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A 12-month pilot study outcomes of vagus nerve stimulation in Crohn's disease

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Abstract

Background: The vagus nerve has anti-inflammatory properties. We aimed to investigate vagus nerve stimulation (VNS) as a new therapeutic strategy targeting an intrinsic anti-inflammatory pathway in a pilot study in Crohn's disease patients. The main objectives addressed the questions of long-term safety, tolerability, and anti-inflammatory effects of this therapy. This study is the continuation of previous reported findings at 6 months.

Methods: Nine patients with moderate active disease underwent VNS. An electrode wrapped around the left cervical vagus nerve was continuously stimulated over 1 year. Clinical, biological, endoscopic parameters, cytokines (plasma, gut), and mucosal metabolites were followed-up.

Key Results: After 1 year of VNS, five patients were in clinical remission and six in endoscopic remission. C-reactive protein (CRP) and fecal calprotectin decreased in six and five patients, respectively. Seven patients restored their vagal tone and decreased their digestive pain score. The patients' cytokinergic profile evolved toward a more "healthy profile": Interleukins 6, 23, 12, tumor necrosis factor α, and transforming growth factorβ1 were the most impacted cytokines. Correlations were observed between CRP and tumor necrosis factor α, and some gut mucosa metabolites as taurine, lactate, alanine, and beta-hydroxybutyrate. VNS was well tolerated.

Conclusion & Inferences: Vagus nerve stimulation appears as an innovative and well-tolerated treatment in moderate Crohn's disease. After 12 months, VNS has restored a homeostatic vagal tone and reduced the inflammatory state of the patients. VNS has probably a global modulatory effect on the immune system along with gut metabolic regulations. This pilot study needs replication in a larger randomized double-blinded control study.

KEYWORDS

cholinergic anti-inflammatory pathway, Crohns disease, cytokines and metabolomics, heart rate variability, vagus nerve stimulation

1 | INTRODUCTION

Crohn's disease (CD) is a chronic inflammatory bowel disease (IBD) involving the distal small bowel and colon, with a remitting/relapsing course, and has a significant impact on the patient's quality of life.[1] Genetic susceptibility, environmental factors, and altered gut microbiota, leading to dysregulated innate and adaptive immune responses, are the core issue of CD.[1] Antitumor necrosis factor (TNF) α therapy has changed the prognosis of IBD, but 30% of patients are primary non-responders and up to 46% of responders will lose their response within 12 months[2] due to antidrug antibody formation in up to 43% of patients.[3] The median cost of 1-year anti–TNF-α therapy raises up to $40 000 for CD patients.[4] These treatments have complex side effects[5] resulting in 30%-50% of non-adherence[6] and patients show growing interest for complementary medicines.[7,8]

There is a bidirectional communication between the brain and the gut through the autonomic nervous system, represented by the parasympathetic nervous system (eg, the vagus nerves), and the sympathetic nervous system. An alteration of these brain-gut interactions is involved in the pathogenesis of IBD.[9] Bioelectronic medicine is based on neuromodulation of the nervous system restoring organ functions and health with less adverse effects than drugs, thus preventing adherence issues.[10] In this context, targeting the vagus nerve (VN) could open new therapeutic avenues in IBD.[11,12] The VN consists of 80% afferent and 20% efferent fibers[13] and is involved in the control of gastrointestinal functions.[14] It has anti-inflammatory properties both through its afferents, activating the hypothalamic-pituitary adrenal (HPA) axis to release corticosteroids, and its efferents, acting within the cholinergic anti-inflammatory pathway (CAIP).[15] An imbalance between the HPA axis and the VN is observed in CD.[16] Its efferent arm has an anti-inflammatory effect (eg, anti–TNF-α) through an inflammatory vagovagal reflex[17] and an interaction with the sympathetic splenic nerve within the celiac ganglion resulting in a TNF-α release inhibition by splenic macrophages.[18] Recent studies also showed that selective stimulation of vagal afferents induces an anti-inflammatory response due to activation in splenic projecting sympathetic fibers and splanchnic nerves activate an anti-inflammatory response in a variety of abdominal organs to reduce lipopolysaccharide (LPS)-induced TNF-α production; the efferent arm has also an anti-inflammatory effect due to sympathetic activation through the splanchnic nerve.[19,20] In addition, the superior mesenteric ganglia were recently identified as a node in the CAIP, also able to activate the splenic sympathetic innervation and mesenteric lymph nodes.[21] Thus, vagus nerve stimulation (VNS) has a potential therapeutic interest for TNF-α-mediated diseases such as IBD and rheumatoid arthritis.

Vagus nerve stimulation is already approved for the treatment of drug-resistant epilepsy with an efficacy of about 50% and is well tolerated with no severe adverse events.[22] We previously showed that VNS improves colitis in rats,[23] and in a translational approach, we then performed a pilot study where VNS improved active CD and restored vagal tone at a 6-month follow-up in five out seven patients.[24] Here, we report the outcomes of this pilot study at 12-month follow-up in nine CD patients with an overview of VNS effects not just on the previous clinical and physiological parameters but also on molecular parameters as plasma and gut inflammatory markers, and gut metabolomic profiles.

Key Points

- A 12-month invasive VNS alleviates the inflammatory status of patients with Crohn's disease.
- VNS impacts specifically the pro-inflammatory cytokines IL6, IL12, IL23, TNFa, in relation with gut mucosa metabolites.
- VNS restores a homeostatic vagal tone.

2 | MATERIALS AND METHODS

The study was approved by the Institutional Ethics Review Board (Identifier 11-CHUG-28), registered in Clinical Trials.gov (NCT01569503), and was conducted in accordance with the principles stipulated in the Declaration of Helsinki and the Good Clinical Practice guidelines of the International Council.

2.1 | Inclusion and exclusion criteria

We prospectively included patients (18 < age < 65 years) with a moderate CD activity index (220 ≤ CDAI ≤ 450) diagnosed for at least 3 months prior to screening, naive of biologic treatment (eg, anti–TNF-α) or despite a stable treatment reference, with a C-reactive protein (CRP) >5 mg/L and/or a fecal calprotectin (FC) >100 μg/g[25,26] and a CD endoscopic index of severity (CDEIS) ≥7.[27] For more details, see ClinicalTrials.gov. All patients signed an informed consent.

2.2 | Vagus nerve stimulation

Implantation of the VNS device was performed under general anesthesia by a single neurosurgeon familiar with this technique, as previously described.[24] Briefly, an electrode (Model 302; Cyberonics) was wrapped around the left cervical VN and connected to a bipolar pulse generator (Model 102). The left cervical vagus was stimulated because of a weak effect on heart rate.[28] Just after implantation, VNS was delivered during preprogrammed and fixed 30 seconds ON and 5 minutes OFF cycles with an initial intensity of 0.25 mA, a pulse width range of 250 or 500 μs (depending on patient tolerance), and a fixed low-frequency stimulation of 10Hz. The intensity was increased gradually from 0.25 mA to the highest tolerable setting. VNS was continuously performed for 12 months.

2.3 | Patient follow-up

Patients were followed up in consultation on inclusion and at 2, 4, and 6 weeks, and 2, 3, 6, 9, and 12 months after VNS implantation, and CDAI, CRP, and FC were monitored. An ileocolonoscopy was performed before the implantation of the device, at month 6 and month 12 of VNS to calculate the CDEIS.[29] Clinical remission was defined as usual by a CDAI ≤ 150.[30] Clinical response was defined as a CDAI improvement from baseline of at least 70 points (CDAI-70). Endoscopic remission was defined as a CDEIS score <6, complete endoscopic remission as a CDEIS score <3, and an endoscopic response by a decrease in more than five points.[31] Tolerance of VNS was evaluated at each consultation as for VNS in epilepsy.[32]

2.4 | Heart rate variability measurement

Heart rate variability (HRV) was monitored within the week before implantation of the device, and after 6 and 12 months of VNS. A continuous electrocardiogram (BIOPAC, Cerom) was recorded (1000 Hz sample frequency) under resting condition, during 30 minutes in a standard sitting limb lead II position, apart from any environmental stressful conditions. HRV was analyzed over periods of 20 minutes, with the Kubios-HRV software according to the European Task Force guidelines (1996).[33] The HRV spectrum (0-0.4 Hz) was calculated on interbeat intervals with FFT-based Welch's periodogram method (256s segment window; 50% overlap). Vagal tone modulation was evaluated by means of the HRV power spectrum including the high (HF, 0.15-0.4 Hz, ms^2), low (LF, 0.03-0.04 Hz, ms^2), and very low (VLF, 0-0.03 Hz, ms2) frequency bands. A normalization was applied on the HF [HF normalized unit (nu) = HF (ms^2)/(total power (ms^2)−VLF(ms^2)) × 100] and LF bands [LFnu = LF (ms^2)/(total power (ms^2)−VLF(ms^2)) × 100].

2.5 | Visceral pain and Hospital Anxiety and Depression scorings

The current perceived digestive pain score was reported on a visual analog scale (VAS) from 0 (no perceived pain) to 10 (intolerable perceived pain).[34]

Anxiety and depression were evaluated with the Hospital Anxiety and Depression (HAD) self-report scale.[35]

2.6 | Cytokine assays on blood and gut samples

Blood and gut biopsy samples were collected before and after 12 months of VNS (at 3-month VNS for the two excluded patients). Cytokine analysis was performed on EDTA-plasma samples and homogenized biopsies, using magnetic bead-based multiplex immunoassays following manufacturers' instructions. Biopsy samples were collected in the inflamed segments, as notified in Table 2 and Figure 5. Multiple cytokine analysis kits were obtained from Merck for interleukin (IL)-1beta, IL2, IL6, IL10, IL12(p70), IL17A, IL21, IL23, macrophage inflammatory protein (MIP-1α), interferon γ (IFNγ), granulocyte macrophage colony-stimulating factor (GM-CSF), and TNF-α (MILLIPLEX MAP High Sensitivity Human Cytokine Magnetic Bead Panel kit -28SK-12) and from Bio-Rad for Transforming Growth Factorβ1 (TGF-β1) (#171W4001M) and Monocyte Chemoattractant Protein 1 (MCP1) (#171BK36MR2). Data from the reactions were acquired using a MagPix system. Levels of each cytokine were compared to those of 10-12 healthy donors obtained on the same Luminex system, as graciously provided by the manufacturers. In order to show on a single plot the global cytokinin profile of patients, multivariate statistics were assessed (SIMCA v14 software; Umetrics AB) and PCA analysis was performed as for metabolomic data (see below).

2.7 | High-resolution magic angle spinning nuclear magnetic resonance (HRMAS NMR)-based metabolomics data acquisition and spectra processing

^{1}H HRMAS NMR experiments were performed on the same gut biopsy samples assessed for cytokine assay (ie, before and 12-month VNS), and biopsies collected at 6-month VNS have been added for the metabolomics study. Spectra were acquired on a Bruker Avance III 500 spectrometer (Bruker Biospin) of IRMaGE facility (CEA Grenoble), at a 500.13 MHz proton frequency. The samples were spun at 4 KHz and temperature maintained at 4°C for all experiments. Metabolites were assigned using standard 2D experiments, standard human metabolome database (HMDB) and according to literature. All NMR spectra were Fourier transformed, phased, and aligned on alanine 1.475 ppm (right peak of the doublet) with the Bruker software Topspin v3.2. Then, they were pre-processed for multivariate statistics using the using the NMRprocflow open source software (http://nmrprocflow.org[36]).

2.8 | Statistics

Due to the small sample size (n = 9 patients) of this pilot study, most of the variables are expressed by the median. Only the cytokines and metabolomics profiles were statistically analyzed as follows:

2.9 | Multivariate analysis

2.9.1 | Metabolomics

The buckets (variables) were imported into the SIMCA v14 software for the multivariate statistical analysis. Each bucket was mean centered

TABLE 1 Pre-VNS and 12-month VNS data recorded and analyzed during the trial study

	Patients	1		3		5		6	
	VNS time	Pre	12M	Pre	12M	Pre	12M	Pre	12M
CLINICAL	CDAI	330	180	221	141	227	−4	233	19
	CRP	7	<3[a]	6	8	2	6	21	<3[a]
	FC	38	24	20	14	3500	<15	3500	126
	CDEIS	11	5	8	0	8	0	8	2.5
Plasma (pg/mL)	TNF-α [NR: 1.9-5.6]	9.0	5.8	5.6	7.5	0.5	8.6	6.4	1.85
	IL23 [NR: 10.1-197]	1186	710	456	434	88	591	67.5	185
	IL17A [NR: 1.2-10.6]	26.6	28.1	19.6	24.8	6.8	23.8	6.6	7.5
	IL1β [NR: 0-2.15]	7.4	6.5	1.9	2.2	0.1	5.3	0.1	1.6
	IFNγ [NR: 0-8.7]	14.7	15.0	11.0	16.1	6.0	5.5	5.6	19.8
	II12p70 [NR: 0.3-3.8]	12	7.2	7.3	7.1	3.3	9.0	0.1	1.0
	IL6 [NR: 0-1.6]	4.0	2.2	2.1	3.5	2.0	4.4	4.3	0.1
CYTOKINES	IL2 [NR: 0-3.7]	4.2	3.5	0.2	0.2	0.2	1.1	0.2	0.2
	IL21 [NR: 0-2.6]	0.2	0.1	0.5	0.7	0.1	3.1	0.1	0.1
	GMCSF [NR: 15-275]	187	152	159	138	133	281	55	149
	MIP-1α [NR: 1.3-19.3]	37	35	23.5	24	15	33	40.5	27
	MCP1 [NR: 2-48]	64.6	31.6	35	28.1	54.1	36.6	31.6	18.8
	IL10 [NR: 0-8.9]	6.4	1.4	24.8	15.4	1.7	33.3	4.1	5.6
	TGF-β1 [NR: 100-2700]	6762	5584	4155	23378	12619	12521	6173	11579
Gut (pg/mg)	TNF-α	ND	ND	17.1	13.6	0.04	0.09	8.2	0.06
	MIP-1 α	ND	ND	40	24.5	29.5	17.1	189	41
	Mean heart rate	68.5	68	84	84.5	68.7	70	82	65
	Total power (ms^2)	1979	3569	1240	1200	3434	5805	1702	2805
HRV	VLF (0-0.04 Hz)	1243	2101	697	708	837	3007	971	1512
	LF (0.04-0.15 Hz)	657	961	484	364	644	1404	599	926
Spectral	HF (0.15-0.4 Hz)	80	507	59	129	1952	1483	132	367
analysis	LF (n u)	89.1	65.4	89.1	73.8	24.8	48.6	82	71.6
	HF (n u)	10.9	34.5	10.9	26.1	75.2	51.4	18	28.4
	LF/HF	8.2	1.9	8.2	2.8	0.3	0.95	4.55	2.5
HAD	Anxiety (0-21)	3	5	11	9	7	5	6	4
	Depression (0-21)	2	2	7	10	7	4	3	0
PAIN	Digestive pain (0-10)	2	1	4	5	2	1	6	0

Note: Red columns: patients removed at 3-mo VNS.

Abbreviation: CRP, C-reactive protein; FC, fecal calprotectin; HAD, Hospital Anxiety and Depression; ND: not determined; [NR]: for normal range values.

[a]Under the specificity limit.

and scaled to unit variance. Unsupervised principal component analysis was first performed to identify outliers. Then, supervised orthogonal partial least square analysis (OPLS) was performed using first the time after VNS as response variable for a discriminant analysis (OPLS-DA). Then clinical or biological parameters collected for all patients (transformed in log): CDAI, CRP, FC, CDEIS, HFnu, HAD, and cytokines were used as response variable. The number of components was determined using the cross-validation procedure, which produces the R2Y and Q2 factors, indicators of the goodness of fit and of predictability of the model. The results were visualized by plotting the scores of individuals in a plan, showing that the metabolic profiles evolve with each clinical/biological parameter separately and by plotting the first loadings. These latter were plotted as covariance (peak intensity) versus spectral buckets, then mimicking a NMR spectrum, but with positive (negative) peaks representing metabolites that increase (or decrease) according to the response variable, and color of peaks representing how discriminant these metabolites are in the statistical model (correlation and covariance between the metabolite level and the model). The metabolites

7		8		9		2		4	
Pre	12M	Pre	12M	Pre	12M	Pre	3M	Pre	3M
264	88	175	50	290	171	358	452	354	279
<3[a]	<3[a]	6	<3[a]	44	10	88	5	166	10
244	61	123	183	1349 2419	1349 2419	1577	222	847	1207
7	0	0	0	7.6	13	30	ND	14	ND
11.6	6.6	4.8	4.3	13.1	8.3	22	9	2.2	1.1
780	389	505	352.5	2970	1446	2243	2304	86	67.5
54.5	44.1	43.7	55.3	53.8	33.8	37.1	25.1	4.1	4.0
5.1	3.8	12.5	15.0	8.1	7.9	6.3	10.9	0.1	0.1
39.0	30.4	18.2	24.0	37.2	27.1	31.6	24.5	5.6	2.5
9.4	7.0	17.4	24.4	18.4	10.2	26.5	32.0	0.1	0.1
4.3	3.1	5.0	6.7	11.8	4.4	18.3	5.0	5.3	1.8
2.3	1.4	7.6	9.9	6.1	3.2	7.6	12.6	0.2	0.2
2.5	1.4	2.6	5.5	3.5	3.0	23.3	27.9	0.1	0.3
498	421	275	309	451	347	408	456	59	31
42	41	35	49	48	41	40	45.3	14	11.5
57.2	33.3	36.6	18.8	35	24.5	51.1	24.5	36.5	43.2
34.3	21.5	54	55	42	31.8	5.1	10.5	2.6	0.1
38998	8740	10410	2229	2245	6467	18913	3840	ND	12711
5	61	0.04	0.05	8.5	17.6	3.05	0.4	57.3	ND
73	84	79.2	29.8	116.6	79.6	231.4	70.7	1199	ND
87.9	79	63	69	85.5	83	64	ND	96	89
2012	2200	7196	3302	1643	1138	3249	ND	116	176
670	886	2584	1636	804	629	1988	ND	91	96
850	817	2429	1030	533	362	1042	ND	22	47
484	480	2182	635	305	147	219	ND	3	33
63.4	62.1	52.7	62	63.6	71.1	82.6	ND	86.3	59
36.1	36.5	47.3	38	36.4	28.9	17.4	ND	13.7	41
1.8	1.7	1.1	1.6	1.75	2.45	4.75	ND	6.3	1.4
14	12	8	6	9	9	13	13	11	8
11	7	5	8	2	2	2	1	11	8
3.5	1	4	0	5	5	4	ND	5	2

with correlation >.5 were considered as important and are discussed in this paper.

2.9.2 | Cytokine profile

The same procedure was used to build the PCA analysis with all cytokines; that is, each cytokine value was mean centered and scaled to unit variance before submitted to unsupervised Principal Component Analysis.

3 | RESULTS

All data are reported in Table 1.

3.1 | Patients

Nine patients (five men and four women; median age: 39 years; range: 20-52 years) were included in the study. The first patient was included in April 2012 and the last one in March 2016. Demographics

TABLE 2 Demography, disease characteristics, and stimulation settings

Demographics disease characteristics	1	2	3	4	5	6	7	8	9
Gender	M	M	M	F	F	M	F	M	F
Smoking habit	No	No	No	No	No	No	Yes	No	No
Age at inclusion (y)	47	30	40	49	52	24	20	52	31
Disease duration (y)	26	0.3	11	0.25	17	1	4.5	1	7
Disease location	Terminal ileum	Ileon colon	Ileon colon	Ileon colon	Colon	Ileon colon	Terminal ileum	Terminal ileum	Colon
Disease behavior	Stricturing	Stricturing	No sticturing no penetrating	No sticturing no penetrating	No sticturing no penetrating	No sticturing no penetrating	Stricturing	No sticturing no penetrating	No sticturing no penetrating
Treatment at inclusion	Aza[a]	None	None	None	None	Aza[a]	None	None	None

Stimulation Settings (duty cycle: 30 s ON/5 min OFF)				
	Post-surgery	Month 3	Month 6	Month 12
1	0.25 mA 250 μs 10 Hz	0.75 mA 500 μs 10 Hz	0.75 mA 500 μs 10 Hz	1 mA 500 μs 10 Hz
2	0.25 mA 500 μs 10 Hz	0.75 mA 500 μs 10 Hz	1.25 mA 500 μs 10 Hz	1.25 mA 500 μs 10 Hz
3	0.25 mA 250 μs 10 Hz	0.5 mA 250 μs 10 Hz	0.5 mA 250 μs 10 Hz	1 mA 250 μs 10 Hz
4	0.25 mA 250 μs 10 Hz	1 mA 500 μs 10 Hz	0.5 mA 500 μs 10 Hz	0.5 mA 500 μs 10 Hz
5	0.25 mA 500 μs 10 Hz	0.75 mA 500 μs 10 Hz	1 mA 500 μs 10 Hz	1 mA 500 μs 10 Hz
6	0.25 mA 500 μs 10 Hz	1.25 mA 500 μs 10 Hz	1.25 mA 500 μs 10 Hz	1.25 mA 500 μs 10 Hz
7	0.25 mA 500 μs 10 Hz	1 mA 500 μs 10 Hz	0.75 mA 500 μs 10 Hz	0.75 mA 500 μs 10 Hz
8	0.25 mA 500 μs 10 Hz	1 mA 500 μs 10 Hz	1.5 mA 500 μs 10 Hz	1.5 mA 500 μs 10 Hz
9	0.25 mA 500 μs 10 Hz	1 mA 500 μs 10 Hz	1 mA 500 μs 10 Hz	1 mA 500 μs 10 Hz

[a]Azathioprine 2.5 mg/kg; red-colored lines: removed patients at month 3 VNS who asked to remain stimulated until the end of the trial.

and Montreal classification[37] are presented in Table 2. Even with slight disease activity (CDAI = 175, CRP = 6, FC = 123), patient 8 was authorized to enter into the trial by the medical staff because of a flu-like syndrome under azathioprine, inducing reluctance to drugs. Patients 2 and 4 were removed from the study after a 3-month follow-up because of a worsened clinical state. P2 underwent an ileocecal resection, and P4 received combination therapy with infliximab and azathioprine. However, at their request, these two patients kept their device ON. None of the seven patients who continued the study received any rescue therapy such as steroids, immunosuppressants, or biologics during the 12 months of follow-up.

3.2 | Vagus nerve stimulation: parameters and tolerance

Intensity, duty cycle, pulse-width, and frequency of the nine patients are detailed in Table 2. The stabilized intensity at the end of the study was 1.25 mA in five patients (P1, P2, P5, P6, and P8), 1 mA in two patients (P3, P9), and 0.75 mA for P7. P2 kept the same stimulation parameters as before surgery and the intensity decreased to 0.5 mA in P4 after starting combination therapy. As reported in our 6-month follow-up study, we did not observed any major side-effects, in particular infections, and the device was well-tolerated by the patients as long as the intensity did not exceed 1.5 mA. No pulse generator or lead was removed. We observed minor effects classically described[24,28] and related to the stimulation (30 seconds/5 minutes) such as voice alteration/hoarseness and throat pain. No relevant cardiac effects were observed at the setting used.

3.3 | Crohn's Disease Activity Index

On *inclusion*, all the patients had a CDAI > 220 except P8 (175) (Figure 1). Median CDAI was 264 [range: 175-358]. After 12 months of VNS, five patients were in clinical remission (CDAI < 150) and two patients (P1, P9) had only slight disease activity (171 and 180, respectively). Median CDAI after 12 months of VNS was 88 [range: 0-180]. CDAI scores were reduced by at least 70 points in the seven patients, and a response primer (five out of nine patients) was observed from 3 months of VNS (Figure 1). It is noted that P4 had reached the CDAI-70 at 3-month VNS.

3.4 | C-reactive protein

On *inclusion*, seven patients had a CRP > 5 mg/L (Figure 2). The two patients with normal CRP (P5 and P7) had over-normal FC (Figure 2). Median CRP was 7 [range: <3-166]. At 12 months, CRP level decreased for four patients, with three patients reaching the normal value, and increased just above the normal value for two patients (P3, P5). P7 remained at a normal value. Median CRP was 3 [range: 0-10]. P2 and P4 dramatically decreased their CRP from 88 and 166 to 5 and 10, respectively, at month 3.

3.5 | Fecal calprotectin

On *inclusion*, seven patients had a FC level above 100 μg/g and two patients (P1,P3) had a value below 100 μg/g, with an over-normal CRP (Figure 2). Median was 847 [range: 20-3500]. After 12 months of VNS, FC level decreased for three patients (P5, P6, P7), two of them reaching the normal level. One patient still had high FC (P9). P1 and P3 remained at a normal level, while P8 stayed below normal level. The median was 61 [range: 14-2419]. Note that the FC level decreased for P2 at month 3, while it increased for P4.

3.6 | Crohn's Disease Endoscopic Index of Severity

On *inclusion*, all the patients had a CDEIS ≥ 7, except P8 with a CDEIS < 1 (Figure 1). Median CDEIS was 8 [range: 0-30]. At 12-month VNS, median CDEIS was 0 [extremes: 0-13]. The endoscopic score decreased below the cutoff level for five patients, increased for P9 while staying below the cutoff level for P8. CDEIS scores were reduced by 60 to 100% in 5 out of 7 patients at 12 months (Figure 1).

3.7 | Digestive pain

The median digestive pain score was 4 [range: 2-6] before VNS, and 1 [range: 0-5] after 12 months of VNS (Figure 2).

3.8 | Heart Rate Variability

Vagus nerve stimulation increased the HFnu component of HRV in three out of seven patients who had a low HFnu on *inclusion* (HFnu = 11; 11; 18); VNS produced a moderate effect on HFnu in three out of seven patients who initially exhibited a medial HFnu (HFnu = 36; 36; 47), and induced a dramatic reduction in the only patient with very high HFnu on inclusion (HFnu = 75). Hence, VNS produced a global modulatory effect on vagal tone according to the initial level on inclusion (Figure 3).

3.9 | Depression and anxiety symptoms

On *inclusion*, the range of HADS anxiety and HADS depression of the patients were 3-14 and 2-11, respectively, with three out of seven patients exhibiting mild anxiety (HADS-A = 11; 14; 9) and one mildly depressive patient (HADS-D = 11; Figure 2). The remaining four patients were under the threshold. At 12-month VNS, the range of HADS-A and HADS-D was 5-12 and 0-10, respectively, the

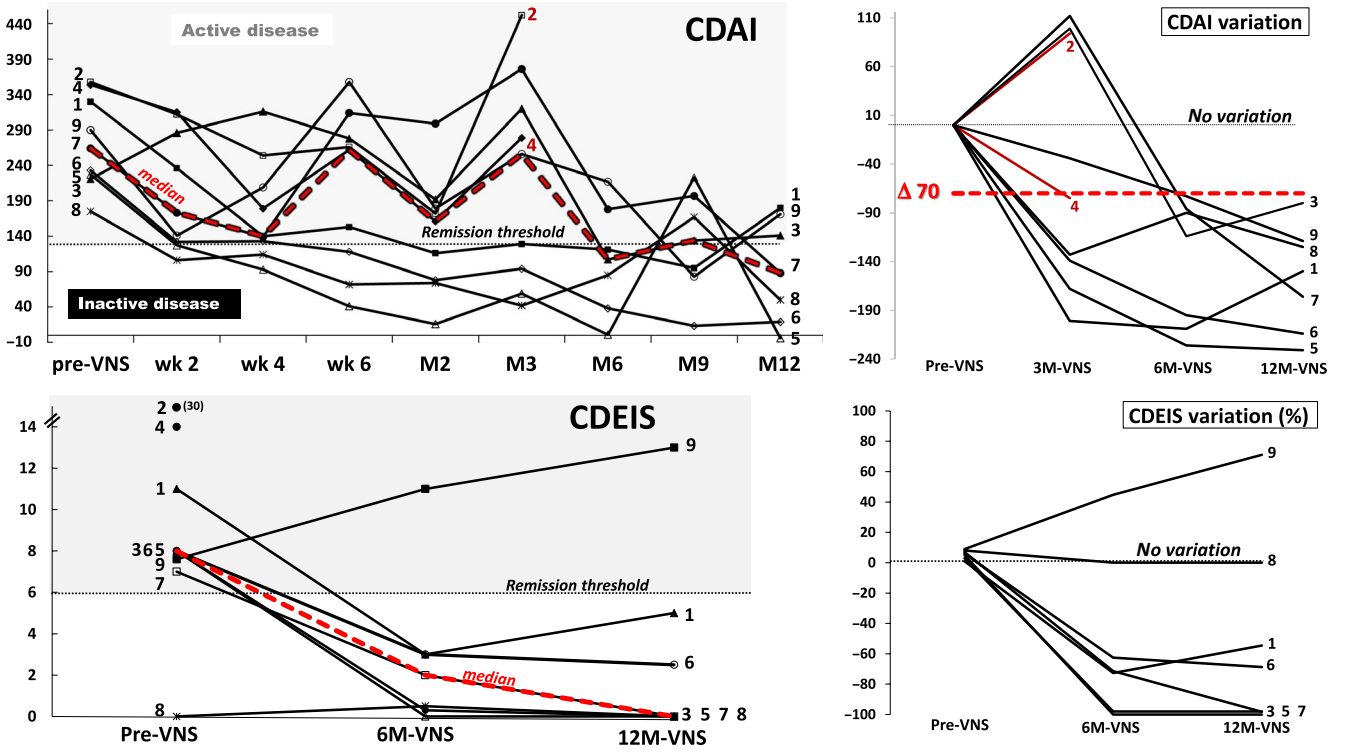


FIGURE 1 CDAI and CD endoscopic index of severity (CDEIS) 12-month follow-up and variations. Red dotted line: median; black thin dotted line: remission threshold level (150 for CDAI and six for CDEIS). For CDAI variation, the clinical response is defined by a decrease of 70 points after 12-mo vagus nerve stimulation (VNS). CDEIS variation is expressed in percentage

proportions of patients exhibiting mild anxiety and depression remained the same as on inclusion.

3.10 | Cytokines

The PCA analysis, using multicytokines assay for all patients, allows representing a plasma cytokinergic profile for controls, before, 6-month, and 12-month VNS (Figure 4). The control values appeared well grouped, while profiles before stimulation were very scattered, indicating that each patient had his/her own cytokinergic profile. After 6 months, and even more after 12 months of VNS, the points are tightened, indicating that cytokine levels evolve through a more "common" profile.

Before VNS , some plasma cytokines stood out from others with 2- to > 10-fold over the normal range for some patients (Figure 5). This is the case for pro-inflammatory ***IL6*** (7/9), ***IL23 and IL17A*** (6/9), ***IL12*** and ***IL1β*** (5/9), and ***IFNγ*** and ***MIP-1α*** (4/9). ***TNF-α*** high levels were observed for three patients, and surprisingly, this cytokine was four times lower than the inferior normal range for P5. For *anti-inflammatory cytokines*, above-ranged levels were observed for ***TGF-β1*** in seven patients and in four patients for ***IL10***, with once again below the inferior normal range for P5 (Figure 5).

After 12 months of VNS , the plasma cytokinergic profile was characterized by a decrease in the following pro-inflammatory cytokines (Figure 5): ***IL6*** (four patients returned to normal); ***IL12*** decreased in four patients (three patients returned to normal); ***TNF-α*** (two patients returned to normal); ***IL23*** decreased in four patients (one patient return to normal); **IFNγ** decreased in two patients. ***MIP-1α*** returned to normal in one patient. Removed patients at 3-month VNS had normalized ***IL6*** (P4) and ***TNF***-α (P2). It should be noted that some pro-inflammatory cytokines increased in four patients. In particular, P5 increased IL12, IL17A, IL1β, IL6, and IL23 (above normal levels), while TNF-α below the normal range at inclusion, was normalized. The cytokinergic profile also evolved for anti-inflammatory cytokines: Five and four patients remained or increased to over-ranged ***TGF-β1 and IL10*** levels, respectively (Figure 5). As for pro-inflammatory cytokines, P5 showed a huge increase for IL10 (1800%) and TGF-β1 (460%). For patients removed at 3-month VNS, ***TGF-β1*** reached normal while ***IL10*** levels remained unchanged.

Because of the scarcity of gut biopsies, only TNF-α and MIP-1α were assayed. TNF-α median level decreased 16 times after 12-month VNS and MIP-1α median was halved (Figure 5).

3.11 | Metabolomics

An untargeted ^{1}H HRMAS NMR-based metabolomics study was performed on all biopsies to investigate whether a distinct metabolic signature could be identified between patients before and

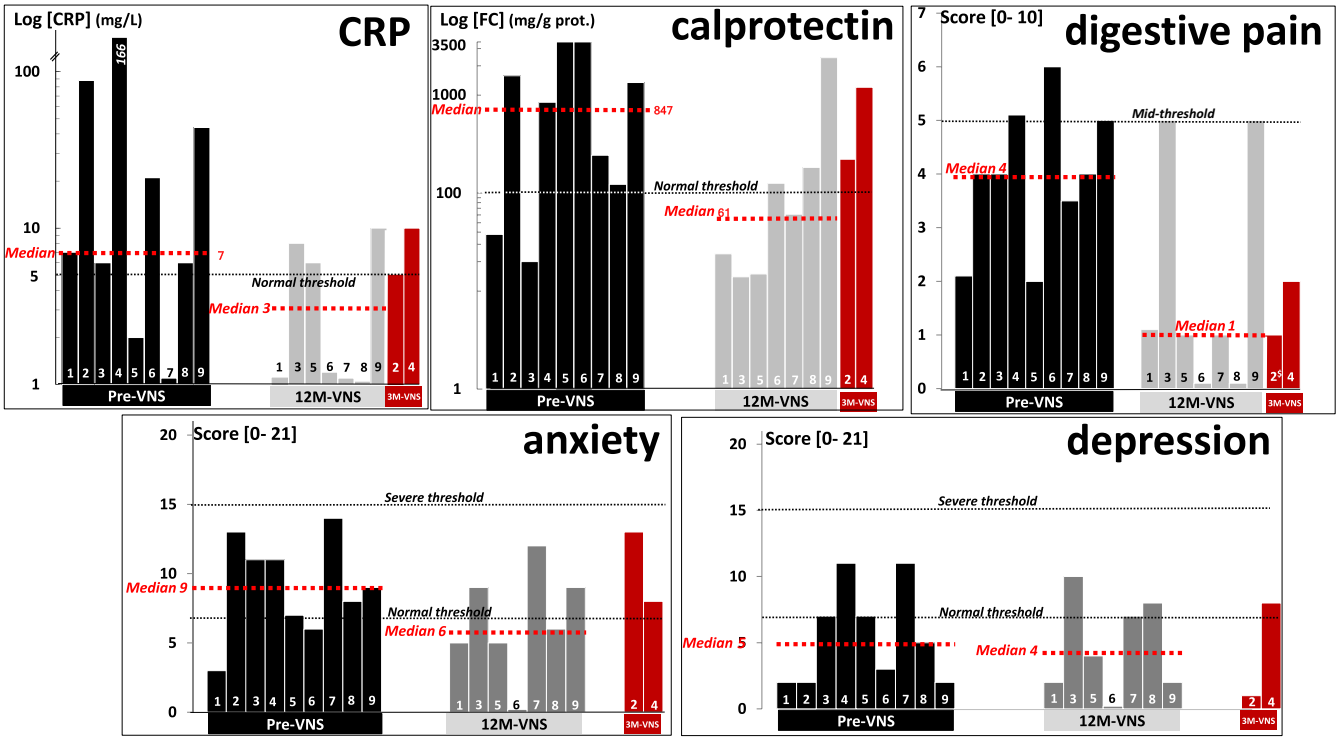
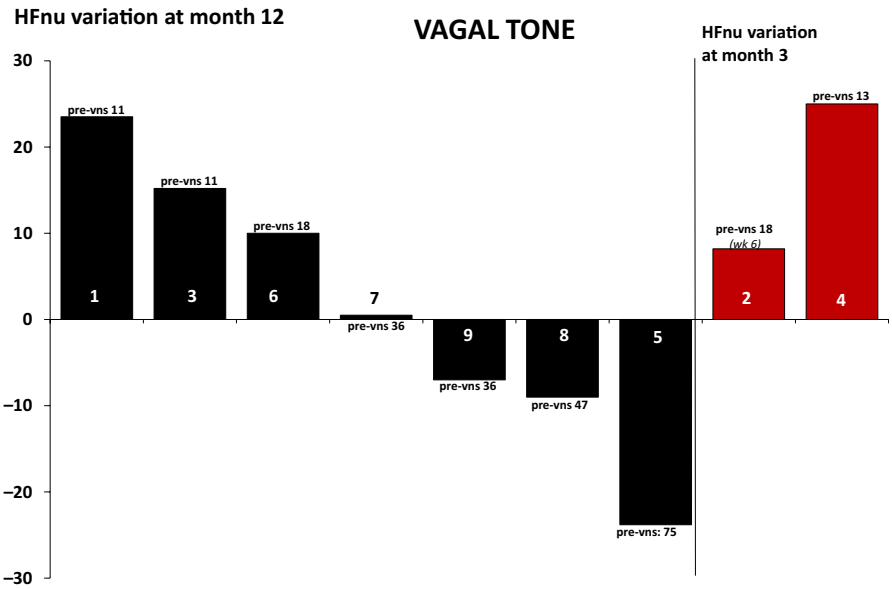


FIGURE 2 Twelve-month vagus nerve stimulation (VNS) effect on C-reactive protein (CRP), fecal calprotectin (FC), digestive pain, and Hospital Anxiety and Depression (HAD). CRP and FC are expressed in mg/L and mg/g proteins, respectively; CRP normal threshold <5 mg/L; CRP <3 is considered to be under the specificity limit; FC normal threshold <100 µg/g; perceived digestive pain score is reported on a visual analog scale (VAS) from 0 (no perceived pain) to 10 (intolerable perceived pain); anxiety and depression were evaluated with the HAD self-report scale: Each sub-score ranges from 0 to 21 with scores of 0-7 being considered normal, 8-10 indicative of mild, 11-14 moderate, and 15-21 severe anxiety/depression symptoms. Red dotted line: median; black thin dotted line: thresholds; $: value at 6 wk; In red: patients removed at 3-mo VNS

FIGURE 3 Twelve-month vagus nerve stimulation (VNS) effect on vagal tone variation. High frequencies are expressed in normal units; HFnu at inclusion are given underneath or on top of the histogram of variation respective to each patient (pre-vns value)



under VNS, at two time points (6 and 12 months). Good resolution and signal-to-noise ratio were obtained allowing for searching metabolic differences between the two conditions (no and active VNS).

The PCA showed no outliers (data not shown). Orthogonal partial least square analysis performed between metabolite data matrix and each time point of VNS treatment (ie, pre-, 6-month and 12-month VNS) did not reveal any statistical separation or between metabolite matrix and clinical/biological parameters, except for CRP and TNF-α. Indeed, a time-independent correlation between metabolic profile and either CRP or TNF-α levels was highlighted (Figure 6A,B). We mainly observed an increase

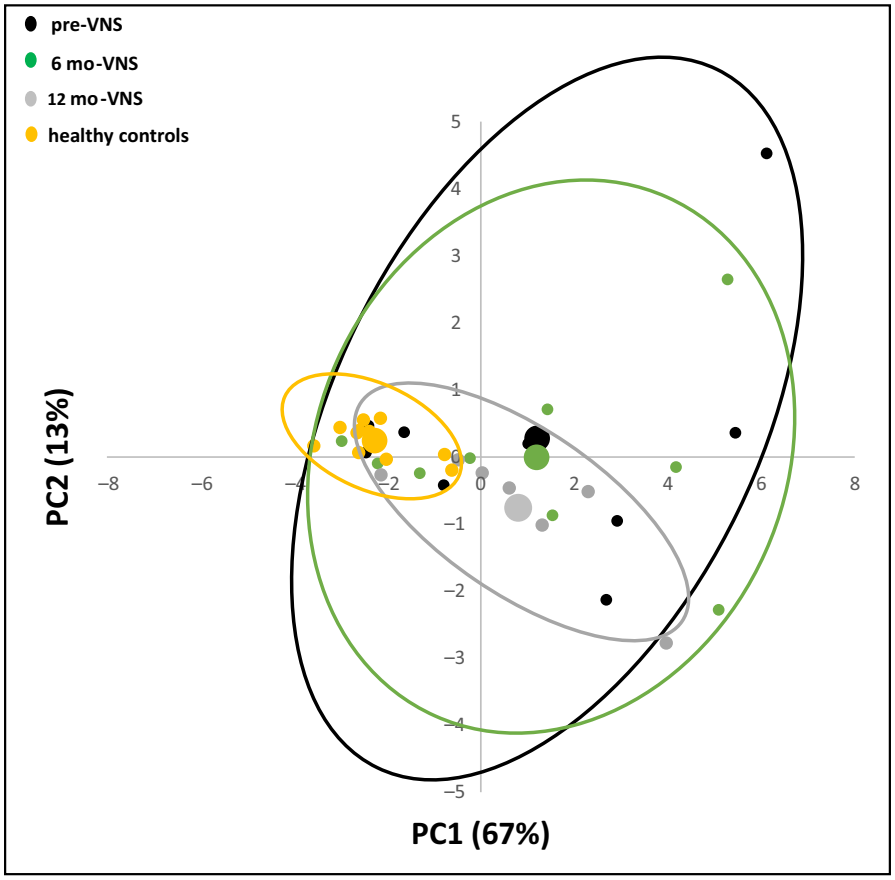


FIGURE 4 Vagus nerve stimulation (VNS) effect on blood cytokinergic profiles at inclusion, 6, 12 mo, and healthy controls. A principal component analysis (PCA) was built with cytokine levels: score plot relative to the two first principal components PC1 and PC2 with their explained variance in percentage, and the Hotelling's T2 ellipse in grey (0.05 significance level). Each point is sample. Black: pre-VNS; green: 6-mo VNS; grey: 12-mo VNS; yellow: healthy control values, furnished by manufacturers, respectively, to cytokine assay. Ellipses centered on the barycenter of each group (ie, the big dots) and which include the most remote point of the corresponding group, have been drawn for a clearer visualization of each group

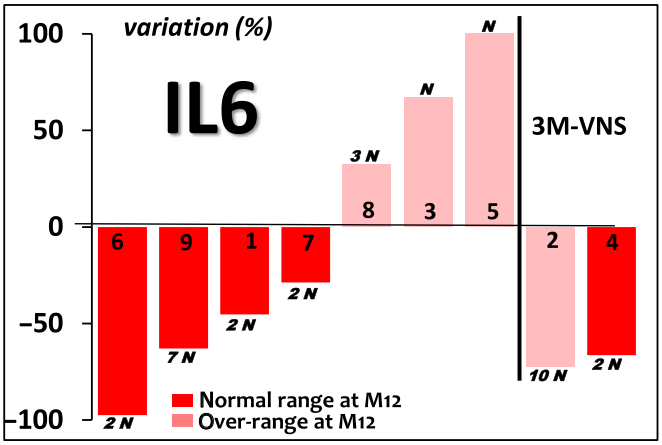
in taurine with CRP increase. TNF-α increase was also correlated with a decrease in lactate and alanine (ALA) and to an increase in beta-hydroxybutyrate (β-HB).

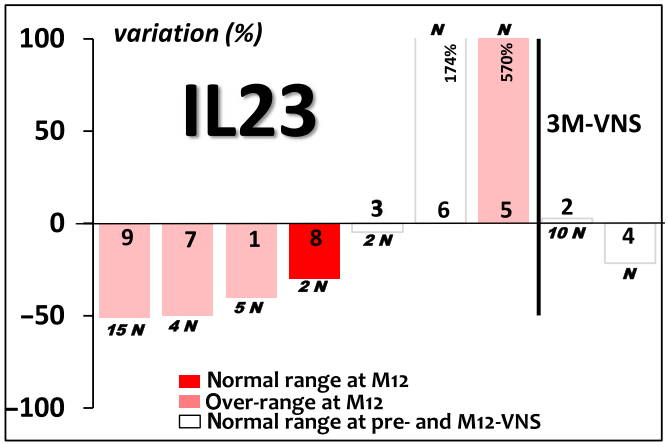
4 | DISCUSSION

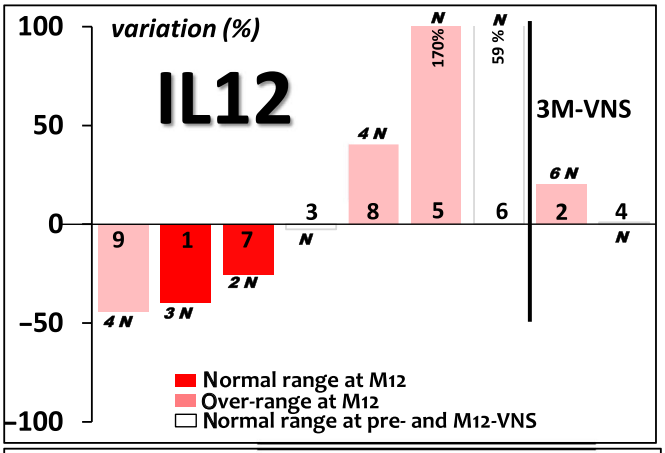
We report, for the first time, the results of a 12-month follow-up pilot clinical trial of VNS in CD patients. We confirm the clinical alleviation of the disease previously observed at 6 months[24] with five patients who went into remission among the seven who completed the 12-month VNS. We observed a modulatory effect of VNS on vagal tone, plasma cytokines, and gut metabolic profiles. In addition, VNS induced a major decrease in pro-inflammatory cytokines IL6, IL12, TNF-α, and IL23. VNS was also able to increase, or keep increased, the anti-inflammatory TGF-β1. No adverse events related to the device were observed except discomfort to the intensity/output current levels provoking voice's hoarseness classically described under VNS.[28,32]

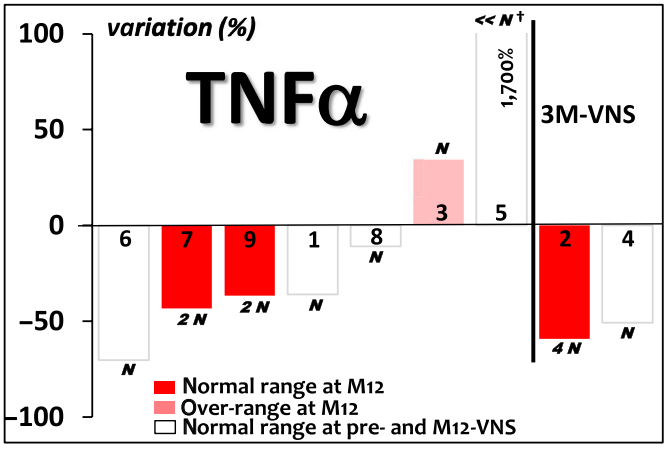
Two patients were removed from the study after 3 months of VNS (three early terminations in D'Haens study in CD patients[38]), probably because of very high inclusion scores for CDAI (>350), CDEIS (>14), and CRP (>88), suggesting that VNS, because of its slow effect as observed for epilepsy,[39] is indicated for mild-to-moderate active CD. Five out of the seven patients who completed the 12-month VNS were in clinical remission, and all the patients reached the CDAI-70 response. Five patients were in endoscopic remission. These results are in accordance with the preliminary results of D'Haens study,[38] published in abstract form, who observed clinical, biomarker, and endoscopic improvement for half of the 16 CD

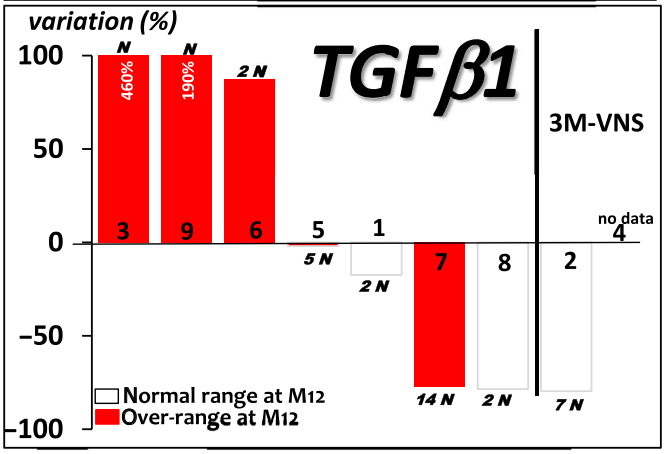
FIGURE 5 Variation of plasma and gut cytokine levels after 12-mo VNS. Pro-inflammatory cytokines: *IL6*; *IL23*; *TNF-α*; *IL23*. In red: cytokine level reaching normal range at 12-mo VNS; pink: cytokine level remaining over-range at 12-mo VNS; white: normal cytokine level at inclusion (pre-VNS) and at 12-mo VNS. Anti-inflammatory cytokines: *TGF-β1*; *IL10*. In red: cytokine level reaching or remaining over-range; white: normal cytokine level at inclusion (pre-VNS) and at 12-month VNS; cytokine levels at inclusion are given underneath or on top of the histogram of variation respective to each patient: **N** for normal value; **xN** for x fold over the normal value; †: <N or «N for below the normal value. *Gut cytokines:* biopsic TNF-α level (pg/mg protein); biopsic MIP-1α level (pg/mg protein); no data for patient 1 (pre- and 12-month VNS) and patient 4 (12-mo VNS). **$**: intestinal biopsy harvested during a usual endoscopic control of the patient 2 (with his consent), 9 month post-surgery and under active VNS. Red bold dotted line: median. Assays were done on inflamed gut segments harvested during the pre-VNS ileocolonoscopy and on the same segments at the 12-mo VNS. Gut segments: *ileon* for patients 3, 7, and 8; *cecum* for patient 6; *ascending colon* for patient 2, *transversal colon* for patient 5; *descending colon* for patients 4 and 9

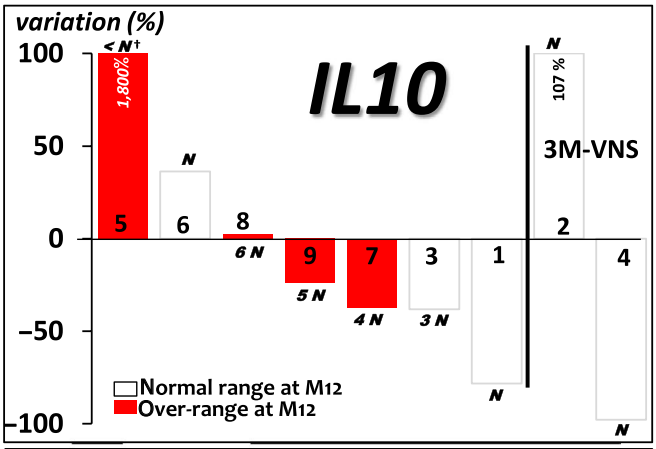

variation (%)
IL6
3M-VNS
Normal range at M12
Over-range at M12

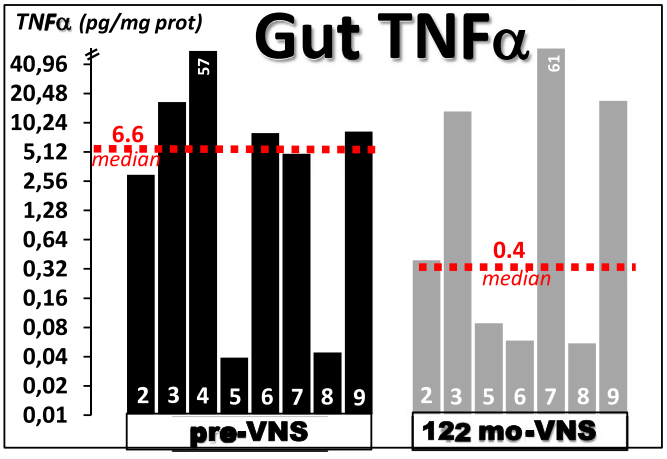

variation (%)
IL23
3M-VNS
174%
570%
Normal range at M12
Over-range at M12
Normal range at pre- and M12-VNS

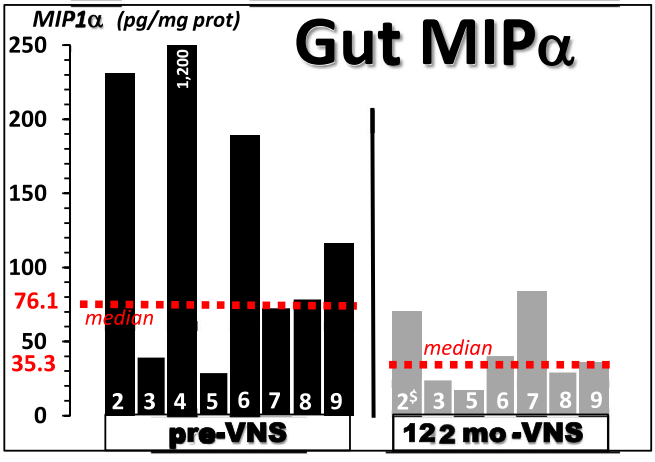

variation (%)
IL12
3M-VNS
170%
59 %
Normal range at M12
Over-range at M12
Normal range at pre- and M12-VNS


variation (%)
TNFα
3M-VNS
1,700%
Normal range at M12
Over-range at M12
Normal range at pre- and M12-VNS


variation (%)
TGFβ1
3M-VNS
460%
190%
no data
Normal range at M12
Over-range at M12


variation (%)
IL10
3M-VNS
1,800%
107 %
Normal range at M12
Over-range at M12


TNFα (pg/mg prot)
Gut TNFα
6.6
median
0.4
median
pre-VNS
122 mo-VNS


MIP1α (pg/mg prot)
Gut MIPα
76.1
median
35.3
median
pre-VNS
122 mo-VNS

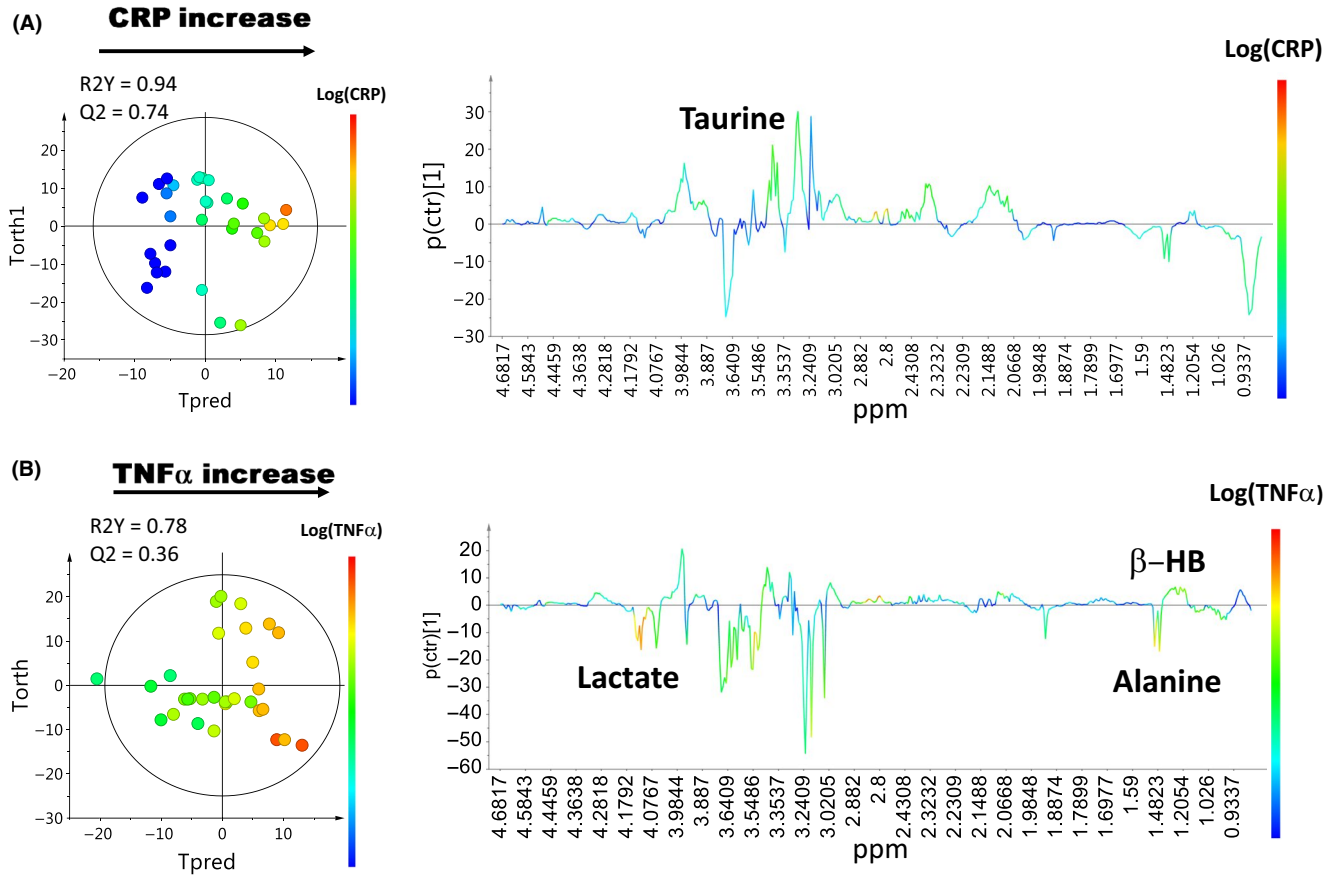


FIGURE 6 Typical ^{1}H HRMAS NMR spectrum of intact colon biopsies. OPLS models built with HRMAS NMR spectra and logCRP (A) and logTNF-α (B) as classification parameter. The score of each sample is plotted relative to the first 2 components of the model (Predictive Tpred and orthogonal Torth). Right: Corresponding 1D loadings showing the metabolites that most contribute to the correlations, with metabolites increased with CRP/TNF-α (positive peaks) or decreased (negative peaks). Taurine is the most important metabolite for CRP, while β-HB is increased and lactate is decreased with TNF-α increase. β-HB, beta-hydroxybutyrate; CRP, C-reactive protein

patients who received either VNS monotherapy (biologics refractory patients) or VNS adjunctive therapy for 4 months.

C-reactive protein is a good marker of remission,[40] and its induction/reduction is positively linked to IL6 production/reduction.[41] After 12-month VNS, four patients decreased their CRP level (three patients reaching normal value), and two others had a CRP level just above the normal value. Interestingly, in our metabolomics study performed in situ on patient gut mucosa, we observed an increase in taurine with CRP increase. This correlation could be explained by their active role during acute inflammation: CRP-activated leucocytes produce taurine that inhibits the overproduction of reactive oxidant species.[42] This is in accordance with the primary role of taurine, which is cytoprotection and homeostatic maintenance of cells involved in acute and chronic inflammatory/oxidative stress.[43]

Fecal calprotectin is useful in monitoring disease activity and response to therapy, and for predicting relapse and post-operative recurrence in CD.[44] Among the seven patients with high FC at inclusion, the level decreased after 12-month VNS in three patients, two of whom were normalized.

Vagus nerve stimulation had no global effect on anxiety and depression probably due to the under thresholds HAD scores on inclusion. Interestingly, VNS reduced the perceived digestive pain score. A restored vagal balance may explain this reduction, especially if we consider the role of the VN in interoception.[45]

Vagus nerve stimulation, used in the treatment of drug-refractory epilepsy, drives a 50% reduction frequency in 40%-60% of the patients, with an increasing efficacy up to 10 years, showing that this treatment is a slow-acting therapy.[39,46] Indeed, we observed that the VNS effect was not immediate but with a response primer after at least 3 months. VNS should therefore not be used alone in patients with severe flares. Indeed, the two patients removed from the study after 3 months of VNS had a more important flare than the other patients (P2 and P4). We used the same VNS parameters as in epilepsy but with a lower frequency stimulation supposed to activate vagal efferents.[28] However, we previously reported that VNS at low frequency (5-10Hz) of stimulation has a central effect.[47,48] VNS may have a placebo effect; however, in clinical trials with anti-TNF drugs in CD, clinical remission during maintenance treatment ranged between 12% and 20% under placebo at 12 months[49,50] while in the present study 71% (five on seven patients) were in clinical remission.

Vagus nerve stimulation has also a modulatory role in vagal tone. We previously reported that a low vagal tone in CD patients was correlated with high serum TNF-α levels and salivary cortisol, thus arguing

for an imbalance between the HPA axis and the autonomic nervous system.[16] Koopman et al[51] reported that autonomic dysfunction precedes the development of rheumatoid arthritis. Most of the CD patients studied here had a low vagal tone on inclusion (except P5 with an abnormally high vagal tone) which was rebalanced to normal in all patients after 12-month VNS. Consequently, vagal tone appears as an interesting marker for monitoring the inflammatory state in CD patients and its normalization appears as a therapeutic goal in VNS treatment.

The different mechanisms of the anti-inflammatory role of the VN, most likely activated by VNS, are exposed in the introduction. In addition, in the intestine, the anti-inflammatory effect of VNS is independent of the splenic T cells, as the VN interacts with cholinergic myenteric neurons in close contact with the muscularis macrophages, suggesting that intestinal muscularis resident macrophages expressing α7nAChR are most likely the ultimate target of the gastrointestinal CAIP.[52] VNS is supposed to activate the anti-inflammatory reflex through, at least, an inhibition of TNF-α production,[53] that is why in our study we used VNS as an alternative therapy to anti–TNF-α. When considering whether other cytokines were impacted by VNS, we observed that each patient had his/her own blood cytokinergic profile, suggesting that either they were not at the same stage of the disease at inclusion or that inflammation was driven by a different subset of immune cells. Our results partially confirmed the pro-inflammatory role of TNF-α, although the cytokine was not high in all patients at inclusion. This could be explained either by a moderate activity of the disease or a former chronic inflammatory state where TNF-α had already played its role, leaving other downstream cytokines acting on the immune cell subset. Whatever the reason, we demonstrated that 12-month VNS reduces its production. In addition, IL6, IL12, and IL23, three other archetypal pro-inflammatory cytokines in CD, were also reduced after 12-month VNS. IL6 and TNF-α reduction has been previously reported in patients with rheumatoid arthritis under VNS.[54] Moreover, IL6 was reduced in experimental sepsis when stimulating the accessory celiac branch of the sub-diaphragmatic[55] or the cervical VN in experimental colitis.[56] VNS was also able to increase or sustain plasma anti-inflammatory TGF-β1 in six patients, probably 1/through its active regulatory role, in combination with IL6, on Th17/Treg balance as demonstrated by Bettelli[57] and Tanaka,[58] and 2/through its regulatory role in monocyte-driven inflammatory responses resulting in a reduction in TNF-α and a production of IL10.[59]

We also observed a mucosal decrease in MIP-1α and TNF-α, supposing the local pro-inflammatory role of these cytokines in the inflammation sites. As these cytokines are mainly produced by innate immune cell subsets in the gut (macrophages, dendritic cells, and innate lymphoid cells), we hypothesize that the effect of VNS on pro-inflammatory cytokines could be due to a regulation on those cells that, secondarily, may regulate an inappropriate adaptive immune response driven by Th1/Th17 on the pro-inflammatory side and by Treg on the anti-inflammatory side.

Interestingly, in our metabolomics study of gut mucosa, the negative correlation between lactate and alanine with TNF-α and the positive one for β-HB could reflect the metabolic shift that occurs during VNS within the inflamed-gut mucosa (epithelial, immune, and neuroendocrine cells). Lactate production results from carbohydrate metabolism of immune cells and is concomitant to an activation of either Treg or Th17, depending on the activation status during inflammation.[60] The negative correlation of lactate with TNF-α could be related partially to an activation of Treg rather than Th17, supported by our results suggesting a role for a high TGF-β1/IL6 ratio on the activation of these cells after VNS. The negative correlation of alanine with TNF-α could be explained by its close metabolic link with glutamine (GLN) as alanine provides high glutamine and glutamate pools directly acting on the redox state of the cells.[61] GLN, a key substrate for enterocytes and immune cells,[62] has a cytoprotective action to inflammation[63] and decreases cytokine release in active CD biopsies via NF-κB and p38MAPK pathways.[64,65] High consumption of GLN during inflammation is associated with a poor clinical outcome[66] that can be reversed by supplemented alanine, improving the redox state of the cells.[61] VNS effect on cellular metabolism could be also demonstrated by β-HB/TNF-α-positive correlation. Indeed, β-HB is a ketone body used as an alternative source of energy when glucose is insufficient in inflammatory conditions. Its positive correlation with TNF-α could be surprising as this ketone has been shown to block the macrophagic NLRP3 inflammasome, thus inhibiting NF-κB-induced-IL1β secretion.[67] Our results are, however, in line with a very recent study showing that β-HB significantly increases MCP1, IL6, and IL1β gene expression in LPS-stimulated endothelial cells.[68] Moreover, β-HB can also induce hepatocyte inflammatory injury and oxidative stress through the NF-κB signaling pathway, by increasing TNF-α, IL6, and IL1β expression.[69]

Besides these correlations, no metabolic signature could be identified between pre-VNS and 6- and 12-month VNS patients, most likely because of the small number of patients. So far, few studies have been able to differentiate metabolic profiles between active and remission states. However, Dawiskiba[70] demonstrated that ^{1}H NMR-based metabolic fingerprinting of human serum allowed distinguishing active and non-active IBD patients.

By correlating two important decreasing CD markers (TNF-α, CRP) with mucosal metabolites, we hypothesize that VNS could modulate innate immune cell subsets, resulting in a fine regulation of bioenergy metabolic pathways of the adaptive immune cells (TH17/Treg axis). This role is in accordance with Willemze et al[71] who recently hypothesized for an active role of VNS on changing phenotype/activity of intestinal ChAT + T cells during inflammation. This metabolic shift has already been discussed,[72-74] arguing for a required metabolic reprogramming of immune cells during inflammation. We also suggest a role of VNS on the intestinal barrier, maybe on the microbiota because the VN is involved in the microbiota-gut-brain axis.[75] Indeed, we know that the development and maintenance of innate lymphoid cells, controlled by cytokines, are directly dependent on nutrients and gut bacteria and they participate in various intestine diseases including infectious, inflammatory diseases, and cancer.[76]

Finally, VNS may act in a holistic manner on a complex network of various interactions between cytokines and various immune cells. Unlike the single cytokine target of biotherapies, VNS acts through a global intrinsic mechanism on innate and adaptive immune cells along with metabolic pathways recovering equilibrated regulation of the gut immune system and assuming a modulatory action according to the cytokine profile of the patient.

In summary, VNS appears as a potential innovative and safe treatment for moderate CD patients, taking advantage of the patient's adherence, a guarantee for the alleviation of the disease. Today, VNS in CD patients is studied by another team[38] and in patients with rheumatoid arthritis.[54] Research on immune regulatory function by neural interfacing seems to provide a powerful tool to enhance remission in IBD patients,[77] and VNS research is currently conducted in order to perform and adapt the device to other diseases.[78] Of course, this pilot study requires confirmation in a larger randomized double-blinded control study and, overall, a long-lasting follow-up of the patients to confirm these promising results.[79]

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CONFLICT OF INTEREST

All authors approved the final version of the article and have no competing interests.

AUTHOR CONTRIBUTIONS

VS was involved in acquisition of data; analysis and interpretation of data; technical and material support; drafting of the manuscript; and critical revision of the manuscript. SP was involved in study concept, design, and supervision; technical and material support; acquisition of data; analysis and interpretation of data; drafting of the manuscript; and critical revision of the manuscript. FF and CT were involved in acquisition of data; technical and material support; and analysis and interpretation of data. DH and LV were involved in critical revision of the manuscript; technical and material support on VNS device; and management. J-LC was involved in study concept and design; critical revision of the manuscript; obtained funding; administrative, technical and material support; and study supervision. OD was involved in study supervision and critical revision of the manuscript. BB was involved in study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript; obtaining funding; administrative, technical, and material support; and study supervision.

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REFERENCES

1. Torres J, Mehandru S, Colombel J-F, Peyrin-Biroulet L. Crohn's disease. *Lancet*. 2017;389(10080):1741-1755.
2. Ben-Horin S, Chowers Y. Review article: loss of response to anti-TNF treatments in Crohn's disease. *Aliment Pharmacol Ther*. 2011;33(9):987-995.
3. Chaparro M, Guerra I, Muñoz-Linares P, Gisbert JP. Systematic review: antibodies and anti-TNF-alpha levels in inflammatory bowel disease. *Aliment Pharmacol Ther*. 2012;35(9):971-986.
4. Targownik LE, Benchimol EI, Witt J, et al. The effect of initiation of anti-TNF therapy on the subsequent direct health care costs of inflammatory Bowel disease. *Inflamm Bowel Dis*. 2019;25(10):1718-1728.
5. Click B, Regueiro M. Managing risks with biologics. *Curr Gastroenterol Rep*. 2019;21(2):1.
6. Chan W, Chen A, Tiao D, Selinger C, Leong R. Medication adherence in inflammatory bowel disease. *Intest Res*. 2017;15(4):434-445.
7. Knox NC, Forbes JD, Van Domselaar G, Bernstein CN. The gut microbiome as a target for IBD treatment: are we there yet? *Curr Treat Options Gastroenterol*. 2019;17(1):115-126.
8. Torres J, Ellul P, Langhorst J, et al. European Crohn's and colitis organisation topical review on complementary medicine and psychotherapy in inflammatory Bowel disease. *J Crohns Colitis*. 2019;13(6):673-685e.
9. Bonaz BL, Bernstein CN. Brain-gut interactions in inflammatory Bowel disease. *Gastroenterology*. 2013;144(1):36-49.
10. Olofsson PS, Tracey KJ. Bioelectronic medicine: technology targeting molecular mechanisms for therapy. *J Intern Med*. 2017;282(1):3-4.
11. Bonaz B, Sinniger V, Pellissier S. Vagus nerve stimulation: a new promising therapeutic tool in inflammatory Bowel disease. *J Intern Med*. 2017;282(1):46-63.
12. Mogilevski T, Burgell R, Aziz Q, Gibson PR. Review article: the role of the autonomic nervous system in the pathogenesis and therapy of IBD. *Aliment Pharmacol Ther*. 2019;50(7):720-737.
13. Prechtl JC, Powley TL. The fiber composition of the abdominal vagus of the rat. *Anat Embryol (Berl)*. 1990;181(2):101-115.
14. Grundy D. Vagal control of gastrointestinal function. *Baillieres Clin Gastroenterol*. 1988;2(1):23-43.
15. Bonaz B, Sinniger V, Pellissier S. The vagus nerve in the neuro-immune axis: implications in the pathology of the gastrointestinal tract. *Front Immunol*. 2017;8:1452.
16. Pellissier S, Dantzer C, Mondillon L, et al. Relationship between vagal tone, cortisol, TNF-alpha, epinephrine and negative affects in Crohn's disease and irritable bowel syndrome. *PLoS One*. 2014;9(9):e105328.
17. Borovikova LV, Ivanova S, Zhang M, et al. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature*. 2000;405(6785):458-462.
18. Rosas-Ballina M, Ochani M, Parrish WR, et al. Splenic nerve is required for cholinergic antiinflammatory pathway control of TNF in endotoxemia. *Proc Natl Acad Sci U S A*. 2008;105(31):11008-11013.
19. Komegae EN, Farmer DGS, Brooks VL, McKinley MJ, McAllen RM, Martelli D. Vagal afferent activation suppresses systemic inflammation via the splanchnic anti-inflammatory pathway. *Brain Behav Immun*. 2018;73:441-449.
20. Martelli D, Farmer DGS, McKinley MJ, Yao ST, McAllen RM. Anti-inflammatory reflex action of splanchnic sympathetic nerves is distributed across abdominal organs. *Am J Physiol Regul Integr Comp Physiol*. 2019;316(3):R235-R242.

21. Murray K, Barboza M, Rude KM, Brust-Mascher I, Reardon C. Functional circuitry of neuro-immune communication in the mesenteric lymph node and spleen. *Brain Behav Immun*. 2019;82:214-223.
22. Boon P, De Cock E, Mertens A, Trinka E. Neurostimulation for drug-resistant epilepsy: a systematic review of clinical evidence for efficacy, safety, contraindications and predictors for response. *Curr Opin Neurol*. 2018;31(2):198-210.
23. Meregnani J, Clarençon D, Vivier M. Anti-inflammatory effect of vagus nerve stimulation in a rat model of inflammatory bowel disease. *Auton Neurosci*. 2011;160(1–2):82-89.
24. Bonaz B, Sinniger V, Hoffmann D, et al. Chronic vagus nerve stimulation in Crohn's disease: a 6-month follow-up pilot study. *Neurogastroenterol Motil*. 2016;28(6):948-953.
25. Limburg PJ, Ahlquist DA, Sandborn WJ, et al. Fecal calprotectin levels predict colorectal inflammation among patients with chronic diarrhea referred for colonoscopy. *Am J Gastroenterol*. 2000;95(10):2831-2837.
26. von Roon AC, Karamountzos L, Purkayastha S, et al. Diagnostic precision of fecal calprotectin for inflammatory bowel disease and colorectal malignancy. *Am J Gastroenterol*. 2007;102(4):803-813.
27. Singh S. Evolution of clinical trials in inflammatory Bowel diseases. *Curr Gastroenterol Rep*. 2018;20(9):41.
28. Bonaz B, Picq C, Sinniger V, Mayol JF, Clarençon D. Vagus nerve stimulation: from epilepsy to the cholinergic anti-inflammatory pathway. *Neurogastroenterol Motil*. 2013;25(3):208-221.
29. Mary JY, Modigliani R. Development and validation of an endoscopic index of the severity for Crohn's disease: a prospective multicentre study. Groupe d'Etudes Therapeutiques des Affections Inflammatoires du Tube Digestif (GETAID). *Gut*. 1989;30(7):983-989.
30. Best WR, Becktel JM, Singleton JW, Kern F. Development of a Crohn's disease activity index. National Cooperative Crohn's Disease Study. *Gastroenterology*. 1976;70(3):439-444.
31. Hebuterne X, Lémann M, Bouhnik Y, et al. Endoscopic improvement of mucosal lesions in patients with moderate to severe ileocolonic Crohn's disease following treatment with certolizumab pegol. *Gut*. 2013;62(2):201-208.
32. Giordano F, Zicca A, Barba C, Guerrini R, Genitori L. Vagus nerve stimulation: surgical technique of implantation and revision and related morbidity. *Epilepsia*. 2017;58(Suppl 1):85-90.
33. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation*. 1996;93(5):1043-1065.
34. Schirbel A. Impact of pain on health-related quality of life in patients with inflammatory Bowel disease. *World J Gastroenterol*. 2010;16(25):3168-3177.
35. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand*. 1983;67(6):361-370.
36. Jacob D, Deborde C, Lefebvre M, Maucourt M, Moing A. NMRProcFlow: a graphical and interactive tool dedicated to 1D spectra processing for NMR-based metabolomics. *Metabolomics*. 2017;13(4):36.
37. Satsangi J. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. *Gut*. 2006;55(6):749-753.
38. D'Haens GR, Cabrijan Z, Eberhardson M, et al. Mo1906 - the effects of vagus nerve stimulation in biologicrefractory Crohn's disease: a prospective clinical trial. *Gastroenterology*. 2018;154(6):S-847.
39. Revesz D, Fröjd V, Rydenhag B, Ben-Menachem E. Estimating long-term vagus nerve stimulation effectiveness: accounting for antiepileptic drug treatment changes. *Neuromodulation*. 2018;21(8):797-804.
40. Vermeire S, Van Assche G, Rutgeerts P. Laboratory markers in IBD: useful, magic, or unnecessary toys? *Gut*. 2006;55(3):426-431.
41. Heinrich PC, Castell JV, Andus T. Interleukin-6 and the acute phase response. *Biochem J*. 1990;265(3):621-636.
42. Marcinkiewicz J, Kontny E. Taurine and inflammatory diseases. *Amino Acids*. 2014;46(1):7-20.
43. Kim C, Cha YN. Taurine chloramine produced from taurine under inflammation provides anti-inflammatory and cytoprotective effects. *Amino Acids*. 2014;46(1):89-100.
44. Mumolo MG, Bertani L, Ceccarelli L, et al. From bench to bedside: fecal calprotectin in inflammatory bowel diseases clinical setting. *World J Gastroenterol*. 2018;24(33):3681-3694.
45. Strigo IA, Craig AD. Interoception homeostatic emotions and sympathovagal balance. *Philos Trans R Soc Lond B Biol Sci*. 2016;371(1708):20160010.
46. Elliott RE, Morsi A, Tanweer O, et al. Efficacy of vagus nerve stimulation over time: review of 65 consecutive patients with treatment-resistant epilepsy treated with VNS > 10 years. *Epilepsy Behav*. 2011;20(3):478-483.
47. Reyt S, Picq C, Sinniger V, Clarençon D, Bonaz B, David O. Dynamic Causal modelling and physiological confounds: a functional MRI study of vagus nerve stimulation. *NeuroImage*. 2010;52(4):1456-1464.
48. Kibleur A, Pellissier S, Sinniger V, et al. Electroencephalographic correlates of low-frequency vagus nerve stimulation therapy for Crohn's disease. *Clin Neurophysiol*. 2018;129(5):1041-1046.
49. Hanauer SB. New steroids for IBD: progress report. *Gut*. 2002;51(2):182-183.
50. Schreiber S, Reinisch W, Colombel JF, et al. Subgroup analysis of the placebo-controlled CHARM trial: increased remission rates through 3 years for adalimumab-treated patients with early Crohn's disease. *J Crohns Colitis*. 2013;7(3):213-221.
51. Koopman FA, Tang MW, Vermeij J, et al. Autonomic dysfunction precedes development of rheumatoid arthritis: a prospective cohort study. *EBioMedicine*. 2016;6:231-237.
52. Matteoli G, Gomez-Pinilla PJ, Nemethova A, et al. A distinct vagal anti-inflammatory pathway modulates intestinal muscularis resident macrophages independent of the spleen. *Gut*. 2014;63(6):938-948.
53. Pavlov VA, Chavan SS, Tracey KJ. Molecular and functional neuroscience in immunity. *Annu Rev Immunol*. 2018;36:783-812.
54. Koopman FA, Chavan SS, Miljko S, et al. Vagus nerve stimulation inhibits cytokine production and attenuates disease severity in rheumatoid arthritis. *Proc Natl Acad Sci U S A*. 2016;113(29):8284-8289.
55. Somann JP, Wasilczuk KM, Neihouser KV, et al. Characterization of plasma cytokine response to intraperitoneally administered LPS & subdiaphragmatic branch vagus nerve stimulation in rat model. *PLoS One*. 2019;14(3):e0214317.
56. Meroni E, Stakenborg N, Gomez-Pinilla PJ, et al. Functional characterization of oxazolone-induced colitis and survival improvement by vagus nerve stimulation. *PLoS One*. 2018;13(5):e0197487.
57. Bettelli E, Carrier Y, Gao W, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature*. 2006;441(7090):235-238.
58. Tanaka T, Kishimoto T. Targeting interleukin-6: all the way to treat autoimmune and inflammatory diseases. *Int J Biol Sci*. 2012;8(9):1227-1236.
59. Lee JC, Espéli M, Anderson CA, et al. Human SNP links differential outcomes in inflammatory and infectious disease to a FOXO3-regulated pathway. *Cell*. 2013;155(1):57-69.
60. Menk AV, Scharping NE, Moreci RS, et al. Early TCR signaling induces rapid aerobic glycolysis enabling distinct acute T cell effector functions. *Cell Rep*. 2018;22(6):1509-1521.
61. Cruzat VF, Tirapegui J. Effects of oral supplementation with glutamine and alanyl-glutamine on glutamine, glutamate, and glutathione status in trained rats and subjected to long-duration exercise. *Nutrition*. 2009;25(4):428-435.

62. Cruzat V, Macedo Rogero M, Noel Keane K, Curi R, Newsholme P. Glutamine: metabolism and immune function, supplementation and clinical translation. *Nutrients*. 2018;10(11):1564.
63. Raizel R, Leite JS, Hypólito TM, et al. Determination of the anti-inflammatory and cytoprotective effects of l-glutamine and l-alanine, or dipeptide, supplementation in rats submitted to resistance exercise. *Br J Nutr*. 2016;116(3):470-479.
64. Lecleire S, Hassan A, Marion-Letellier R, et al. Combined glutamine and arginine decrease proinflammatory cytokine production by biopsies from Crohn's patients in association with changes in nuclear factor-kappaB and p38 mitogen-activated protein kinase pathways. *J Nutr*. 2008;138(12):2481-2486.
65. Coeffier M, Claeyssens S, Hecketsweiler B, Lavoinne A, Ducrotté P, Déchelotte P. Enteral glutamine stimulates protein synthesis and decreases ubiquitin mRNA level in human gut mucosa. *Am J Physiol Gastrointest Liver Physiol*. 2003;285(2):G266-G273.
66. Rodas PC, Rooyackers O, Hebert C, Norberg Å, Wernerman J. Glutamine and glutathione at ICU admission in relation to outcome. *Clin Sci (Lond)*. 2012;122(12):591-597.
67. Youm YH, Nguyen KY, Grant RW, et al. The ketone metabolite beta-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. *Nat Med*. 2015;21(3):263-269.
68. Chriett S, Dąbek A, Wojtala M, Vidal H, Balcerczyk A, Pirola L. Prominent action of butyrate over beta-hydroxybutyrate as histone deacetylase inhibitor, transcriptional modulator and anti-inflammatory molecule. *Sci Rep*. 2019;9(1):742.
69. Shi X, Li X, Li D, et al. beta-Hydroxybutyrate activates the NF-kappaB signaling pathway to promote the expression of pro-inflammatory factors in calf hepatocytes. *Cell Physiol Biochem*. 2014;33(4):920-932.
70. Dawiskiba T, Deja S, Mulak A, et al. Serum and urine metabolomic fingerprinting in diagnostics of inflammatory bowel diseases. *World J Gastroenterol*. 2014;20(1):163-174.
71. Willemze RA, Brinkman DJ, Welting O, et al. Acetylcholine-producing T-cells augment innate immune driven colitis but are redundant in T-cell driven colitis. *Am J Physiol Gastrointest Liver Physiol*. 2019;317(5):G557-G568.
72. Gruenbacher G, Thurnher M. Mevalonate metabolism governs cancer immune surveillance. *Oncoimmunology*. 2017;6(10):e1342917.
73. Lai B, Wang J, Fagenson A, et al. Twenty novel disease group-specific and 12 new shared macrophage pathways in eight groups of 34 diseases including 24 inflammatory organ diseases and 10 types of tumors. *Front Immunol*. 2019;10:2612.
74. O'Neill LAJ, Pearce EJ. Immunometabolism governs dendritic cell and macrophage function. *J Exp Med*. 2015;213(1):15-23.
75. Bonaz B, Bazin T, Pellissier S. The vagus nerve at the interface of the microbiota-gut-brain axis. *Front Neurosci*. 2018;12:49.
76. Bostick JW, Zhou L. Innate lymphoid cells in intestinal immunity and inflammation. *Cell Mol Life Sci*. 2016;73(2):237-252.
77. Brinkman DJ, ten Hove AS, Vervoordeldonk MJ, Luyer MD, de Jonge WJ. Neuroimmune interactions in the gut and their significance for intestinal immunity. *Cells*. 2019;8(7):670.
78. Mertens A, Raedt R, Gadeyne S, Carrette E, Boon P, Vonck K. Recent advances in devices for vagus nerve stimulation. *Expert Rev Med Devices*. 2018;15(8):527-539.
79. Cheng J, Shen H, Chowdhury R, Abdi T, Selaru F, Chen JDZ. Potential of electrical neuromodulation for inflammatory Bowel disease. *Inflamm Bowel Dis*. 2019:izz289. https://doi.org/10.1093/ibd/izz289. [Epub ahead of print].

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