118)**	*p*
FC (μg/g), mean ± SD	156.89 ± 105.9	519.56 ± 437.24	<**0.001**
CRP (mg/L), mean ± SD	13.32 ± 5	23.21 ± 18.7	<**0.001**

Significant differences have been highlighted in bold.
CR, clinical remission; CRP, C-reactive protein; FC, fecal calprotectin; MH, mucosal healing; TH, transmural healing.
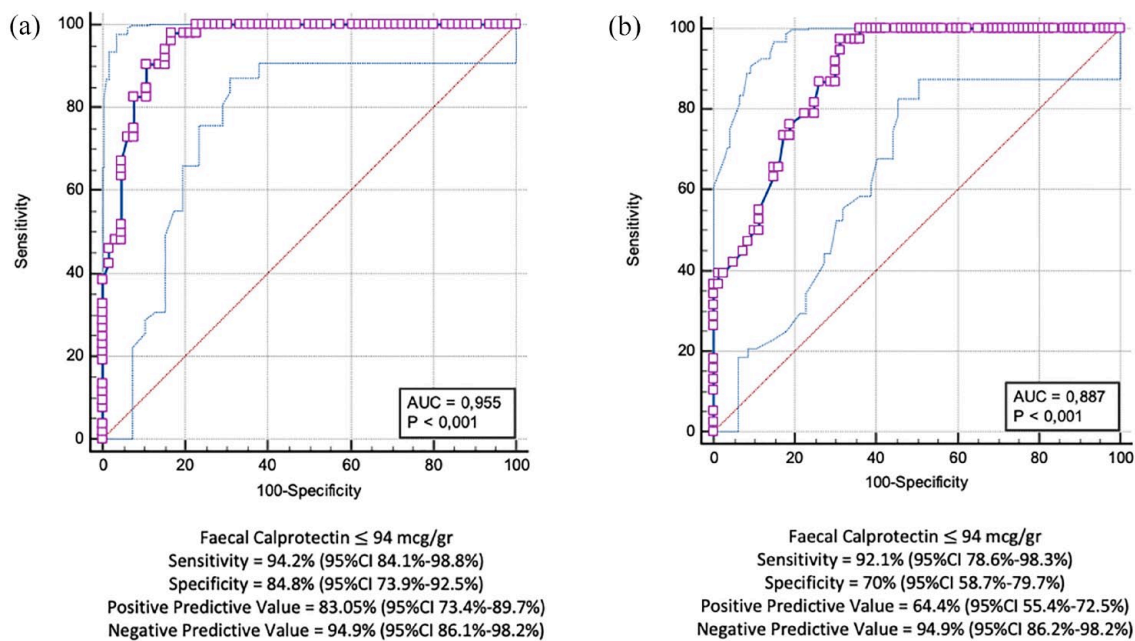
Figure 1. Receiver operating characteristic (ROC) curve showing the best fecal calprotectin cut-off value for MH (a) and TH (b).
AUC, area under the curve; 95% CI, 95% confidence interval; MH, mucosal healing; TH, transmural healing.

clinical and endoscopic remission and that values higher than 150–200 μg/g are mainly associated with disease relapse.[31,32] Recently, the STRIDE-II has identified an FC cut-off value of 150 μg/g associated with MH,[4] but has considered a gray zone for FC values ranging from 150 to 250 μg/g. A *post hoc* analysis from the CALM study[33] demonstrated that an FC cut-off <250 μg/g was a useful surrogate marker for MH in CD, with a sensitivity of 70–74%, a specificity of 62–64%, and an AUC of 0.63–0.68. In accordance with this previous evidence, we found that an FC cut-off value of 94 μg/g
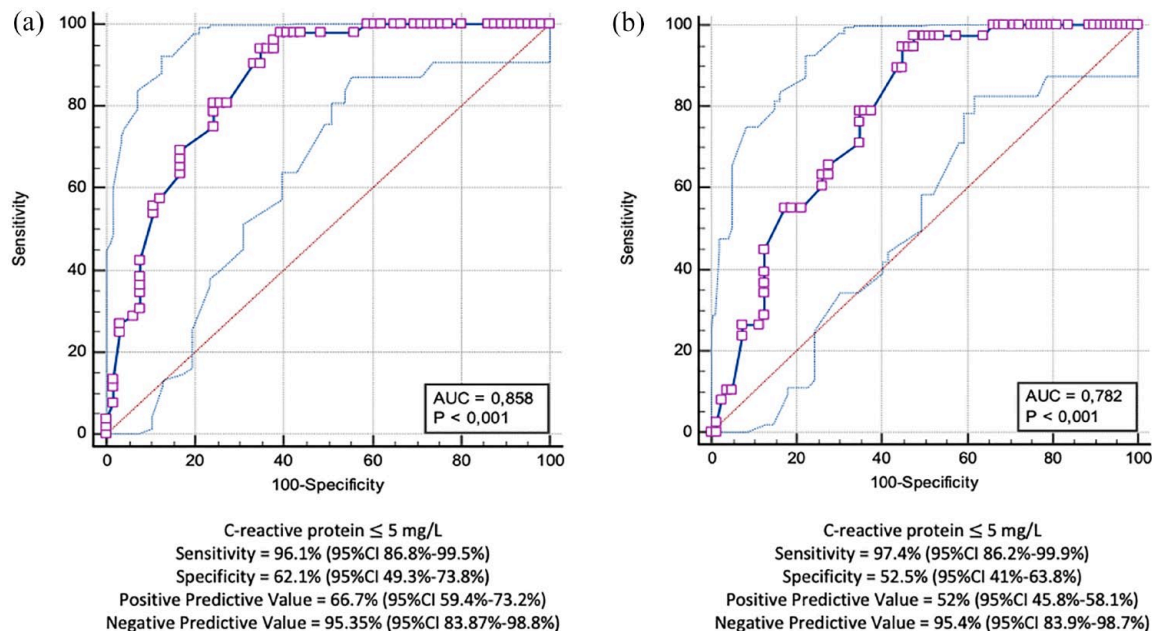
Figure 2. Receiver operating characteristic (ROC) curve showing the best CRP cut-off value for MH (a) and TH (b).
AUC, area under the curve; 95% CI = 95% confidence interval; MH, mucosal healing; TH, transmural healing.
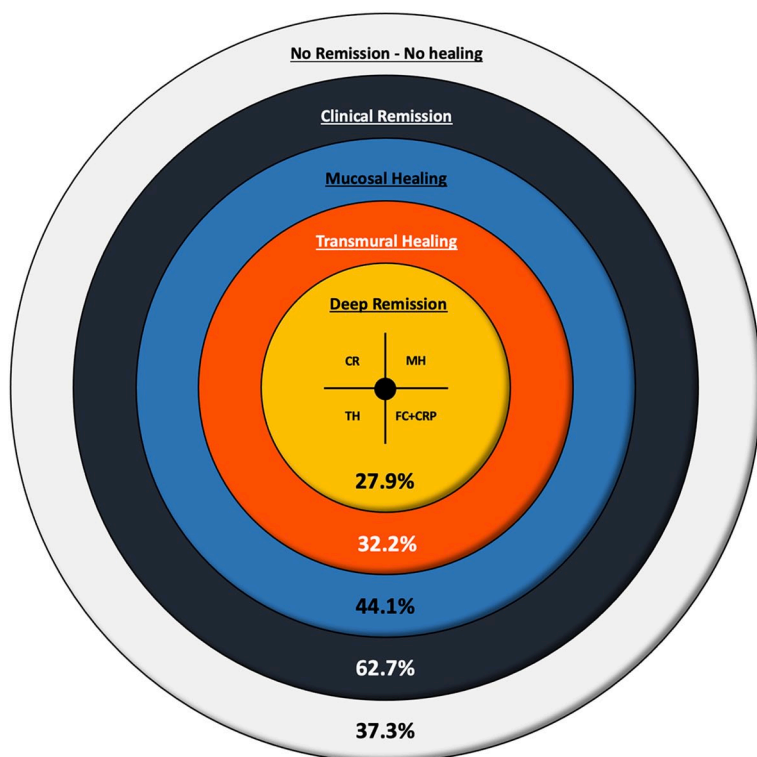

Figure 3. The new concept of DR, expressed as evolution of outcomes from CR to MH and TH in accordance with the treat-to-target strategy.
CR, clinical remission; DR, deep remission; MH, mucosal healing; TH, transmural healing.

was strongly and significantly associated with MH (sensitivity: 94.2%, specificity: 84.8%, PPV: 66.7%, NPV: 95.35%, AUC: 0.95), with an excellent FC/MH agreement ($k = 0.81$).

Some studies have explored using the combined cut-off values of FC and CRP to predict MH.

Bondjemah *et al.* [34] found that in adult active CD, FC < 200 µg/g or CRP < 5 mg/L could predict CDEIS < 6 with 83% sensitivity and 71% specificity. When assessing 355 patients with CD in CR, the combination of CRP < 10 mg/L and FC < 200 µg/g predicted CDEIS < 3 with 78% sensitivity and 58% specificity. In our study, we found that all subjects with FC < 94 µg/g also had CRP < 5 mg/L, with an excellent/good FC/CRP agreement for both MH ($k = 0.82$) and TH ($k = 0.74$). Thus, the 'normal' value of CRP should be included in the new definition of DR.

Although FC has been extensively evaluated in ulcerative colitis, and in association with MH in IBD in general, little is known about the relationship between FC and TH in CD.

A recent systematic review[35] explored the association between TH and biomarkers: among five

studies that included a total of 742 patients, a normal CRP level was statistically associated with TH in two studies, and a good concordance ($p = 0.02$) between CRP levels and TH was observed in two other studies. In effect, only two studies (one of which was conducted in a pediatric setting[36] and the other in an adult setting[37]) evaluated the association between TH and FC.

With regard to the relationship between FC and active transmural disease, previous studies have shown that FC correlates with transmural inflammation measured by magnetic resonance enterography (MRE), with a correlation coefficient range of 0.46–0.72.[38–40] Domachevsky *et al.*[41] and Bertani *et al.*[42] arbitrarily chose an FC cut-off >150 mg/g to associate FC with inflammation documented by PET/MRE and MRE, respectively. Cerrillo *et al.*[43] demonstrated that an FC cut-off of 166.5 mg/g predicted transmural inflammation (i.e., MaRIA score ≥7), with an AUROC of 0.914.

Current knowledge about TH and FC is even more limited. In a recent multicenter Italian study by Calabrese *et al.*[44] to evaluate the role of ultrasonographic tight control and monitoring in CD during biological therapies, it was found that after 3, 6, and 12 months a significant proportion of patients achieved CR and normalization of CRP and FC compared with the baseline. After 12 months, patients who achieved clinical and biochemical remission had a higher rate of TH or improved lesions than the unchanged/worsened lesion group, although specific FC or CRP cut-off values were not found in the study.

A recent pediatric study[36] evaluating the role of biomarkers (FC and CRP) in predicting MH and TH found that an FC cut-off value <100 µg/g predicted deep healing (MH + TH), with a sensitivity of 71%, a specificity of 92%, and an AUC of 0.93. Moreover, the best cut-off value of CRP to predict deep healing was <3 mg/L (72% sensitivity, 68% specificity, and 0.81 AUC).

Noh *et al.*[37] evaluated the usefulness of FC for predicting deep healing (MH + TH) in 268 adult Korean patients with CD receiving anti-TNF therapy, and found that subjects with deep healing (achievable in 28.7% of anti-TNF-treated patients) displayed lower FC values than those with 'only' MH (56.5 *versus* 160.5 mg/kg, $p < 0.01$). The FC cut-off value of 81.1 mg/kg

Table 5. Treatment received by 33 subjects achieving concomitant CR, biochemical remission (FC level <94 µg/g and CRP < 5 mg/L), MH, and TH.

	No. of patients treated (entire population)	No. of patients achieving DR
Adalimumab	75	21 (28%)
Infliximab	10	3 (30%)
Vedolizumab	18	5 (27.7%)
Ustekinumab	15	4 (26.7%)

CR, clinical remission; CRP, C-reactive protein; DE, Deep remission; FC, fecal calprotectin; MH, mucosal healing; TH, transmural healing.

showed 62.3% sensitivity, 81.7% specificity, 57.8% PPV, and 84.3% NPV for predicting deep healing, with an AUC of 0.767.

In the present study, we found that the FC cut-off value of 94 µg/g was associated with TH (sensitivity 92.1%, specificity 70%, PPV 64.4%, NPV 94.9%, AUC 0.88), with a TH/FC agreement of 0.58 ($p < 0.01$), even though we did not find any significant difference in FC concentration between the TH and MH groups. By contrast, CD patients with only CR had significant higher FC levels than patients with MH/TH, confirming and strengthening the few previous results.

In accordance with Noh *et al.*,[37] we would like to underline the high NPV of FC < 94 µg/g in predicting both MH (94.92%) and TH (94.9%), confirming the hypothesis that FC is an excellent biomarker that should be taken into account in the assessment of DR in CD.

To the best of our knowledge, this is the first prospective study that is directly focused on the association of FC levels and both MH and TH, with the aim of incorporating this biochemical marker in the definition of DR, so that the definition would combine clinical, biochemical, endoscopic, and transmural remission. This new endpoint, achievable in about 30% of CD patients treated with biologics, follows the concept of the treat-to-target strategy, in order to guide clinicians in the management of CD, as demonstrated in the CALM trial.[45] Further studies are needed to compare the long-term outcome of CD patients with DR and that of patients with only MH or TH. Similarly, current data are scarce to make suggestions on when, if ever, biologic therapy can be stopped/de-escalated in patients with IBD achieving TH. Therefore, the

decision to discontinue or de-escalate a biologic drug is typically made case by case on the basis of considerations around benefits, risks, and cost-effectiveness.[10] Further studies with therapeutic strategic finality are needed to better define the potential advantages to stop or de-escalate biologic therapy in CD patients achieving all combined outcome of DR.

Our study has some limitations. First, it should be considered as a 'per-protocol' study with a consequent 'positive' selection bias and probable overestimation of the rates of CR, MH, and TH. Moreover, TH was assessed only by BS, and a cross-sectional evaluation (MRE) of TH was not routinely performed. However, previous studies have demonstrated an excellent agreement between BS and other imaging techniques (about 90%), such as magnetic resonance and computed tomography enterography, in the detection of bowel lesions.[2,3]

Although a formal definition of sonographic TH is not currently available, most previous studies used BWT alone to define TH, whereas some combined BWT with doppler signal. The recent consensus by Ilvemark *et al.*[46] suggests including the negative color doppler signal alongside the BWT, even though the prognostic role of negative doppler plus BWT *versus* BWT alone has yet to be demonstrated.

We also recognized that a BWT ⩽ 4 mm of the colon can be normal for some patients, especially if diverticula are present. However, very recent consensus[46] recommends defining TH as BWT ⩽ 3 mm for both small and large bowel.

It is known that FC can significantly vary in individual patients on a day-to-day basis or even in two different samples taken on the same day.[47] We never use FC alone in clinical practice for IBD management: the FC value of 94 μg/g has to be associated with predictive values of TH and MH.

Furthermore, this study was not designed to evaluate a specific treatment or therapeutic strategy, but rather to assess outcomes and associate them to biomarkers, regardless of the medication used. Similarly, it is important in clinical practice to establish the prognostic role of such new definition: a study evaluating the prognostic role of DR *versus* TH alone or MH alone is ongoing.

Moreover, the baseline CRP level is significantly influenced by the underlying coding gene. The CRP non-producers may exist in 15–20% of IBD population. In other words, CRP may not be universally used as an inflammation marker and may not be applicable to everyone.[48]

Conclusion

In conclusion, an FC < 94 μg/g and a normal CRP are associated with CR, MH, and TH and could be included in the definition of DR in association. So, by definition, if applying the combination of our outcomes, DR could be achieved in a little percentage of CD patients treated with biologics for at least 2 years (particularly when considering the 'per protocol' nature of the study). However, this definition of DR could be considered a new and more inclusive concept of clinical, biochemical, endoscopic, and transmural remission.

Declarations

Author contribution(s)
Fabiana Castiglione: Conceptualization; Investigation; Methodology; Project administration; Supervision; Writing – review & editing.

Nicola Imperatore: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Resources; Software; Supervision; Validation; Visualization; Writing – original draft; Writing – review & editing.

Anna Testa: Data curation; Methodology; Supervision; Validation; Writing – review & editing.

Roberto de Sire: Data curation; Validation.

Olga Maria Nardone: Validation.

Simona Ricciolino: Investigation; Validation.

Imma Di Luna: Validation; Visualization.

Marta Patturelli: Validation; Visualization.

Guido Daniele Villani: Validation; Visualization.

Oriana Olmo: Validation; Visualization.

Antonio Rispo: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Supervision; Validation; Writing – review & editing.

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