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Brain-immune axis regulation is responsive to cognitive behavioral therapy and mindfulness intervention: Observations from a randomized controlled trial in patients with Crohn's disease☆

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ABSTRACT

Background and aims: Crohn's disease (CD) is a chronic inflammatory bowel disease associated with psychological stress that is regulated primarily by the hypothalamus-pituitary-adrenal (HPA) axis. Here, we determined whether the psychological characteristics of CD patients associate with their inflammatory state, and whether a 3-month trial of cognitive-behavioral and mindfulness-based stress reduction (COBMINDEX) impacts their inflammatory process.

Methods: Circulating inflammatory markers and a wide range of psychological parameters related to stress and well-being were measured in CD patients before and after COBMINDEX. Inflammatory markers in CD patients were also compared to age- and sex-matched healthy controls (HCs).

Results: CD patients exhibited increased peripheral low-grade inflammation compared with HCs, demonstrated by interconnected inflammatory modules represented by IL-6, TNFα, IL-17, MCP-1 and IL-18. Notably, higher IL-18 levels correlated with higher score of stress and a lower score of wellbeing in CD patients. COBMINDEX was accompanied by changes in inflammatory markers that coincided with changes in cortisol: changes in serum levels of cortisol correlated positively with those of IL-10 and IFNα and negatively with those of MCP-1. Furthermore, inflammatory markers of CD patients at baseline predicted COBMINDEX efficacy, as higher levels of distinct cytokines and cortisol at baseline, correlated negatively with changes in disease activity (by Harvey-Bradshaw Index) and psychological distress (global severity index measure) following COBMINDEX.

Conclusion: CD patients have a characteristic immunological profile that correlates with psychological stress, and disease severity. We suggest that COBMINDEX induces stress resilience in CD patients, which impacts their well-being, and their disease-associated inflammatory process.

; IBD, Inflammatory bowel disease; CD, Crohn's disease; HPA, Hypothalamus-pituitary-adrenal; COBMINDEX, Cognitive-behavioral and mindfulness-based stress reduction; HC, Healthy controls.

☆ The institutional ethics committees of Soroka Medical Center and Rabin Medical Center approved the trial. All participants were given a detailed written and oral description of the research project and provided their written informed consent.

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1. Introduction

Crohn's disease (CD) is a common form of inflammatory bowel disease (IBD) characterized by progressive and destructive chronic inflammation of the gastrointestinal tract (Roda et al., 2020; Gajendran et al., 2018; Petagna et al., 2020). Several factors are implicated in the causation of CD, including a dysregulated immune system, altered microbiota, genetic susceptibility, and environmental factors, but the etiology remains unknown (Petagna et al., 2020; Leppkes and Neurath, 2020).

Recent studies suggest the existence of a regulatory cytokine network that has important implications for the progression of CD (Leppkes and Neurath, 2020). A defective gut barrier and microbial dysbiosis induce a local immune response, which results in a pro-inflammatory cytokine loop that overrides anti-inflammatory signals and causes chronic intestinal inflammation (Neurath, 2019). Well-characterized inflammatory responses which evolve in CD include the IL-1 pathway, pro-inflammatory cytokine (e.g., IL-6, IL-18 and TNFα) release (Huang and Chen, 2016), and an enhanced T helper 17 (Th17) cell response (Velikova et al., 2020; Kuwabara et al., 2017). Recent studies highlight IL-18 as a pro-inflammatory cytokine with a main role in intestinal diseases (Williams et al., 2019). These cytokine responses seem to have a crucial role in the pathogenesis of CD and its complications, and therefore regulating them has a beneficial impact on CD patients (Schoultz and Å., 2019; Ballegeer et al., 2018). The first line medical treatment of CD patients at relapse often includes corticosteroids which effectively block the inflammatory response in the gut (Sulz et al., 2020). Immunosuppressive medication (thiopurines, methotrexate) are then used to prevent and/or reduce the severity of relapses (Adegbola et al., 2018). Currently, the treatment scheme of patients with CD has been extended to include biological treatments, such as anti-TNFα medications (Adegbola et al., 2018; Ding et al., 2016; Osterman et al., 2014) anti-integrins, anti-IL-12 and JAK inhibitors (Cushing and Higgins, 2021).

Individuals living with CD experience numerous psychological symptoms (Wynne, 2019). Psychological dysfunction may be causally associated with both the onset and progression of IBD (Wynne, 2019). The effect of psychological distress can be beneficial or harmful, at least in part, due to its ability to modulate inflammation (F.S., 2009; Reed and Raison, 2016) through pathways involving the sympathetic nervous system (SNS), and the hypothalamus-pituitary-adrenal (HPA) axis (Reed and Raison, 2016). The SNS triggers the release of catecholamines (mainly epinephrine and norepinephrine) from the adrenal gland, which may act to increase or decrease the production of circulating proinflammatory cytokines including IL-1β, IL-6, TNF-α and IL-8 (Johnson et al., 2019; Rea et al., 2017; Chavan et al., 2017). Concurrently with activation of the autonomic nervous system, the brain stimulates the production of neuropeptides, corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP), which together turn on the HPA axis (Reed and Raison, 2016), a key regulatory pathway in stress response and various homeostatic processes (Reed and Raison, 2016). The product of this pathway is cortisol, which is secreted in a pulsatile pattern that increases with environmental and psychological stressors (Spencer RLD, 2017; Makris et al., 2020). Disturbances in normal HPA axis activity profiles are associated with a variety of physiological and mental health disorders (Makris et al., 2020; Sylvia and Demas, 2018). Usually, cortisol exerts major suppressive effects on the immune system by reducing the activity of circulating inflammatory cells and inhibiting the production of proinflammatory mediators (Reed and Raison, 2016; Rea et al., 2017). Additionally, cortisol engenders negative feedback to the HPA axis to ultimately turn down or turn off the axis (Heyner et al., 2019). Whereas the daily circadian rhythm of cortisol regulates inflammation, chronic stress may alter the response of cells to cortisol, producing the so-called steroid resistance (Chavan et al., 2017; Ince et al., 2019), a process facilitating chronic inflammation and reducing the efficacy of corticosteroid drugs (Harpaz et al., 2013).

The understanding that psychosocial interventions enhance immunity and improve immune-related health outcomes is grounded in research showing that immune system processes are influenced by social, neurocognitive, and behavioral factors (Shields et al., 2020). Several treatments are available in clinical practice for reducing psychological dysfunction (including stress, anxiety and depression) in IBD patients (Wynne, 2019). Previous studies have performed cognitive behavioral therapy (CBT) sessions in IBD patients, and reported mixed effects of these psychosocial interventions on disease activity and wellbeing (Gracie et al., 2017; Mikocka-Walus et al., 2015, 2017). Furthermore, a systematic review concluded that bidirectional effects of the gut-brain axis may influence both the natural history of IBD and its psychological manifestations (Fairbrass et al., 2021). More recent studies have demonstrated that psychosocial interventions did not improve the inflammatory process in CD patiens (Gracie et al., 2017; Mikocka-Walus et al., 2015, 2017), suggesting that a more specific and personally tailored treatment along with ongoing self-practice is needed to achieve efficacy. In line with this concept, we recently reported a randomized parallel-group physician-blinded trial of cognitive behavioral and mindfulness-based stress reduction (COBMINDEX) on health-related quality-of-life (QoL) and psychological symptoms in adults with mild-moderate CD. Our study demonstrated that COBMINDEX attenuates emotional stress and mental health symptoms, which in turn contribute to improved QoL (Goren et al., 2021) (Table 1).

In the present study we aimed to determine whether COBMINDEX impacts a range of immune and endocrine markers that regulate the inflammatory process in patients with CD by examining 1) the inflammatory state of CD patients compared to healthy controls (HC), 2) associations between psychological variables and inflammatory markers of disease activity at baseline, 3) associations between psychological outcomes of COBMINDEX and inflammatory mediators, and 4) inflammatory markers as predictors of COBMINDEX efficacy.

2. Materials and Methods

2.1. Cohort

CD patients were recruited from July 2018—July 2020 by advertising at participating hospitals (Soroka Medical Center at Beer Sheva, and Rabin Medical Center at Petah Tikva, Israel) and social media (Goren et al., 2021); the study was completed in November 2020. There were no study-related untoward effects. Patients aged ≥18 years with Harvey-Bradshaw Index (HBI) of disease activity in the range 5–16 were eligible. Clinical social workers performed initial screening, and gastroenterologists enrolled patients meeting inclusion/exclusion criteria (Goren et al., 2021). Exclusion criteria included: age <18 years, no diagnosis of CD, <1 year of follow-up since diagnosis, change of diagnosis in study period, new medication started in past 3 months, surgery in past 6 months, planned surgery, acute surgery during study, pregnancy, planned pregnancy in study period, present/past psychiatric disease/medication, irritable bowel syndrome, not fluent in Hebrew. Patients' electronic medical and pharmacy records were reviewed by the study gastroenterologists to determine if they had any of the exclusion criteria. Irritable bowel syndrome was excluded on clinical grounds (according to Rome 4 criteria). Patients were then randomized to COBMINDEX or wait-list controls; study physicians and laboratory workers were blinded as to patient allocation. Randomization was carried out using the cluster random sampling method (Goren et al., 2021), with proportionate allocation strategy where the fractions were defined by their sex, to COBMINDEX (taught by clinical social workers per standardized protocol on Skype™ over three months) or wait-list (control group). The study was carried out between the time points designated as

Table 1
Demographic, medical, and psychological characteristics of Crohn's disease (CD) patients in COBMINDEX and wait-list experimental groups.

	CD COBMINDEX (n=55)	CD Wait-List (n=61)
Age, mean yr (SD)	33.6 (13)	32.4 (11)
Women, N (%)	38 (69)	37 (61)
Economic status:		
Low, N (%)	6 (11)	23 (38)
Middle-high, N (%)	49 (89)	38 (62)
Working, N (%)	40 (73)	48 (79)
Current smoking, N (%)	6 (11)	8 (13)
Body mass index, median (Min-Max)	21.1 (19.6–25.1)	22.4 (20.0–25.4)
Illness duration, mean years (SD)	9.1 (8.8)	8.9 (8.1)
Harvey-Bradshaw Index:		
Mild disease (5-7), N (%)	25 (45)	29 (48)
Moderate disease (8-16), N (%)	30 (55)	32 (52)
Medications		
Steroids, N (%)	1 (2)	6 (10)
Immunomodulators, N (%)	12 (22)	10 (16)
Biologics, N (%)	24 (44)	23 (38)
Opiates, N (%)	0	0
PSS-4 median and IQR (interquartile range)	T1: 7.00(5.0–8.00) T2: 4.00(3.0–6.00) *P value*<.001	T1: 8.00 (6.0–10.00) T2: 8.00 (5.0–10.00) *P value= 0.675*
HBI median and IQR	T1:8.00 (7.0–10.00) T2: 4.00 (2.00–5.00) *P* value < 0.001	T1:8.00 (6.50–10.50) T2: 7.00 (4.50–9.00) *P* value = 0.005
SIBDQ median and IQR	T1: 41.00 (34.00–46.00) T2: 50 (42.00–54.00) *P* value < 0.001	T1: 38.00 (33.00–46.50) T2: 41.00 (32.00–50.00) *P* value =0.016
SF-12PH median and IQR	T1: 44.44 (38.36–48.31) T2: 47.38 (41.87–47.38) *P* value < 0.001	T1: 41.76 (34.44–45.27) T2: 43.88 (37.28–47.22) *P* value =0.004
SF-12MH median and IQR	T1: 39.12 (36.61–42.45) T2: 42.36 (39.49–45.00) *P* value = 0.001	T1: 39.83 (35.28–43.57) T2: 38.20 (36.07–42.16) *P* value =0.353
GSI median and IQR	T1: 0.98 (0.72–1.55) T2: 0.70 (0.45–0.98) *P* value < 0.001	T1: 1.08 (0.68–1.95) T2: 0.98 (0.58–1.63) *P* value =0.046
FACIT-F median and IQR	T1: 26.00 (20.00–31.00) T2: 33.00 (24.00–41.00) *P* value < 0.001	T1: 25.00 (15.50–32.00) T2: 24.00 (17.50–37.00) *P* value =0.106

Psychological scores at baseline (T1) and after COBMINDEX (T2), as previously reported (Goren et al., 2021). PSS-4, Perceived Stress scale [0-4]; HBI, Harvey-Bradshaw Index [remission 0-4; mild 5-7; moderate 8-16; severe >16]; SIBDQ, Short Inflammatory Bowel Disease Questionnaire [possible range 10–70], SF-12 PH. Short Form Physical Health Composite [0-100]; SF-12 MH, Short Form Mental Health Composite [0-100]; GSI, Global Severity Index [0-4]; FACIT-F, Functional Assessment of Chronic Illness Therapy-Fatigue [0-52]. Wilcoxon signed-rank test was used to test the difference between repeated measurement, $p < 0.001$ between COBMINDEX and Wait-list groups.

baseline T1, and study completion after three months T2. Physicians remained blinded to randomization throughout the study. The nature of the study precluded patient participation in protocol-design. All patients remained on medical follow-up, and participation in the study did not affect their treatment.

Our cohort included 171 individuals, in three groups; (1) age- and sex-matched healthy controls (HC) (n = 55, mean age (SD) 30.6 (8.4) years, 57% females), (2) CD patients taught COBMINDEX (n = 55, mean age 34.0 (13) years, 67% females), (3) CD patients on wait-list (n = 61, mean age 33.4 (11) years, 62% females). COBMINDEX and wait-list patients were similar in age, sex, disease activity and treatments (Table 1).

All the samples and data analyzed and presented in the following study were collected prior to the outburst of the COVID-19 pandemic.

Trial registration: Ministry of Health, Israel. https://my.health.gov.il/CliniTrials/Pages/MOH_2020-02-24_008721.aspx.

ClinicalTrials.gov Identifier: NCT05085925.

2.2. Medical and psychological data

At baseline (T1) and after 3 months (T2) we assessed the patients using the following measures (Goren et al., 2021):

Harvey-Bradshaw Index (HBI): This questionnaire evaluates disease activity in five questions pertaining to the past day's symptoms of well-being. Responses are summed to provide HBI. A HBI ≤4 indicates disease remission, 5–7 mild disease, 8–16 moderate disease, >16 severe disease.

Short Inflammatory Bowel Disease Questionnaire (SIBDQ): This disease-specific quality of life (QoL) questionnaire relates to the past two weeks' symptoms, general feeling and mood in ten items graded on a 7-point Likert scale (1 = all the time, 7 = never). A higher score indicates better QoL.

MOS 12-Item Short Form Survey Instrument (SF-12): This generic QoL measure has twelve items assessing physical functioning and general health. Scores are summed to yield physical health (SF-12PH) and mental health (SF-12MH) composites, with range 0–100. A higher score indicates better QoL.

Functional Assessment of Chronic Illness Therapy- Fatigue (FACIT-F): This tool assessing mental fatigue comprises 13 questions related to the past seven days. Responses are scored on a 5-point Likert scale, a higher score means less fatigue. It is a valid instrument in CD patients.

Brief Symptom Inventory (BSI): This instrument (al'Absi, 2018) measures psychological symptoms in the past month. Its 53 questions assess nine dimensions (depression, somatization, obsession-compulsive, interpersonal sensitivity, anxiety, hostility, phobic anxiety, paranoid ideation, psychoticism) on a 0–4 Linkert scale (0 = not affected, 4 = extremely affected). A higher score implies more severe symptoms. The BSI yields a score for each dimension and a summary score called the Global Severity Index (GSI), all with range 0–4.

Perceived stress scale (PSS-4): Self-reported questionnaire that measures a person's evaluation of stressful situations in the previous 1 month of his or her life. The instrument contains 14 statements which measure how unpredictable, and overloaded respondents feel their lives are. Respondents rate how often they experience stressful situations on a 5-point Likert scale ranging from 'never' (0) to 'very often' (4). Higher scores are correlated to more stress.

2.3. Biological data collection

Biological samples (peripheral blood) for measuring inflammation and hormones were drawn from patients at baseline (T1) and after 3 months (T2), and from HC at T1. Blood samples were taken during morning hours (08:00–13:00) and serum was isolated from Vacuette tube 8 ml with gel (Greiner, Kremsmünster, Austria) and stored in aliquots at −80 °C until analysis. The profiling of cytokines/chemokines in serum samples was performed with the CytoFLEX instrument (Beckman Coulter, Brea, CA, USA) using the LEGENDplex™ (BioLegend, San Diego, CA, USA) Human Inflammation Panel 1 (with assay coefficients of variation) [13-plex: IL-1β (13.2%), IFN-α2 (23.9%), IFN-γ (12.6%), TNF-α (11.1%), MCP-1/CCL2 (8.3%), IL-6 (20.5%), IL-8 (CXCL8- 11%), IL-10 (9.1%), IL-12p70 (11.5%), IL-17A (21.4%), IL-18 (7.6%), IL-23 (9.3%), and IL-33 (19.4%)] according to the manufacturer's instructions. Samples loading into each 96-well plate was randomized to include 20% HC, 40% COBMINDEX CD (20% T1 and 20% T2) and 40% wait-list CD (20% T1 and 20% T2). Data were analyzed with the LEGENDplex™ Data Analysis Software Version 8.0. The validity and reliability of the high-sensitivity multiplex assays have been tested in previous studies, overall demonstrating that circulating serum and plasma concentrations of some

cytokines correlated accurately with ELISA results (Richens et al., 2010; Breen et al., 2011). The multiplex assays were found reliable, particularly in the context of smaller studies performed in a small scale and/or when all samples were collected and can be analyzed at a single time and place (Breen et al., 2011). Hormones were measured in serum samples using IMMULITE 2000 (Siemens, Erlangen, Germany).

2.4. Statistical analyses

Changes in values between T1 and T2 are denoted "Delta"Δ (T2-T1). In data-sets correlating deltas to deltas, changes were calculated as relative values (T2-T1/T1) as indicated in the table legends, denoted as "relative delta". For certain analyses, the COBMINDEX group was sorted according to a "Responsiveness Score" among patients treated with a biological treatment e.g., infliximab. We excluded patients on corticosteroids and immunomodulatory drugs. The responsiveness score is based on the three key parameters that were found to be improved in our recent study- HBI, GSI, and SIBDDQ (Goren et al., 2021). We calculated for each patient the Relative HBI changes between T_1 and T_2 (RelΔHBI), Relative GSI changes between T_1 and T_2 (RelΔGSI) and Relative SIBDQ changes between T_1 and T_2 (RelΔSIBDQ):

$$RelΔHBI = \frac{HBI(T_2) - HBI(T_1)}{HBI(T_1)}$$

$$RelΔSIBDQ = \frac{SIBDQ(T_2) - SIBDQ(T_1)}{SIBDQ(T_1)}$$

$$RelΔGSI = \frac{GSI(T_2) - GSI(T_1)}{GSI(T_1)}$$

$$Responsivness\ Score = RelΔSIBDQ - RelΔHBI - RelΔGSI$$

Categorical variables were summarized using frequencies and percentages. Quantitative variables are presented as mean and standard deviation (SD) if normally distributed, or median and range from minimal to maximal (Min-Max) if not-normally distributed. For univariate analysis, statistical differences were calculated with the Mann-Whitney test for non-normal distributed variables. Correlation coefficients were determined by Spearman rho. For multivariate analysis a quantile regression analysis was created for 0.1, 0.25, 0.5, 0.75 and 0.9 quantiles for target parameters, age, and gender. $P < 0.05$ was considered statistically significant; $0.1 > p > 0.05$ was considered as borderline significant. Analyses were performed using IBM SPSS Statistics 26 for Windows (IBM Corp: Armonk, NY).

A network graph portraying correlations between psychological, inflammatory, and clinical parameters was produced using the graph R package (see Supplementary Data 1) (Epskamp et al., 2012). Variables are displayed as nodes and the correlations between them are displayed as edges, with varying width according to the magnitude of the correlation. Heatmaps depicting cytokine correlations were produced using Python. Correlation p-values were corrected with family-wise error rate (FWER) using the Bonferroni-Holm method for multiple hypothesis adjustment and are displayed using asterisks. CD patients' cytokines at T1 were grouped to modules using the CytoMod approach (Cohen et al., 2019), which utilizes hierarchical clustering to form clusters of cytokines whose signals correlate across a cohort of individuals.

2.5. Ethical considerations

The institutional review boards of Soroka Medical Center (Beer Sheva, Israel) and Rabin Medical Center (Petah Tikva, Israel) approved the trial. Participants were given a detailed written and oral description of the research project and provided informed consent.

3. Results

There were 55 COBMINDEX subjects and 61 wait-list controls. All patients supplied blood samples at T1. However, 13 COBMINDEX and 11 wait-list subjects did not supply the second blood samples, the reasons being pregnancy (1 patient), and inability to draw blood or unwillingness to supply the blood sample (23 patients).

3.1. Crohn's disease patients exhibit increased peripheral low-grade inflammation compared with healthy controls

To characterize an inflammatory pattern among CD patients, we first compared the levels of circulating cytokines, measured with multiplex ELISA, in a cohort of CD patients (n = 92) with that of HCs (n = 55; cohort detailed Table 1). In line with previous studies (Martinez-Fierro et al., 2019), CD patients demonstrated significantly higher levels of the circulating proinflammatory cytokines IL-6 (p=0.018) and IL-18 (p=0.01), as compared with HCs (Fig. 1). While IL-6 was not detected in the serum of all the patients, serum levels of IL-18 were detectable in almost all individuals, and in markedly higher levels [median 213.84 (0–1668.42) n=92 and 157.95 (27.18–991.3) n=55 in CD patients and HCs, respectively], compared to IL-6 [median 0 (0–53.14) n=92 and 0 (0–9.4) n=55 in CD patients and HCs, respectively] (Table S1). In addition, a quantile regression analysis revealed a significant difference between CD patients and HCs for IL-18 [percentiles 25 (141.42 vs. 90.23), p=0.002 and 50 (213.84 vs. 157.95), p=0.054] and IL-6 [percentiles 75 (5.24 vs. 2.67), p=0.049 and 90 (9.98 vs. 3.52), p=0.026]. A significant difference between CD patients and HCs was observed also for the proinflammatory cytokines IFNγ [percentile 75 (2.62 vs. 0.7), p=0.006] and IL-12p70 [percentile 75 (3.14 vs. 1.2), p=0.006]. IL-17, IL-1β, IFNα, IL-8, IL-23 and IL-33 were not significantly different between CD patients and HCs (Table S1), findings that may indicate a variety in the disease phase at the time of sampling and/or subtypes of inflammatory profiles among CD patients. To further analyze variation in cytokine profiles in CD patients, we used CytoMod, an unsupervised method for identifying cytokine modules – clusters of cytokines with highly correlated expression levels. In CD patients at T1, we found cytokine clustering into 5 different modules: M1) IL-1, IFNα, IFNγ, Il-6, IL-12, IL-23, IL-10, and IL-33, M2) TNFα, M3) IL-8 and IL-17, M4) MCP-1, and M5) IL-18 (Fig. 2). Notably, there are strong correlations among cytokines between modules, such between IL-17, IL-23 and IL-12, showing their adjoint actions in the pathogenesis of CD.

3.2. Inflammatory patterns correlate with psychological markers in CD patients

Whereas the low-grade systemic inflammation in CD patients is often linked to the inflammatory process in the gut, the extent to which systemic inflammation represents disease perception, stress and wellbeing is yet unknown. Fig. 3A shows the main psychological parameters of CD patients evaluated at T1 (before COBMINDEX) and their association with parameters of low-grade inflammation (Fig. 3B and Supplementary Data 1). Increased levels of MCP-1 (rho=0.217, p=0.037), IL-8 (rho=0.197, p=0.05) and IL-18 (rho=0.247, p=0.018) positively correlated with PSS-4 (Fig. 3B, Table 2). Levels of IL-10 (rho=−0.266, p=0.01), IL-18 (rho=−0.212, p=0.043), IL-23 (rho=−0.212, p=0.043) and IL-12p70 (rho=−0.242, p=0.002) negatively correlated with scores of the SF-12MH (Fig. 3B, Table 2). Notably, IL-18 was found to be associated with most clinical and psychological measurements obtained at T1: thus, it correlated positively with HBI (rho=0.221, p=0.034), GSI (rho=0.267, p=0.01), PSS-4 (rho=0.247, p=0.018), and the BSI measures of somatization (rho=0.206, p=0.049), hostility (rho=0.256, p=0.014), paranoid ideation (rho=0.267, p=0.01), and psychoticism (rho=0.257, p=0.013). Additionally, it correlated negatively with SIBDQ (rho=−0.298, p=0.004), SF12-PH (rho=−0.217 p=0.037), SF12-MH (rho=−0.212, p=0.043) and FACIT (rho=−0.309, p=0.003) (Figs. 3B

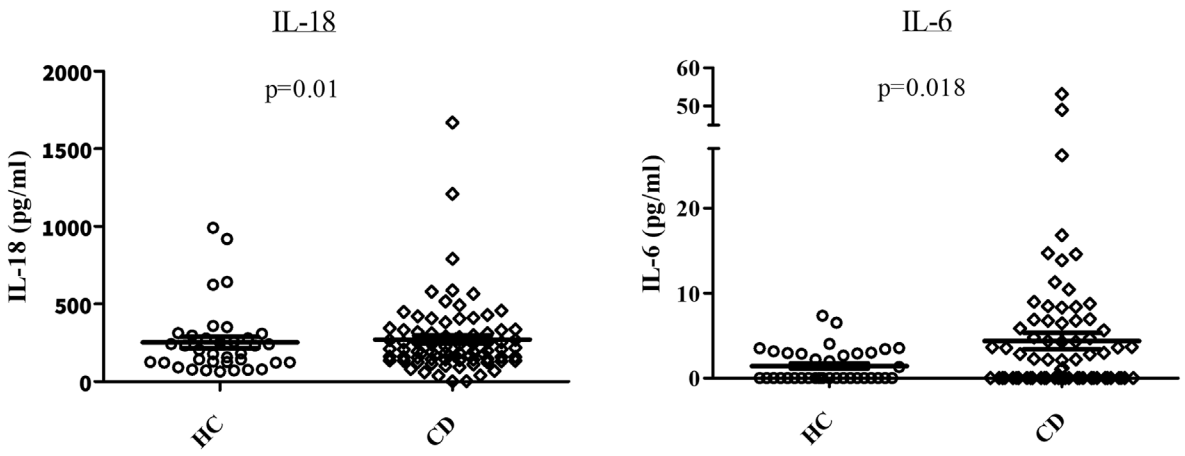

Fig. 1. CD patients demonstrate higher levels of the inflammatory cytokines IL-6 and IL-18. Increased levels of IL-6 (A) and IL-18 (B) in serum samples of CD patients (n=92) in comparison to healthy controls (n=55). p-values were calculated with Mann-Whitney Test.

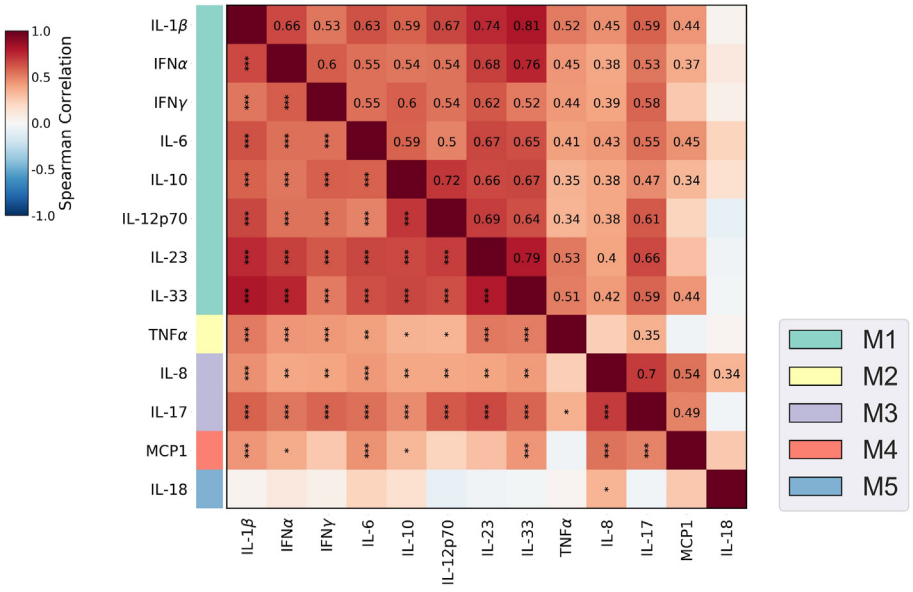

Fig. 2. Cytokine interactions modules among CD patients at baseline. A heatmap portraying Spearman correlations and their p-values corrected using family-wise error rate (FWER) for multiple hypothesis adjustment (Methods). Numbers indicate the correlations and asterisks indicate the FWER-adjusted p-values of these correlations: *p < 0.05, **p < 0.005 and *** for p < 0.0005. The colored stripes represent the assignment of cytokines to modules (M1-M5) by their correlations using the CytoMod algorithm (Cohen et al., 2019).

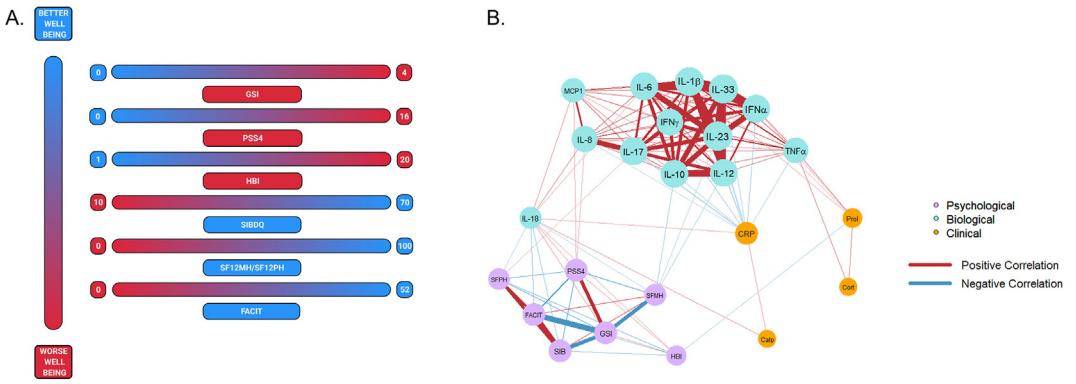

Fig. 3. Psychological, biological and clinical variables are interconnected in CD patients. A. Score ranges of medical and psychological characteristics: visualization of lowest and highest potential scores of 6 main parameters (HBI, GSI, PSS4, SIBDQ, SF12MH and FACIT, details in Materials and Methods) as they relate to the patients' well being. **B.** A network graph visualizing the correlations between inflammatory and psychological variables. Each variable is represented by a node and each correlation by a line, with varying of width according to the correlation magnitude. SFMH=SF12MH, SFPH=SF12PH, SIB=SIBDQ.

and 4, Tables S2–S3). Overall, our results indicate interactions between inflammation and the individual's psychological adjustment with the disease process and suggest that, at least in part, inflammation does not only represent gut pathology, but that it may also associate with the

Table 2
Cytokine levels correlate with PSS-4 and SF-12MH scores in CD patients at baseline (T1).

Cytokine	PSS-4		SF-12MH	
	Rho	p-value	rho	p-value
MCP-1	0.217	**0.037**	−0.067	0.526
IL-8	0.197	**0.059**	−0.156	0.137
IL-10	0.155	0.141	−0.266	**0.011**
IL-18	0.247	**0.018**	−0.212	**0.043**
IL-23	0.124	0.24	−0.212	**0.043**
IL-12p70	0.081	0.441	−0.242	**0.002**

Perceived stress scale (PSS-4), 12-Item Short Form Survey Instrument mental health (SF-12MH), Crohn's disease (CD). Correlations were calculated by Spearman rho.

psychological manifestations of the disease.

3.3. Psychological outcomes of COBMINDEX in CD patients correlate with inflammatory markers

COBMINDEX teaching over 3 months achieved a significant improvement of QoL measures such as SIBDQ, SF-12 and GSI (Goren et al., 2021) (Table 1). We sought to determine whether these psychological outcomes are accompanied by an inflammatory effect measured at T2. Since the serum levels of the cytokines measured at T1 did not significantly differ between T1 and T2 (data not shown), we correlated their changes with changes in psychological parameters between the two time points in both COBMINDEX and wait-list groups. Changes in SF12MH positively correlated with changes in IFNα in the COBMINDEX but not in wait-list group (rho=0.372, p=0.015, n=42 vs rho=0.116, p=0.431, n=50 in COBMINDEX and wait-list, respectively; see also Table S4 for data distribution among quantiles). Furthermore, changes in FACIT fatigue positively correlated with changes in IL-17 in wait-list but not in the COBMINDEX [rho=0.457, p=0.001, n=50 vs rho=0.027 p=0.864, n=42 in wait-list and COBMINDEX, respectively]. Together, these results suggest that psychological outcomes of COBMINDEX are accompanied by inflammatory changes in CD patients.

3.4. Changes in inflammatory markers in CD patients coincide with changes in the HPA axis

The HPA axis with one of its end products, cortisol, may become compromised in the course of CD due to chronic exposure to stress and inflammation (Gajendran et al., 2018). We thus postulated that COBMINDEX may recover, partially, the immunoregulatory role of the HPA axis as reflected by correlations between changes in cortisol and changes in cytokines. Serum levels of cortisol among CD patients at T1 showed only a non-significant increase (p=0.19, n=92) compared to HCs [median CD=8.72 μg/ml (1.01–37.15), n=92; HCs= 7.82 μg/ml (1.82–21.62), n=55). Following COBMINDEX, cortisol levels in serum samples of wait-list did not differ from the COBMINDEX [10.915 μg/ml (3.92–34.87, n=50) vs 9.43 μg/ml (0.32–32.17, n=42)] but were significantly higher than HCs [(8.95 μg/ml (1.01–37.15, n=55)] at T1 (p=0.024, n=42). Furthermore, changes in the serum levels of cortisol correlated positively with changes in serum levels of the regulatory cytokines IFNα (rho=0.326, p=0.046) and IL-10 (rho=0.332, p=0.039) and negatively with changes in serum levels of MCP1 (rho=−0.324, p=0.044) in COBMINDEX (Table 3). Changes in cortisol levels correlated negatively with changes in SIBDQ (rho=−0.305, p=0.039) and positively with changes in PSS-4 (rho=0.553, p=0+), in wait-list, but not in COBMINDEX (Tables 3 and S4). Taken together, these data suggest that, compared with wait-list controls, CD patients responding to COBMINDEX become more resilient to stress as reflected by the changes in serum levels of cortisol that coincide with a more balanced cytokine profile and

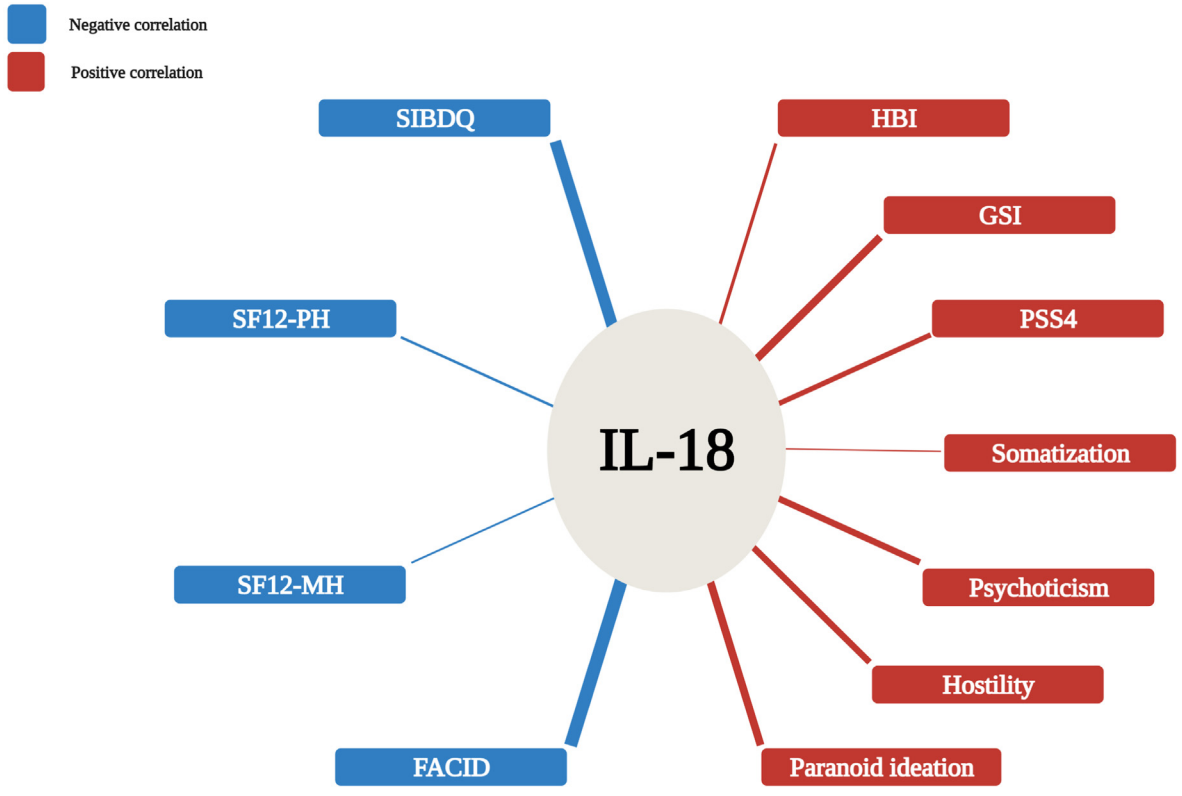

Fig. 4. IL-18 as a biomarker for declined well-being in CD patients. Visualization of the overall interactions between IL-18 levels and the psychological parameters of CD patients at baseline (Table S2). The red line denotes the negative correlation coefficients, the blue line denotes a positive correlation coefficients, and the thickness of the line stands for the correlation coefficients that are significant at the level of 5% ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Changes in cortisol levels correlate with changes in cytokine levels and the psychological parameters SIBDQ and PSS-4, among COBMINDEX and wait-list control CD patients.

		Delta Cortisol		
		CD	Wait-list	COBMINDEX
Delta IFNα	*rho*	−0.024	−0.18	0.326
	p-value	0.83	0.237	**0.046**
	N	92	50	42
Delta IL-10	*rho*	0.124	0.052	0.332
	p-value	0.261	0.737	**0.039**
	N	92	50	42
Delta MCP-1	*rho*	−0.156	0.033	−0.324
	p-value	0.158	0.828	**0.044**
	N	92	50	42
Delta SIBDQ	*rho*	−0.258	−0.305	−0.097
	p-value	**0.017**	**0.039**	0.555
	N	92	50	42
Delta PSS-4	*rho*	0.355	0.553	0.074
	p-value	**0.001**	**0+**[a]	0.656
	N	92	50	42

[a] 0+ positive number close to 0. Data are shown for the whole cohort (CD), wait-list or COBMINDEX patients. Correlations were calculated by Spearman rho.

psychological manifestations.

3.5. Inflammatory markers of CD patients predict COBMINDEX efficacy

To determine whether the cytokines, chemokines or cortisol measured at T1, predict which patients will benefit from COBMINDEX, the inflammatory markers obtained at T1 were correlated with the changes in clinical and psychological parameters obtained at T2. Higher serum levels of the cytokines IFNα (rho=−0.306, p=0.046), IFNγ (rho=−0.438, p=0.003), IL-10 (rho=−0.45, p=0.002), IL-23 (rho=−0.343, p=0.025), IL-33 (rho=−0.394, p=0.009) and IL-12p70 (rho=−0.318, p=0.037) observed at T1, correlated negatively with changes in HBI scores in COBMINDEX, but not in wait-list (Table 4 and S5). In addition, basal levels of circulating cortisol at T1 correlated negatively with changes in GSI in COBMINDEX (rho=−0.334, p=0.035), but not in wait-list (Table S6). Thus, higher baseline levels of cortisol lead to an improvement in GSI (psychological symptoms), stressing that it is a positive prognostic factor for COBMINDEX in CD patients. Finally, calprotectin, correlated positively with changes in IL-18 in wait-list (rho=0.36, p=0.027) (Table S6) and may indicate, at least in part, psychological manifestations of the disease.

Table 4
Baseline levels of cytokines correlate with changes in HBI in CD patients subjected to COBMINDEX.

		Delta HBI	
Cytokine		COBMINDEX	Wait-list
IFNα T1	Correlation Coefficient	−0.306	−0.029
	Sig. (2-tailed)	**0.046**	0.842
	N	42	50
IFNγ T1	Correlation Coefficient	−0.438	−0.004
	Sig. (2-tailed)	**0.003**	0.979
	N	42	50
IL-10 T1	Correlation Coefficient	−0.45	0.085
	Sig. (2-tailed)	**0.002**	0.562
	N	42	50
IL-23 T1	Correlation Coefficient	−0.343	0.064
	Sig. (2-tailed)	**0.025**	0.66
	N	42	50
IL-33 T1	Correlation Coefficient	−0.394	0.043
	Sig. (2-tailed)	**0.009**	0.77
	N	42	50
IL-12p70 T1	Correlation Coefficient	−0.318	−0.071
	Sig. (2-tailed)	**0.037**	0.627
	N	42	50

Data are shown for the whole cohort (CD), wait-list or COBMINDEX patients. Correlations were calculated by Spearman rho. Harvey-Bradshaw Index (HBI).

3.6. TNFα has a role in determining COBMINDEX effectiveness

When examining the inflammatory pattern in CD patients subjected to COBMINDEX at T2, we found no significant changes in the mean levels of cytokines compared to T1 (data not shown). We thus sought to find a correlation between the individual's responsiveness score and inflammatory mediators in a list of patients subjected to COBMINDEX who did not take corticosteroids and immunosuppressive drugs (n=29). Thus, we generated a combined individual responsiveness score based on the measures of SIBDQ, GSI and HBI at T1 and T2 (Methods). Notably, in these patients, we found a significant negative correlation between the responsiveness score and the circulating levels of TNFα. The correlation was observed at both T1 (rho=−0.488, p=0.007, n=29) and T2 (rho=−0.365, p=0.052, n=29) time points, indicating not only that TNFα plays a key pathogenic role in CD but that it may serve a negative biomarker for COBMINDEX efficacy.

4. Discussion

The present study evaluated serum samples of CD patients, before and after COBMINDEX, compared with wait-list CD patients and HCs. We demonstrated a characteristic inflammatory profile among CD patients, which concurs with psychological markers of stress and wellbeing. Furthermore, the patients' positive psychological outcomes following COBMINDEX were correlated with inflammatory changes and with changes in the main stress hormone, cortisol. Finally, both psychological and biological parameters were identified as predictors of successful COBMINDEX.

We found a distinct inflammatory pattern among CD patients consisting of higher levels of IL-6, IL-18, IFNγ and IL-12p70 like the characteristic inflammatory profile shown in previous studies (Martinez-Fierro et al., 2019). For other cytokines and chemokines tested, we did not observe a significant increase compared with HCs—a phenomenon that may signify the complexity of measuring inflammatory markers in patients with different histories in terms of disease onset, phenotype and treatments and enrolled into the research at different phases of the disease. Nonetheless, the cytokines measured as a group indicated strong interconnections and connections to psychological characteristics, demonstrating the undeniable impact they have on the progression of CD. Notably, the cytokines exhibit distinct clustering patterns, which may reflect their role in the pathogenesis of CD. While zooming into this network of connections, IL-18 is highlighted as an anchor, correlating with all aspects of the psychological status of the patients at the time, stressing its distinct role among inflammatory markers. These connections may reflect disease severity (Williams et al., 2019) or the involvement of IL-18 in depression and anxiety. Whereas IL-18 levels correlated with one of the main clinical biomarkers of CD, calprotectin (Karczewski et al., 2015), recent data also indicates associations of IL-18 with depressive symptoms in diabetes (Herder et al., 2018) and in depressive disorders in general (Fan et al., 2017). These studies found that chronic stress result in induction of IL-18 in the basolateral amygdala and in depressive-like behavior (Herder et al., 2018). Thus, IL-18—released via activation of the Nod-like receptor pyrin containing 3 (NLRP3) inflammasome in leukocytes and in neural cells—may play a role in linking environmental stimuli reflected by cellular stress with the development of depressive symptoms (Fan et al., 2017). Additionally, although serum levels of the inflammatory cytokines MCP-1 and IL-8 did not significantly increase in average in CD patients at T1, as previously described (Martinez-Fierro et al., 2019), they did correlate with PSS-4, similarly to IL-10, IL-18, IL-23 and IL-12p70 that correlated negatively with SF12-MH, thus further suggesting a connection between the disease process and emotional effects. Overall, whereas the cytokines measured reflect the extent and type of

inflammation associated with the process of CD (Ilias et al., 2020; Abautret-Daly ÁDempsey et al., 2017), how they impact stress, depression and wellbeing in CD requires further research.

As reported, a three-month COBMINDEX trial caused a significant improvement in psychological outcomes of CD patients (Goren et al., 2021). Although a previous study which compared standard care (SC) of CD patients with cognitive behavioral therapy (CBT) + SC failed to show a significant effect on CRP, disease activity scores, white cell count and mental health (Mikocka-Walus et al., 2017), we sought to determine whether the improved psychological outcomes in our study (Goren et al., 2021) were accompanied by changes in more specific markers of inflammation as measured at T1. Whereas the average mean levels of cytokines in serum did not show significant changes between T1 and T2, the levels of several cytokines did change in parallel with the psychological changes. Increase in physical health (SF12PH) was associated with an increase in IFNα, a cytokine that can have both pro- and anti-inflammatory functions (Chen et al., 2017). Its correlation with improved SF12PH scores suggests strongly that COBMINDEX promotes a regulatory role in the inflammatory process of CD patients (Andreou et al., 2020). Additionally, the increase in fatigue among patients (FACIT) in wait-list patients, but not COBMINDEX (where it decreased considerably), was associated with a significant increase in IL-17, a key pro-inflammatory cytokine in the pathogenesis of CD (Kuwabara et al., 2017; Bianchi and Rogge, 2019). IL-17 plays a key role in inflammatory diseases (Hohenberger et al., 2018), as it is produced by certain subsets of innate lymphoid cells and the pro-inflammatory Th17 cells (Hohenberger et al., 2018). Specifically, in the intestine, release of IL-23 induces the expression of IL-17 which then induces the expression of additional inflammatory mediators that, together, contribute to epithelial cell damage seen in IBD (Hohenberger et al., 2018). Notably, fatigue is both more frequent and more severe in patients with IBD than the general population (Schreiner et al., 2021). Thus, the consistency of low fatigue scores (indicating worse fatigue) in wait-list was possibly caused, in part, by increased levels of IL-17 due to its strong impact on pathogenic inflammation. Overall, it is evident that although COBMINDEX did not change the mean levels of cytokines among the entire cohorts of both wait-list and COBMINDEX—presumably given the variability in samples described above—it ameliorated the psychological manifestations which were markedly associated with the inflammatory markers we measured.

An important question in our study is whether the increased inflammatory profile in CD patients is, at least in part, related to cortisol dysregulation, cortisol being a key regulator of both stress (Rainer and Straub, 2016) and inflammatory responses (Yeager et al., 2011; Silverman and Sternberg, 2012). Under normal physiologic conditions, cortisol regulates inflammation primarily by inhibiting synthesis, release, and signaling of cytokines and other mediators that promote immune reactions (al'Absi, 2018). Additionally, cortisol may inhibit antigen presentation and thereby reduce activation and proliferation of T and B cells, causing a change in trafficking and function of peripheral cells and specifically leukocytes (Allolio, 2015). As such, the cortisol analogues methylprednisolone and dexamethasone are highly effective anti-inflammatory drugs used in numerous diseases including CD (Dorrington et al., 2020). Whereas COBMINDEX in our trial did not affect the serum levels of cortisol, it did result in positive correlations between serum levels of cortisol and the regulatory cytokines IL-10 and IFNα, as compared with wait-list (Martinez-Fierro et al., 2019). Furthermore, serum levels of cortisol correlated with the main psychological stress parameters in the study, negatively with changes in SIBDQ and positively with changes in PSS-4 among wait-list, but not COBMINDEX (Tables 3 and S4). It is thus plausible that the chronic stress that CD patients experience causes desensitization of the glucocorticoid (GC) receptor in various cellular components including gut epithelium and leukocytes, a process referred to as GC resistance (al'Absi, 2018; Merkulov et al., 2017). Decreased sensitivity to GCs is a characteristic features of various stress-induced psychopathologies (Chavan et al., 2017) and other inflammatory conditions like rheumatoid arthritis and asthma. Among CD patients, GC resistance is a known phenomenon which physicians fear due do its common complication of adrenal insufficiency (Ibrahim et al., 2017). While the detailed mechanism is unclear, several studies suggest altered GC sensitivity among Th17 cell-related diseases (Banuelos et al., 2017). Based on the correlations of cortisol with SIBDQ and PSS4 which were observed in wait-list, but not in COBMINDEX, we suggest that COBMINDEX may cause stress resilience manifested by the improved immunoregulatory role of cortisol. Thus, we suggest that exercising COBMINDEX during disease remission, which diminishes everyday psychological stress, may act to decrease the inflammatory tone during subsequent relapse.

Inflammatory diseases, specifically CD, have a variety of clinical manifestations among different individuals depending on genetic background and environmental factors. Understanding distinct baseline parameters among CD patients that may interfere or intervene with therapy is critical to improve efficacy. We thus aimed to uncover psychological or inflammatory markers that can predict COBMINDEX efficacy. Higher baseline levels of both pro-inflammatory and regulatory cytokines are indicative of a smaller change in disease activity (HBI), stressing that a worse inflammatory state reduces COBMINDEX efficacy (Abautret-Daly ÁDempsey et al., 2017). In contrast, our results show that a higher level of serum cortisol is a positive predictive factor for COBMINDEX success in CD patients, as it predicts improvement in general health (low delta of GSI score). Since activating the HPA axis is known to induce a dynamic range of cortisol response (Heyner et al., 2019), higher levels of circulating cortisol indicate that these individuals may be in a tense distress episode and thus are more likely to benefit from COBMINDEX (Ince et al., 2019; Mikocka-Walus et al., 2015).

When focusing on a combined COBMINDEX responsiveness score, we aimed to find additional specific parameters that determine which patients are at a better position to benefit from COBMINDEX. TNFα, known for its involvement in the pathological processes in CD, and a key target for biologic therapy, was found to be an important negative prognostic factor in the efficacy of COBMINDEX. CD has a characteristic T-cell mediated response, while the hallmark of its pathogenesis is a transmural inflammation, which is facilitated by increased proinflammatory cytokines such as TNFα (Adegbola et al., 2018). TNFα is an early potent pro-inflammatory cytokine in inflammatory processes underlying CD, meaning that it represents the state of inflammation during the disease (Adegbola et al., 2018). Accordingly, in our study higher levels of basal TNFα signified patients who were at a worse inflammatory state, were evidentially more stressed, and did not benefit as well from treatment. Therefore, our results suggest that a longer COBMINDEX period may be needed for CD patients with advanced disease to overcome their lengthy inflammatory state. Interestingly, our data imply that CD patients responding to anti-TNFα medications would demonstrate positive effects on wellbeing as well as in measures of disease activity.

In this study, as stated, we excluded patients with irritable bowel syndrome, which is a more common malady with a marked psychological component (Bianchi and Rogge, 2019). The classic association of increased or reduced bowel frequency related to change in the form of the stools over a six-month period was absent in all our patients. Patients with CD may have ongoing diarrhea and abdominal pain even when the CRP and HBI measurements decrease with medication. These patients have a persistent intestinal inflammation and have not reached histological remission. We emphasize the exclusion of irritable bowel syndrome to affirm that the results shown here characterize our cohort with CD.

We note the limitations of cohort size due to heterogeneity among patients which can result from 1) disease mechanism, phase, and severity, 2) different medications and/or 3) treatment compliance. A larger cohort with a high-resolution inflammatory mediators and cortisol evaluation over several time points, and in multiple samples of serum, saliva, and/or hair, may better represent the changes in the inflammatory response to stress and COBMINDEX in CD patients over time.

5. Conclusions

CD patients have a characteristic immunological profile that correlates with well-being, stress, and the disease process. COBMINDEX targets a reduction of everyday psychological stress and causes significant changes in patients' wellbeing. Our study demonstrates that these changes are correlated with inflammatory and stress biomarkers, which indicate that COBMINDEX may affect stress resilience, and thereby could improve the immunoregulatory role of cortisol. Therefore, exercising COBMINDEX during relapse would likely act to reduce the inflammatory tone. Finally, we identified several psychological and inflammatory markers that may predict COBMINDEX efficacy and can help in creating a personalized treatment plan for the individual CD patient.

Author statement

Nemirovsky Anna; Performed experiments and wrote the manuscript. Ilan Karny; Performed experiments and wrote the manuscript. Lerner Livnat; Performed experiments. Cohen-Lavi Liel; Performed data analysis. Schwartz Doron; Designed the study and recruited patients. Goren Ganit; Performed the cognitive behaviroal and mindfulness intervention. Sergienko Ruslan; Performed data analysis. Greenberg Dan; Designed the study. Slonim-Nevo Vered; Designed the CBI-mindfulness study. Sarid Orly; Designed the CBI-mindfulness study. Friger Michael; Performed data analysis. Regev Shirley; Performed data analysis. Odes Shmuel; Designed the study and wrote the manuscript. Hertz Tomer; Performed data analysis. Monsonego Alon; Designed the study, analyzed experiments and wrote the manuscript. All authors critically reviewed the manuscript.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Doron Schwartz reports a relationship with Takeda Pharmaceutical Co Ltd that includes: consulting or advisory and speaking and lecture fees. Doron Schwartz reports a relationship with AbbVie Inc that includes: consulting or advisory and speaking and lecture fees. Doron Schwartz reports a relationship with Pfizer Inc that includes: consulting or advisory. Doron Schwartz reports a relationship with Janssen Pharmaceuticals Inc that includes: speaking and lecture fees. Doron Schwartz reports a relationship with Ferring Pharmaceuticals Inc that includes: speaking and lecture fees. Doron Schwartz reports a relationship with Neopharm Labs Inc that includes: speaking and lecture fees. Ganit Goren reports a relationship with Ferring Pharmaceuticals Inc that includes: speaking and lecture fees.

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Appendix A. Supplementary data

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