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How many biomarker measurements are needed to predict prognosis in Crohn's disease patients under infliximab?—A prospective study

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Abstract

Background: Timely stratification of Crohn's disease (CD) is essential for patients' management. The use of noninvasive accurate biomarkers is key to monitor treatment and to pursue mucosal healing, the ultimate treatment endpoint in CD.

Objective: We aimed to evaluate the performance of readily available biomarkers and develop risk matrices to predict CD progression.

Methods: Data from 289 CD patients receiving infliximab (IFX) maintenance therapy for 2 years was collected; those patients were included in DIRECT, a prospective multicenter observational study. Disease progression was evaluated using two composite outcomes incorporating clinical and drug-related factors, the first including IFX dose and/or frequency adjustments. Univariate and multivariable logistic regressions were used to calculate the odds ratios (OR) and to develop risk matrices.

Results: The isolated presence of anemia at least once during follow-up was a significant predictor of disease progression (OR 2.436 and 3.396 [$p \leq 0.001$] for composite outcomes 1 and 2, respectively) regardless of confounding factors. Isolated highly elevated C-reactive protein (CRP; >10.0 mg/L) and fecal calprotectin (FC; >500.0 μg/g) in at least one visit were also significant predictors, while milder elevations (3.1–10.0 mg/L and 250.1–500.0 μg/g) were only relevant when detected in at least two visits (consecutive or not). The combination of biomarkers in risk matrices had good ability to predict progression; patients simultaneously presenting anemia, highly elevated CRP and FC at least once had 42%–63% probability of achieving the composite outcomes.

Conclusion: The combined evaluation of hemoglobin, CRP, and FC in at least one time point and their incorporation into risk matrices seems to be the optimal strategy for CD management, as data from additional visits did not meaningfully influence the predictions and may delay decision-making.

KEYWORDS
biomarkers, calprotectin, Crohn's disease, infliximab

INTRODUCTION

Crohn's disease (CD) is a chronic inflammatory bowel condition with a relapsing-remitting course. An essential aspect of its management consists of anticipating flares and avoiding complications to improve the prognosis and quality of life of the patients.[1] CD symptoms correlate poorly with the levels of mucosal inflammation underlying disease progression. Therefore, considerable efforts have been made in the last decades to identify representative biomarkers for disease burden that can be used to improve therapeutic management.[2] Although some genetic and serologic tests have shown good correlation with disease activity, they are considered too expensive and time-consuming to be adopted as standards for everyday clinical practice,[3] and non-invasive, reliable, and easily accessible biomarkers for CD are still an unmet need. Potential candidates include fecal calprotectin (FC) and C-reactive protein (CRP).[4,5] Ferritin concentration and transferrin saturation (TS) have also been proposed as potentially useful parameters for CD diagnosis and monitoring.[6] Recent studies have emphasized that combinations of several biomarkers could further enhance the accuracy of the predictions.[7,8]

In the present study, we intended to evaluate the performance of easily available biomarker tests in the prediction of CD prognosis, evaluated through composite outcomes. A combination of individual variables allows for the optimization of resources and increases the accuracy and statistical power, and therefore composite outcomes have been increasingly favored in both interventional and observational studies aimed at exploring the effectiveness of a given therapeutic strategy.[9] We performed an analysis of the data from the DIRECT, a 2-year, prospective multicenter study, with the primary aim of identifying the most relevant parameters for the prediction of CD progression in patients under maintenance therapy with infliximab. Based on these results, we developed two different risk matrices for the quick assessment of disease prognosis.

MATERIALS AND METHODS

Study design and participants

The DIRECT study enrolled inflammatory bowel disease (IBD) patients from eight Portuguese specialized healthcare centers between May 2016 and October 2019. Inclusion criteria for the study were (i) 18 years of age or above; (ii) belonging to the Portuguese IBD group (GEDII) registry; (iii) moderate-to-severe active CD or ulcerative colitis (UC); and (iv) treatment with vedolizumab or tumor necrosis factor alpha (TNF-α) blockers (adalimumab, golimumab or infliximab). The study was approved by all the relevant local ethics committees and by the National Data Protection Authority and was conducted according to the principles of the Declaration of Helsinki. The present analysis included all patients diagnosed with CD who had started maintenance therapy with infliximab (IFX, 5–10 mg/kg) at least 14 weeks before enrollment in the study. Patients on IFX plus azathioprine (AZA) for at least 6 weeks before the study baseline were considered on combined therapy, while those on IFX alone or IFX plus AZA but for less than 6 weeks before baseline were considered on monotherapy.

Key Summary

Summarize the established knowledge on this subject

- Crohn's disease (CD) symptoms poorly correlate with inflammation and disease progression.
- Genetic and serologic tests are suboptimal, expensive, and time-consuming.
- Identification of readily available prognostic biomarkers is important.

What are the significant and/or new findings of this study?

- The combined evaluation of fecal calprotectin, C-reactive protein, hemoglobin, and transferrin saturation allows predicting CD progression even after a single measurement.
- The use of matrices based on widely available biomarkers allows rapid prediction of CD progression, improving patients' monitoring and therapeutic management.

Data collection

Patients were evaluated before each IFX infusion (every 4, 6, or 8 weeks) for 24 months. The clinical data collected included body mass index (BMI), comorbidities, disease location and behavior (according to the Montreal classification), concomitant therapies, adjustments on biological therapy (dose, interval, switch, or swap), and CD-related hospitalizations or surgeries. In addition, blood and stool samples, and patient-reported outcomes (PRO) questionnaires referring to the previous 7 days were collected at each infusion appointment. Serum CRP concentrations were determined using a highly sensitive assay (Konelab™, Thermo Scientific, Finland). FC and IFX levels were measured using the Quantum Blue® fCAL and IFX quantitative assays, respectively (Bühlmann Laboratories, Switzerland), while anti-IFX antibody levels were quantified with a validated in-house ELISA (anti-human lambda chain assay, 1.2–100 μg/mL; values below 1.7 μg/mL were considered negative).[10,11] All determinations were performed in a central laboratory. The doctors in charge of the patients were blinded to central labs' results to avoid undesired influence on treatment decisions.

Outcome definitions

Two composite outcomes were used, both reflecting disease progression. The first (composite outcome 1) included clinical-related items (first occurrence of IBD-related surgery or hospitalization, or new fistulae, abscess or stricture) and drug-related items (first prescription with at least one course of oral corticosteroids or more than 10 mg of prednisolone per day, or de novo azathioprine [AZA] or methotrexate, or swap/switch of biological therapy [to adalimumab, golimumab, vedolizumab, or ustekinumab], or AZA dose increase other than by weight fluctuation, or IFX dose escalation or interval reduction). The second outcome (composite outcome 2) was identical to the first except for the exclusion of IFX dose escalation or interval reduction as one of the individual items. These composite endpoints were adapted from previous studies.[9,12]

Statistical analysis

Categorical variables were summarized by absolute and relative frequencies. Continuous variables were described as median plus 1st quartile-3rd quartile range (Q1–Q3). Mann-Whitney U tests were used to test for associations between composite outcomes and continuous variables. Categorical variables were compared using either the Chi-square or Fisher's exact test. Logistic regressions were applied to determine the relationship between clinical and demographic factors, and composite outcomes. Odds ratios (OR) were estimated with 95% confidence intervals (CI) and adjusted for confounding factors (sex, body mass index, disease location, and behavior, presence of perianal disease, and age at diagnosis). Receiver operating characteristic (ROC) analysis and areas under the curve (AUC) were used to determine the ability of individual variables to discriminate between composite outcomes. A sensitivity analysis was performed using the different items included in the two composite outcomes: (i) clinical-related items, corresponding to the first occurrence of IBD-related surgery or hospitalization, or new fistulae, abscess or stricture (included in both endpoints), (ii) drug-related items including IFX dose and/or frequency adjustments

(comprised in composite outcome 1), and (iii) drug-related items without IFX adjustments (included in composite outcome 2).

All *p*-values were two-sided, with the significance level set at 5%. Data were processed using IBM SPSS Statistics software (version 28.0; IBM, Armonk, USA). Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were followed for the reporting.

RESULTS

Patients' characteristics

Out of the 429 patients that were originally included in the DIRECT study 332 had a diagnosis of CD and were treated with IFX. Of these, 289 received IFX for at least 14 weeks (maintenance therapy) and were included in the present analysis (Figure 1). The characteristics of these patients at baseline are summarized in Table 1. The sex ratio was balanced (51.2% men vs. 48.8% women) and the patients had a median age of 25 (20–34); 44.4% had disease affecting both the ileum and the colon (L3, Montreal classification); perianal disease was present in 34.3% of the cases, and extraintestinal manifestations in 57.4%. Most of the patients (61.2%, $n = 177$) received IFX (5 mg/kg) every 8 weeks, and 41.9% ($n = 121$) were under combination therapy with AZA. Only 14 patients (4.8%) exhibited complications such as fistulas, abscesses, or strictures (Supplementary Table S1). At baseline, symptoms (loose/liquid stools, abdominal pain, general well-being) were present in 20.1% of the patients, while 58.8% developed symptoms during follow-up. Regarding the optimization of IFX, the dose was increased in 24.2% of the cases, while 24.9% of the patients required an increase in the frequency of administration. Biologic therapy was changed in 20 patients (6.9%) during follow-up.

Achievement of the composite outcomes

Composite outcome 1 was achieved in 100 patients, while composite outcome 2 was attained in 57. Conversely, 180 individuals did not achieve either of the endpoints. The clinical-related items of those outcomes (first occurrence of IBD-related surgery or hospitalization, new fistulae, abscess or stricture) were registered in 9.3% of the patients during follow-up (8/289), while drug-related items with and without adjustment of infliximab posology occurred in 31.5% (91/289) and 13.1% (38/289), respectively. The two composite endpoints were more common in patients with higher FC and CRP levels and in those with anemia (hemoglobin <12 or 13 g/dL for women or men) (Supplementary Figure S1a,b). Furthermore, elevations in FC and CRP and low hemoglobin were associated with quicker development of the progression-related outcomes (Supplementary Figure S2a,b). Tables 2 and 3 present the proportions of patients achieving each endpoint who presented values above the cut-offs in at least one, two (consecutive or not) and three visits during maintenance therapy.

Values outside the reference range in at least one visit

During the follow-up, 32.8% of the patients presented anemia in at least one visit, 8.0% of whom only once (Supplementary Table S2). These patients had significantly higher endpoint achievement (47.9 vs. 28.0% and 32.3 vs. 13.5%, for composite outcomes 1 and 2,

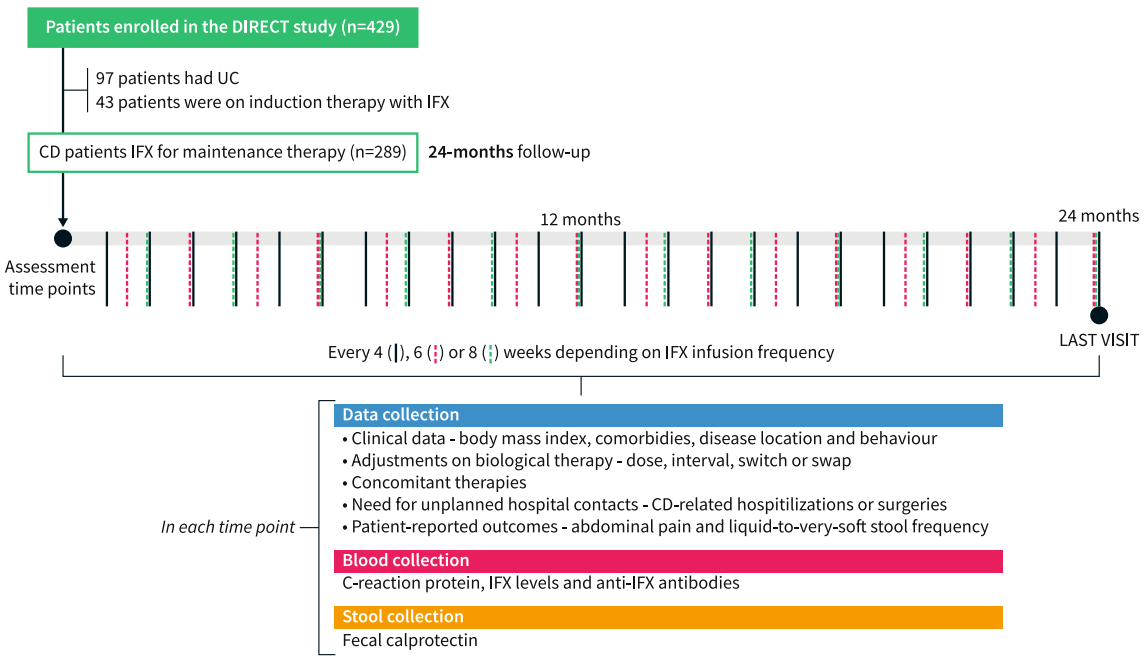

FIGURE 1 Flowchart of patient enrollment and schematic representation of the monitoring schedule. CD, Crohn's disease; IFX, infliximab; UC, ulcerative colitis.

TABLE 1 Characteristics of Crohn's disease patients receiving infliximab as maintenance therapy (n = 289).

Patients' characteristics at baseline		
Sex	Male—*n* (%)	148 (51.2)
	Female—*n* (%)	141 (48.8)
Age at diagnosis	Years—median (Q1–Q3)	25 (20–34)
Age at inclusion	Years—median (Q1–Q3)	42 (32–51)
Smoking habits	Never smoked—*n* (%)	149 (51.6)
	Less than 10/day—*n* (%)	42 (14.5)
	Between 10 and 20/day—*n* (%)	21 (7.3)
	More than 20/day—*n* (%)	7 (2.4)
	Former smoker—*n* (%)	70 (24.2)
Crohn's disease location	L1—*n* (%)	111 (38.5)
	L2—*n* (%)	49 (17.0)
	L3—*n* (%)	128 (44.4)
	L4—*n* (%)	44 (15.3)
Crohn's disease behavior	B1—*n* (%)	144 (49.8)
	B2—*n* (%)	56 (19.4)
	B3—*n* (%)	89 (30.8)
	Perianal disease—*n* (%)	99 (34.3)
Disease duration	Years—median (Q1–Q3)	10 (6–18)
History of IBD-related surgery	Yes—*n* (%)	153 (52.9)
Symptoms at baseline	Yes—*n* (%)	58 (20.1)
Extraintestinal manifestations	Psoriasis—*n* (%)	57 (19.7)
	Arthralgia—*n* (%)	44 (15.2)
	Arthralgia + psoriasis—*n* (%)	15 (5.2)
	Erythema nodosum—*n* (%)	2 (0.6)
	Pyoderma gangrenosum—*n* (%)	1 (0.3)
	Sclerosing cholangitis	1 (0.3)
	Total—*n* (%)	166 (57.4)
Infliximab levels	<3 μg/mL	91 (31.5)
	≥3 μg/mL	195 (67.5)
	≤7 μg/mL	177 (61.2)
	>7 μg/mL	109 (37.7)
Anti-infliximab antibody levels	<1.7 μg/mL	248 (86.7)
	≥1.7 μg/mL	38 (13.3)

respectively) (Table 2). The presence of anemia in at least one time point was a significant predictor of disease progression (OR 2.436 [95% CI 1.408–4.215, p = 0.001] and 3.396 [95% CI 1.770–6.510, p < 0.001] for composite outcomes 1 and 2 after adjusting for sex, body mass index, disease location and phenotype, perianal disease, and age at diagnosis) (Supplementary Table S3; Table 4). On the other hand, isolated rises in CRP and FC in at least one visit were only significant predictors when their concentration was high (above 10.0 mg/L [OR 3.187, 95% CI 1.456–6.979] and 500.0 μg/g [OR 3.069, 95% CI 1.462–6.443; Table 4). Patients with TS below 15.1% in at least one time point (n = 159) had a significant OR for achieving only composite outcome 1 (OR 1.844 [95% CI 1.099–3.123, p = 0.023]), while no significant differences were detected depending on ferritin or IFX through levels. The sensitivity analysis showed a similar pattern for drug-related items (with and without adjustments on infliximab posology) (Supplementary Table S4); the results for clinical-related items lacked statistical significance.

Values outside the reference range in at least two or three visits

The proportion of outcomes' achievement remained quite stable when anemia or low TS was detected more frequently but rose steadily with sustainedly elevated CRP (Tables 2 and 3). Persistently elevated CRP was the only parameter that significantly predicted the occurrence of surgery, hospitalization, new fistulae, abscesses, or strictures (clinical items of the composite outcomes) (Supplementary Table S4). Regarding FC, intermediate elevations (250.1–500.0 μg/g) in at least two consecutive visits were associated with greater outcomes' achievement, while only a marginal increase was observed for repeated measurements above 500.1 μg/g. In line with this, the OR for developing the composite outcomes rose in response to repeated elevated measurements of CRP and FC, while it did not increase as anemia or low TS got more frequent during follow-up (Table 4). Indeed, the OR for disease progression was significantly higher when the CRP concentration was between 3.1 and 10.0 mg/L in at least two non-consecutive (OR 2.459 [95% CI 1.239–4.880, p = 0.010]), two consecutive (OR 2.890 [95% CI 1.306–4.423, p = 0.012]), or three non-consecutive (OR 3.510 [95% CI 1.901–6.489, p < 0.001]) visits (values for composite outcome 1). Regarding FC, intermediate concentrations (250.1–500.0 μg/g) were only associated with higher odds of achieving the composite outcomes when obtained in two consecutive or in three different visits (OR 5.607 [95% CI 1.586–19.780] and OR 2.036 [95% CI 1.029–4.027], respectively), while levels above 500.1 μg/g were associated with higher OR even when registered only once (Table 4). Regarding IFX through levels, composite outcome 1 was more frequent only for concentrations below 3.0 μg/mL in at least two consecutive or three non-consecutive visits (Tables 2 and 3).

Risk matrices for outcome prediction

The variables found to be significant in the multivariable analysis were then considered as risk factors and used for the development of

TABLE 2 The proportion of patients achieving the two composite outcomes with values above the defined cut-offs for each parameter in at least one or two non-consecutive visits during follow-up.

		Values in at least one visit				Values in at least two non-consecutive visits			
		Composite outcome 1		Composite outcome 2		Composite outcome 1		Composite outcome 2	
Parameters		Proportion of patients achieving the outcome	*p*-value	Proportion of patients achieving the outcome	*p*-value	Proportion of patients achieving the outcome	*p*-value	Proportion of patients achieving the outcome	*p*-value
CRP	≤3.0 mg/L	20.4% (10/49)	0.002	12.2% (6/49)	0.043	19.8% (16/81)	<0.001	11.1% (9/81)	0.007
	3.1–10.0 mg/L	28.0% (28/100)		15.0% (15/100)		35.2% (43/122)		18.0% (22/122)	
	≥10.1 mg/L	44.3% (62/140)		25.7% (36/140)		47.7% (41/86)		30.2% (26/86)	
Fecal calprotectin	≤250.0 µg/g	20.0% (11/55)	0.002	10.9% (6/55)	0.002	22.2% (22/99)	<0.001	12.1% (12/99)	0.040
	250.1–500.0 µg/g	27.0% (17/63)		9.5% (6/63)		33.3% (22/66)		15.2% (10/66)	
	≥500.1 µg/g	43.1% (72/167)		26.9% (45/167)		46.7% (56/120)		29.2% (35/120)	
Anemia (Hg < 12 g/dL for women, <13 for men)	Yes	47.9% (46/96)	<0.001	32.3% (31/96)	<0.001	49.3% (36/73)	0.002	34.2% (25/73)	<0.001
	No	28.0% (54/193)		13.5% (26/193)		29.6% (64/216)		14.8% (32/216)	
Ferritin	≤30.0 µg/L	33.3% (4/12)	0.988	33.3% (4/12)	0.046	22.7% (5/22)	0.231	22.7% (5/22)	0.883
	30.1–100 µg/L	35.1% (40/114)		18.4% (21/114)		39.1% (52/133)		20.3% (27/133)	
	≥100.1 µg/L	34.4% (56/163)		19.6% (32/163)		32.1% (43/134)		18.7% (25/134)	
Transferrin saturation	≤15.0%	40.3% (64/159)	0.026	22.0% (35/159)	0.279	42.0% (47/112)	0.036	24.1% (27/112)	0.136
	≥15.1%	27.7% (36/130)		16.9% (22/130)		29.9% (53/177)		16.9% (30/177)	
IFX through levels <3.0 µg/mL	Yes	38.4% (84/219)	0.049	21.0% (46/219)	0.333	38.2% (71/186)	0.086	19.4% (36/186)	0.833
	No	27.1% (19/70)		15.7% (11/70)		28.2% (29/103)		20.4% (21/103)	
Anti-IFX antibodies >1.7 µg/m	Yes	41.7% (55/132)	0.021	22.7% (20/132)	0.239	39.1% (34/87)	0.294	24.1% (21/87)	0.216
	No	28.7% (45/157)		17.2% (27/157)		32.7% (66/202)		17.8% (36/202)	

two matrices to predict the occurrence of the composite outcomes, the first using CRP and FC values, and anemia (Figure 2); and the second using CRP, FC, and TS concentrations (Supplementary Figure S3). The areas under the ROC curve ranged between 0.695 and 0.728 for the first matrix (Supplementary Figure S4; good predictive ability), and from 0.692 to 0.702 for the second matrix (Supplementary Figure S5; moderate-to-good).

Parameters in at least one visit

The first risk matrix showed that the probabilities of achieving the two composite outcomes increased significantly when low hemoglobin and elevated CRP or FC were detected (Figure 2). For example, when anemia was the only finding (normal CRP and FC), the probabilities of developing the composite outcomes 1 and 2 were 22.0% and 13.0%, respectively. On the other hand, the probability of presenting disease progression increased significantly as more parameters were concomitantly out of the normal range. Indeed, when CRP and FC were simultaneously highly elevated (above 10 mg/dL and 500.0 µg/g) and anemia was also detected, the probabilities of achieving the endpoints reached 58.0% and 42.0% for composite outcomes 1 and 2, respectively. The pattern observed for the second matrix was similar (Supplementary Figure S3). When patients presented only mildly elevated CRP or FC levels or TS below 15.1%, the risk for developing the composite endpoints was between 6% and 15%. When the three biomarkers were simultaneously altered in at least one timepoint the probabilities rose, up to 51.0% and 32.0%, respectively.

Parameters in at least two visits

When patients presented with anemia, CRP above 10.0 g/L or FC above 500.0 µg/g at least twice during follow-up (without concomitant elevations in the other parameters) the probabilities of reaching the composite outcomes were between 13.0% and 32.0% (Figure 2). Differently, the risks for disease progression increased sharply when

TABLE 3 The proportion of patients achieving the two composite outcomes with values above the defined cut-offs for each parameter in at least two consecutive or three visits during follow-up.

		Values in at least two consecutive visits				Values in at least three visits			
		Composite outcome 1		Composite outcome 2		Composite outcome 1		Composite outcome 2	
Parameters		Proportion of patients achieving the outcome	*p*-value	Proportion of patients achieving the outcome	*p*-value	Proportion of patients achieving the outcome	*p*-value	Proportion of patients achieving the outcome	*p*-value
CRP	≤3.0 mg/L	19.1% (18/94)	0.020	8.5% (8/94)	0.014	18.0% (20/111)	<0.001	10.8% (12/111)	<0.001
	3.1–10.0 mg/L	36.4% (20/55)		23.6% (13/55)		42.9% (54/126)		20.6% (28/126)	
	≥10.1 mg/L	-		-		50.0% (26/52)		36.5% (19/52)	
Fecal calprotectin	≤250.0 µg/g	20.0% (21/105)	0.007	9.5% (10/105)	0.620	25.2% (33/131)	0.004	14.5% (19/131)	0.086
	250.1–500.0 µg/g	53.8% (7/13)		15.4% (2/13)		40.4% (23/57)		22.8% (13/57)	
	≥500.1 µg/g	-		-		45.4% (44/97)		25.8% (25/97)	
Anemia (Hg < 12 g/dL for women, <13 for men)	Yes	50.7% (34/67)	0.002	34.3% (23/67)	<0.001	49.1% (26/53)	0.014	34.0% (18/53)	0.004
	No	29.7% (66/222)		15.4% (34/222)		31.4% (74/236)		16.5% (39/236)	
Ferritin	≤30.0 µg/L	19.2% (5/26)	0.068	19.2% (5/26)	0.930	32.6% (14/43)	0.413	27.9% (12/43)	0.342
	30.1–100 µg/L	39.0% (39/100)		20.0% (20/100)		38.5% (52/135)		18.5% (25/135)	
	≥100.1 µg/L	-		-		30.6% (34/111)		18.0% (20/111)	
Transferrin saturation	≤15.0%	45.2% (23/73)	0.028	27.4% (17.1%)	0.058	43.4% (36/83)	0.047	24.1% (20/83)	0.236
	≥15.1%	31.0% (67/216)		17.1% (37/216)		31.1% (64/206)		18.0% (37/206)	
IFX through levels <3.0 µg/mL	Yes	26.4% (37/140)	0.005	19.5% (29/149)	0.909	44.2% (65/147)	<0.001	21.1% (31/147)	0.553
	No	42.3% (63/149)		20.0% (28/140)		24.6% (35/142)		18.3% (26/142)	
Anti-IFX antibodies >1.7 µg/m	Yes	45.2% (28/62)	0.049	29.0% (18/62)	0.053	39.3% (24/61)	0.381	26.2% (16/61)	0.150
	No	31.7% (72/227)		20.2% (46/227)		33.3% (76/228)		18.0% (41/228)	

more variables were simultaneously above the normal range: patients with anemia, CRP levels above 10 mg/L and FC levels above 500.0 µg/g demonstrated probabilities of attaining composite outcomes 1 and 2 as high as 63.0% and 50.0%, respectively. The results obtained for the second matrix were similar (Supplementary Figure S3).

DISCUSSION

Early flare prediction is essential for the timely management of CD, allowing optimal use of the available therapeutic toolbox and decreasing disease burden. However, no consensus has been reached to date on relevant predictive factors, while the appropriate cut-off values for some biomarkers (such as fecal calprotectin) remain elusive.[12] To shed some light on this important topic, we evaluated the ability of a combination of readily available biomarkers to predict CD prognosis using data from a prospective cohort study that enrolled IBD patients under IFX maintenance therapy. In this analysis, disease progression was monitored using two composite outcomes, both evaluating clinical and drug-related factors, but the first one included data on IFX dose escalation or interval reduction. The choice to differentiate the endpoints was made to accommodate the needs of healthcare institutions that cannot easily monitor IFX trough levels and anti-drug antibodies and where IFX posology cannot, therefore, be readily adjusted.

Although the first composite outcome was more frequently achieved, both outcomes were significantly more common in patients with higher FC and CRP levels and presenting anemia during the follow-up.

The presence of anemia was more effective in the prediction of disease progression when compared with single moderate elevations of FC (250.1–500.0 µg/g) or CRP (3.1–10.0 mg/L) levels, and this was evident in the multivariable analysis, even when anemia was detected in at least one visit. Previous studies have already reported that anemia is significantly correlated with disease progression, higher relapse, and hospitalization rates, need for surgical intervention and lower quality of life.[13,14] However, to the best of our knowledge, the quantitative impact of anemia on CD had not been formally investigated.

TABLE 4 Multivariable logistic regression—odds ratios (OR) for each of the two composite outcomes after adjusting for confounding factors (sex, body mass index, disease location and behavior, presence of perianal disease, and age at diagnosis).

		Values in at least one visit	Values in at least two non-consecutive visits	Values in at least two consecutive visits	Values in at least three non-consecutive visits
Composite outcome 1					
CRP	≤3.0 mg/L	*Reference*			
	3.1–10.0 mg/L	1.587 (0.687–3.666, $p = 0.280$)	**2.459** (1.239–4.880, $p = 0.010$)	**2.890** (1.306–4.423, $p = 0.012$)	**3.510** (1.901–6.489, $p < 0.001$)
	≥10.1 mg/L	**3.187** (1.456–6.979, $p = 0.004$)	**3.783** (1.845–7.757, $p < 0.001$)	-	**4.504** (2.1123–9.559, $p < 0.001$)
Fecal calprotectin	≤250.0 µg/g	*Reference*			
	250.1–500.0 µg/g	1.495 (0.624–3.579, $p = 0.367$)	1.935 (0.947–3.955, $p = 0.070$)	**5.607** (1.586–19.780, $p = 0.007$)	**2.036** (1.029–4.027, $p = 0.041$)
	≥500.1 µg/g	**3.069** (1.462–6.443, $p = 0.003$)	**3.357** (1.804–6.247, $p < 0.001$)	-	**2.789** (1.550–5.020, $p = 0.001$)
Anemia (Hg < 12 g/dL for women, <13 for men)	Yes	**2.436** (1.408–4.215, $p = 0.001$)	**2.312** (1.288–4.150, $p = 0.005$)	**2.403** (1.306–4.423, $p = 0.005$)	**2.070** (1.086–3.947, $p = 0.027$)
	No	*Reference*			
Transferrin saturation	≤15.0%	**1.844** (1.099–3.123, $p = 0.023$)	**1.815** (1.074–3.066, $p = 0.026$)	**1.920** (1.063–3.469, $p = 0.031$)	1.736 (0.988–3.051, $p = 0.055$)
	≥15.1%	*Reference*			
Composite outcome 2					
CRP	≤3.0 mg/L	*Reference*			
	3.1–10.0 mg/L	1.306 (0.462–3.691, $p = 0.614$)	1.962 (0.830–4.637, $p = 0.124$)	**3.831** (1.321–11.108, $p = 0.013$)	2.091 (0.982–4.451, $p = 0.056$)
	≥10.1 mg/L	**2.613** (1.012–6.749, $p = 0.047$)	**3.591** (1.522–8.475, $p = 0.004$)	-	**4.938** (2.113–11.541, $p < 0.001$)
Fecal calprotectin	≤250.0 µg/g	*Reference*			
	250.1–500.0 µg/g	0.880 (0.264–2940, $p = 0.836$)	1.529 (0.602–3.886, $p = 0.372$)	2.222 (0–3886–12.801, $p = 0.371$)	1.764 (0.773–4.029, $p = 0.178$)
	≥500.1 µg/g	**3.267** (1.265–8.435, $p = 0.014$)	**3.639** (1.668–7.937, $p = 0.001$)	-	**2.486** (1.234–5.009, $p = 0.011$)
Anemia (Hg < 12 g/dL for women, <13 for men)	Yes	**3.396** (1.770–6.510, $p < 0.001$)	**3.277** (1.660–6.468, $p < 0.001$)	**3.077** (1.547–6.124, $p = 0.001$)	**2.735** (1.348–5.549, $p = 0.005$)
	No	*Reference*			
Transferrin saturation	≤15.0%	1.280 (0.685–2.392, $p = 0.439$)	1.553 (0.814–2.813, $p = 0.190$)	1.677 (0.851–3.303, $p = 0.135$)	1.289 (0.662–2.504, $p = 0.454$)
	≥15.1%	*Reference*			

Note: The values within brackets represent the 95% confidence interval and the *p*-value for each OR.

CRP values above 10.0 mg/L in at least one visit were significant predictors of disease progression, whereas milder elevations were only significant when detected in at least two visits (consecutive or not). FC values between 250.1 and 500.0 µg/g only predicted the occurrence of composite outcome 1 when recorded in at least two consecutive or three non-consecutive visits. However, these intermediate concentrations were not good predictors of composite outcome 2. On the other hand, when FC values were above 500.0 µg/g—even in at least one measurement—a significant association with both composite outcomes was detected. A recent study[15] reported that increased FC concentrations were associated with higher risk for CD progression, also estimated using a composite endpoint (but with no drug-related items). In that report, the FC cut-off associated with progression was lower (115 µg/g). However, all these patients were asymptomatic, most had an inflammatory phenotype and less than 20% were under anti-TNF treatment, suggesting that the disease was milder in that study population.

In our study, ferritin, IFX trough levels and anti-IFX antibody concentrations were not significant in the multivariable analysis after adjusting for previously described[16,17] confounding factors. In the

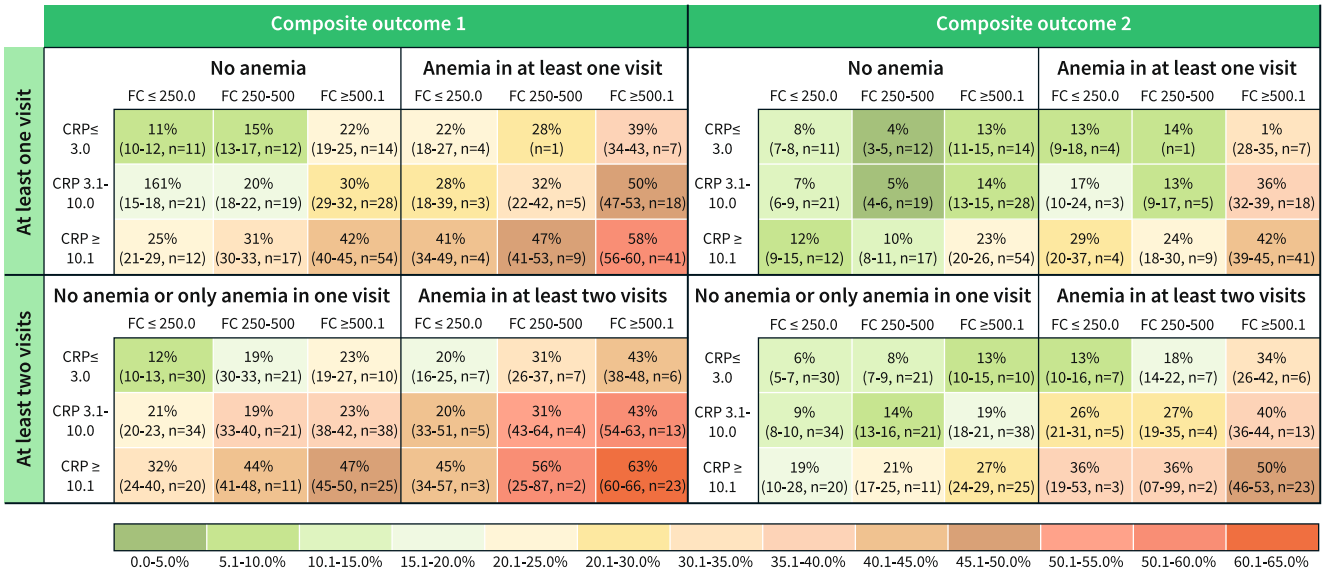

FIGURE 2 Risk matrix with presence of anemia, C-reactive protein (CRP) and fecal calprotectin (FC) levels considered as variables. The mean probability (percentage) of achieving the two composite outcomes depending on the presence or absence of anemia and on whether the CRP and FC levels were above or below the defined cut-off values is depicted. Individual squares are color-coded according to the risk category for achieving the outcome. The number of patients and 95% confidence intervals for individual probabilities are shown in brackets.

case of anti-IFX antibodies, the lack of significance may be associated with the cut-off utilized (considered as positive if ≥ 1.7 μg/mL). Indeed, in a recent study, higher thresholds (between 16 and 45 μg/mL) modestly correlated with treatment failure, hospitalizations, need for steroids and drug exchange[18] Concerning IFX concentrations, widely variable cut-offs have been reported depending on the week of measurement and the target endpoint,[19,20] suggesting that this parameter is difficult to include in a risk matrix, at least in a dichotomous way.

The sensitivity analysis, which performed clinical and drug-related items separately, showed similar findings for the latter. The assessment of the ability of the parameters to predict only clinical items (surgery, hospitalization, new fistulae, abscess or stricture) is biased by their low prevalence in our cohort (9.3%).

In contrast with individual parameters, the combination of two or more had a better predictive ability. Indeed, patients who simultaneously exhibited CRP values above 10.0 mg/L, FC values above 500.0 μg/g and anemia demonstrated probabilities of achieving the composite outcomes that ranged between 42% and 63%. The incorporation of data collected in at least one or two time points to the risk matrices seems to represent the optimal strategy, since adding data from additional visits did not meaningfully influence the predictions derived from the risk matrices and is likely to simply delay decision-making.

This is one of the first studies developing risk matrices to predict the occurrence of composite outcomes in CD patients, contributing to validate these endpoints and providing an easily applicable tool for CD clinical management. The results obtained in this analysis suggest that: (i) the isolated presence of anemia, TS bellow 15.1%, FC values above 500.0 μg/g, or CRP values above 10.0 mg/L even during a single visit during maintenance therapy are good predictors of CD progression in patients under IFX treatment and should be considered as a red flag; (ii) intermediate elevations of FC or CRP values are not accurate predictors by themselves, unless observed in at least two consecutive or in at least three visits during follow-up; (iii) the predictive ability of these biomarkers is noticeably improved when they are combined in a risk matrix, allowing for an accurate prediction of CD prognosis even when data from a single time point is used. These results corroborate the findings of the CALM trial,[2] in which biomarkers were shown to be crucial for the timely management of CD patients under adalimumab therapy.

The use of risk matrices as point-of-care tools has emerged as a sound strategy for the clinical management of CD patients, allowing for closer monitoring of those individuals with higher probability of progression, and aiding therapeutic decisions. Based on the current state of knowledge, the best strategy is probably to maintain FC as the cornerstone of IBD monitoring[21] but to increase its diagnostic accuracy by incorporating additional laboratory parameters (the presence of anemia and CRP levels, in particular). Even though some previous studies have emphasized the superiority of multiple measurements of FC and CRP in predicting IBD prognosis,[22,23] our study suggests that a single measurement above the cut-off values is enough to predict progression, particularly when both parameters are simultaneously abnormal and are accompanied by anemia.

The main strength of this report is being a real-world study with high visit compliance, central monitoring, centralized laboratory measurements, long-term follow-up, and the involvement of almost 300 patients. Moreover, the use of readily available and cost-effective parameters to generate the risk matrices allows for their application in a wide variety of settings. Nevertheless, this analysis has some limitations, including the lack of imaging, endoscopic or histologic assessment. Also, the size of the study population was not

large enough to allow combining the results of the four different parameters into a single predictive matrix, and no separate derivation and validation cohorts were included. Even though we acknowledge that this strategy may have reduced the external validity, dividing the patients into two cohorts would have decreased the number of patients that simultaneously had concentrations of hemoglobin, TS, CRP, and FC within a given range, thus decreasing the power of the analysis and therefore its internal validity. Future studies focused on different cohorts of CD patients are needed to confirm the generalizability of the risk matrices.

AUTHOR CONTRIBUTIONS

Fernando Magro coordinated study design, was involved in data obtention and interpretation and in manuscript drafting; Maria Manuela Estevinho was involved in data analysis and interpretation and manuscript drafting; Marta Patita, Bruno Arroja, Paula Lago, Isadora Rosa, Helena Tavares de Sousa, Paula Ministro, Irina Mocanu, Ana Vieira, Joana Castela, Joana Moleiro, Joana Roseira, Eugénia Cancela, Paula Sousa, Francisco Portela and Luís Correia were involved in data collection and interpretation; Mafalda Santiago and Sandra Dias were involved in study monitoring and data collection; Joana Afonso, Silvio Danese and Laurent Peyrin-Biroulet were involved in data interpretation and manuscript drafting, Cláudia Camila Dias coordinated data analysis. All authors performed a critical review and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

Fernando Magro served as a speaker and received honoraria from Abbvie, Biogen, Falk, Ferring, Hospira, Janssen, Laboratórios Vitoria, Lilly, Pfizer, Merck Sharp & Dohme, Sandoz, Takeda, UCB and Vifor. Helena Tavares de Sousa received fees for serving as a speaker for Takeda, AbbVie, Janssen, Pfizer, Ferring and Biogen. Isadora Rosa reports personal fees and/or non-financial support from Abbvie, Ferring, Pharmakern, Janssen and Takeda, outside the submitted work, as well as research grants from Abbvie and Ferring. Silvio Danese served as a speaker, consultant and advisory board member for Schering Plough, Abbott (AbbVie) Laboratories, Merck, UCB Pharma, Ferring, Cellerix, Millenium Takeda, Nycomed, Pharmacosmos, Actelion, Alfa Wasserman, Genentech, Grunenthal, Pfizer, AstraZeneca, Novo Nordisk, Cosmo Pharmaceuticals, Vifor and Johnson and Johnson. Laurent Peyrin-Biroulet reports personal fees from Merck, Abbvie, Janssen, Genentech, Mitsubishi, Ferring, Norgine, Tillots, Vifor, Hospira/Pfizer, Celltrion, Takeda, Biogaran, Boerhinger- Ingelheim, Lilly, HAC- Pharma, Index Pharmaceuticals, Amgen, Sandoz, Forward Pharma GmbH, Celgene, Biogen, Lycera, and Samsung Biosepsis. All other authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data will be shared on reasonable request to the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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