Autonomic Dysfunction Correlates with Clinical and Inflammatory Activity in Patients with Crohn's Disease

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Background: Autonomic dysfunction has been implicated in Crohn's disease (CD). We aimed to investigate heart rate variability (HRV) as a marker of possible autonomic imbalance in patients with CD.

Methods: Thirty patients with CD and 30 age- and gender-matched healthy controls were enrolled in a prospective cohort study and underwent HRV study. Anxiety level was scored using the STAI questionnaire and CD clinical activity was assessed by Harvey–Bradshaw index. Blood tests including inflammatory markers were obtained for all participants.

Results: CD subjects had lower mean blood pressure (85.51 ± 11.07 mm Hg, 91.51 ± 6.99, $P = 0.015$) and albumin and significantly higher CRP and IL-6 compared with controls ($P < 0.002$ for all comparisons). Mean HRV values of very low-frequency power and low-frequency power components were significantly lower among CD subjects ($P = 0.038$ and 0.027, respectively), implying a predominant sympathetic tone. Anxiety level scores were significantly higher among patients with CD for both state anxiety ($P = 0.001$) and trait anxiety ($P < 0.0001$). However, patients with active disease had similar anxiety scores as patients in remission, yet had a significantly lower BMI, lower albumin level, and higher CRP and IL-6 levels ($P < 0.05$ for all comparisons). Moreover, despite similar anxiety scores, patients with active disease had higher pulse rate ($P = 0.02$) and lower HRV indexes, which correlated with albumin levels($r = 0.7$, $P = 0.001$).

Conclusions: Although patients with CD have higher anxiety levels compared with controls, they exhibit depressed HRV independent of this anxiety state and in direct correlation with disease activity and inflammatory markers. These observations suggest an inherent imbalance of autonomic function associated with active inflammation.

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Key Words: IBD, Crohn's disease, autonomic nervous system, autonomic dysfunction, heart rate variability, anxiety, stress, inflammation

Crohn's disease (CD) is an inflammatory bowel disease (IBD) characterized by transmural inflammation of the gastrointestinal (GI) tract. It may involve any part of the GI tract from the mouth to the perianal area and typically manifests a relapsing and remitting course.[1] The etiology remains uncertain, but it is hypothesized that genetic, environmental, endogenous gut bacteria, and immunological factors play important roles.[2–9]

The autonomic nervous system (ANS) modulates GI motility, secretion and mucosal immunity. Psychological stress and anxiety were postulated to possibly trigger IBD activity by inducing changes in motor, sensory and secretory GI function, increasing intestinal permeability, and modulating immune function, possibly through vagus-mediated cholinergic activation inhibiting proinflammatory cytokine secretion.[4–11] There is increasing evidence that stress and autonomic abnormalities may be a possible target for intervention in IBD.[12–17] In this context, fluctuations in heart rate beat-to-beat intervals, designated as heart rate variability (HRV), is known to be governed by vagal and sympathetic tone cross-interaction, making HRV a well-recognized parameter reflecting ANS activity and vagal tone.[18–20] Decreased HRV has been associated with elevations in circulating levels of C-reactive protein (CRP), interleukin (IL)-6, tumor necrosis factor, and other inflammation markers, known to be associated with CD etiogenesis.[7,21,22] However, there are scant data on the correlation between different HRV parameters and CD activity and inflammatory markers.

Therefore, the primary goal of this study was to investigate the profile of HRV parameters in patients with CD compared with healthy controls to gain insight on possible autonomic imbalance unique to patients with CD.

PATIENT AND METHODS

This was a prospective cohort study. Thirty patients with CD and 30 healthy, gender-, and age-matched controls were enrolled. The patient group was recruited from our tertiary center outpatient IBD clinic and included patients in various states of disease activity. Control subjects were healthy volunteers recruited from among hospital staff or from individuals accompanying patients to a clinic visit. All subjects were older than 18 years.

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Individuals with known cardiac disease such as congenital disorders, valvular dysfunction, conduction dysfunction, ischemic heart disease, or congestive heart failures at any degree were excluded. Individuals with endocrine dysfunction including thyroid dysfunction and diabetes mellitus and individuals with systemic inflammatory disease other than CD were excluded. Individuals with known neuropathy, diabetic, or other were excluded. The use of medications affecting cardiovascular system and conduction including antiarrhythmic of any group, beta blockers, calcium channels blockers, or any sympathomimetic medication were also an exclusion criteria. Patients younger than 18 years were also excluded.

The study was conducted in accordance with the ethical principles originating from the Declaration of Helsinki, in compliance with Good Clinical Practice and according to local regulations. The study protocol was approved by Sheba Medical Center Institutional Review Board. All patients enrolled in the study provided written informed consent. The study was conducted from November 2012 to April 2013.

Study Procedures

Demographic information, medication, and medical history were collected from questioners filled by all participants. Vital signs such as heart rate, blood pressure, weight, and height were measured for each participant. Body mass index was calculated.

Anxiety level was assessed and scored using the State and Trait Anxeity Inventory questionnaire,[23,24] which was filled by all participants. Clinical disease activity was assessed by the CD Harvey–Bradshaw index.[25]

Autonomic Testing Procedures

HRV was analyzed by time and frequency domain methods. Time domain analysis refers to statistics that are derived directly from the measurement of the beat-to-beat intervals and statistics calculated from the differences between successive N-N intervals. Simplest variable to calculate is the SD of the N-N intervals. Short-term HRV can be evaluated using the mean of the SD of the N-N intervals derived for each 5-minute period and root mean squared square SD, which denotes the square root of the mean squared differences of successive N-N intervals. Analysis of HRV in the frequency domain, calculated from short-term recordings of 2 to 5 minutes, is a widely used tool in the investigation of the autonomic system.[18] Three oscillatory components are usually differentiated in the spectral profile: the high-frequency (HF) band (0.15–0.40 Hz), the low-frequency (LF) band (0.04–0.15 Hz), and the very low-frequency (VLF) band (<0.04 Hz). The measurement of VLF, LF, and HF power components is made in absolute values of power (in square milliseconds).[18] Vagal activity is the major contributor to the HF component. LF is modulated by baroreflexes with a combination of sympathetic and parasympathetic efferent nerve. VLF reflects parasympathetic activity. LF/HF ratio has been applied in an attempt to better estimate sympathetic activity and is generally understood as a measure of the balance between sympathetic and parasympathetic nervous system activity.[19]

All participants underwent HRV study performed by a trained physician. All studies were performed in our outpatient clinic during morning hours, in a fixed environment as possible, controlled in terms of temperature, lighting, and background. All studies were performed in a comfortable seating position. Initial measurement of HRV indexes was taken for 5 minutes without any stimulation. Thereafter, a sympathetic stimulation was performed (siren horn), and additional 5 minutes' measurement was recorded. Participants were then asked to stand up, and 3 more minutes of measurement were performed. HRV indexes were measured and analyzed using ProComp biofeedback system and BioGraph Infiniti software by Thought Technology, Ltd. Two types of physiological sensors were used to detect heartbeats: electrocardiography (EKG/ECG) and blood volume pulse (also named photoplethysmography). In addition, respiration sensor was used to detect and record breathing rate. Data were stored for subsequent review, artifact rejection, and calculation. Spectral power analyses and time domain analyses were performed in accordance with published standards,[16–18] yielding frequency domain measures such as VLF power, LF power, HF power, and LH to HF ratio and time domain indexes such as SD of the N-N intervals (R-R) and root mean squared square SD were calculated.

Blood samples were taken after the HRV study from all participants. Blood count, chemistry including albumin (g/dL), CRP (mg/L), and interleukin 6 (IL-6) (pg/mL) were measured. For the quantitative determination of human IL-6 concentrations in serum, we used Quantikine HS ELISA (R&D Systems Inc., Minneapolis, MN).

Statistical Analyses

Data were analyzed using SPSS 22.0 for Windows and are presented as mean ± SD or as percentages. The level of significance was set at $P \leq 0.05$ (2-tailed). Before analyses, raw values of all variables were examined for deviations from normality by the Kolmogorov–Smirnov test. Subanalyses were performed to assess differences between the controls and patients using *t* test. Pearson's and Spearman's correlation coefficients were used to compute bivariate relationships between variables for normally and nonnormally distributed variables, respectively.

RESULTS

Sixty subjects were included. The background disposition of the CD (n = 30) and control (n = 30) groups were similar in terms of demographics such as gender, age, and origin without significant difference between groups (Table 1).

CD subjects had significantly lower systolic, diastolic, and mean blood pressure compared with controls. Patients with CD also tended to be numerically leaner than controls but without a statistical significance (Table 2).

No significant differences were found in mean white blood cell count, red blood cell count, or hemoglobin values, but CRP and IL-6 were significantly higher among subjects with CD disease, and albumin level was significantly lower (Table 2).

TABLE 1. Background Characteristics of the Entire Cohort with Breakdown to the CD and the Control Group

	Total (n = 60)	Control (n = 30)	CD (n = 30)	Odds Ratio (95% CI)	*P*
Age, mean ± SD, yr	33 ± 11.38	32.7 ± 11.47	33.2 ± 11.29		0.840
Women, n (%)	31 (51.6)	15 (50)	16 (53.3)	1.0 (0.3–2.7)	0.999
Smoker, n (%)	10 (16.6)	3 (10)	7 (23.3)	2.7 (0.6–11.8)	0.229
BMI, mean ± SD, kg/m^2	22.44 ± 4.06	23.08 ± 3.32	21.79 ± 4.65		0.223
Pulse, mean ± SD, beats per minute	75.65 ± 10.9	73.66 ± 8.89	77.63 ± 12.44		0.161
CD duration, mean ± SD, yr			12.2 ± 9.1		
Operation, n (%)			15 (50%)		
Extra GI manifestation			5 (16.6%)		
Montreal classification of CD		Age at diagnosis	A1-11 (36.6%)		
			A2-18 (60%)		
			A3-1 (3.3%)		
		Location	L1-13 (43.3%)		
			L2-5 (16.6%)		
			L3-11 (36.6%)		
			L4-1 (3.3%)		
		Behavior	B1-6 (20%)		
			B2-14 (46.6%)		
			B3-10 (33.3%)		
			P-3 (3.3%)		
Steroids, n (%)			1 (1.6)		
Anti-tumor necrosis factor, n (%)			17 (56)		
Immunomodulators, n (%)			17 (56)		
Thiopurine, n (%)			14 (46)		
MTX, n (%)			3 (10)		
5-ASA, n (%)			4 (13.3)		
Iron, n (%)			5 (16.6)		
HBI score, mean ± SD (range)			5.36 ± 4.88 (0–18)		
HBI score≥ 5			13 (43%)		

ASA, aminosalicylic acid; CI, confidence interval; HBI, Harvey–Bradshaw index; MTX, methotrexate.

TABLE 2. Inflammatory and Anxiety Indexes in the Entire Cohort and Its Breakdown to the CD and the Control Group

	Total (n = 60)	Control (n = 30)	CD (n = 30)	*P*
Systolic blood pressure, mean ± SD, mm Hg	116.53 ± 12.09	120.33 ± 7.81	112.73 ± 14.37	**0.015**
Diastolic blood pressure, mean ± SD, mm Hg	74.5 ± 9.38	77.1 ± 7.37	71.9 ± 10.52	**0.031**
Mean blood pressure, mean ± SD, mm Hg	88.51 ± 9.66	91.51 ± 6.99	85.51 ± 11.07	**0.015**
WBC, mean ± SD, 10^3/mL	7.08 ± 2.14	6.75 ± 2.17	7.42 ± 2.5	0.234
RBC, mean ± SD, 10^6/mL	4.59 ± 0.51	4.56 ± 0.51	4.62 ± 0.54	0.662
Hemoglobin, mean ± SD, g/dL	13.38 ± 1.41	13.67 ± 1.23	13.09 ± 1.52	0.112
CRP, mean ± SD, mg/L	8.52 ± 16.37	2.41 ± 4.53	14.62 ± 21.23	**0.002**
IL-6, mean ± SD, pg/mL	1.95 ± 4.57	1.17 ± 4.72	2.74 ± 4.26	**<0.0001**
Albumin, mean ± SD, g/dL	4.37 ± 0.53	4.57 ± 0.35	4.16 ± 0.61	**0.002**
State anxiety score, mean ± SD	32.45 ± 11.31	27.66 ± 7.27	37.23 ± 12.58	**0.001**
Trait anxiety score, mean ± SD	37.18 ± 9.93	32.56 ± 8.84	41.8 ± 8.73	**<0.0001**

CI, confidence interval.
Bold values indicate the level of significance was set at $P < 0.05$ (2-tailed).

TABLE 3. HRV Indexes in the Entire Cohort and Its Breakdown to CD and Control Groups

	Total (n = 60)	Control (n = 30)	CD (n = 30)	*P*
Heart rate, mean ± SD, beats per minute	79.31 ± 11.72	76.74 ± 9.86	81.89 ± 13	0.089
SDRR, mean ± SD	121.55 ± 110.03	134.50 ± 131.2	108.61 ± 91.93	0.156
rMSSD, mean ± SD	10.27 ± 4.02	10.8 ± 4.47	9.74 ± 3.76	0.156
VLF total power, mean ± SD	418.68 ± 737.84	535.31 ± 961.67	302.06 ± 434.89	**0.038**
LF total power, mean ± SD	1027.15 ± 2288.48	1301.46 ± 2984.24	752.83 ± 1349.94	**0.027**
HF total power, mean ± SD	1088.81 ± 2897.3	1398.194 ± 2873.683	779.42 ± 1718.518	0.110
LF/HF ratio, mean ± SD	2.8 ± 2.28	2.9 ± 2.3	2.90 ± 2.3	0.813

CI, confidence interval; RMSSD, root mean squared square SD; SDNN (SDRR), SD of the N-N intervals (R-R).

Anxiety level scores were significantly higher among patients with CD compared with healthy controls for both state anxiety ($P < 0.001$) and trait anxiety ($P < 0.0001$) (Table 2).

As shown in Table 3, HRV testing revealed significantly lower values for mean LF, VLF, SD of the R-R intervals, and root mean squared square SD among CD subjects compared with controls, implying a predominant sympathetic tone among CD subjects (Table 3 and Fig. 1A, B).

CD subjects in clinical remission, defined by Harvey–Bradshaw index score <5, had a lower pulse rate ($P = 0.02$), higher body mass index ($P = 0.01$), higher albumin concentration ($P = 0.023$), lower IL-6 levels ($P = 0.005$), and lower CRP levels ($P = 0.04$) compared with patients with active disease (Table 4). Interestingly, state and trait anxiety scores were not significantly different between patients in remission and those with active disease although state anxiety score was numerically lower among the remission group (Table 4). HRV indexes tended to be lower among subjects with active disease compared with patients in remission. However, only the LF and VLF HRV achieved statistical significance (Table 5) and correlated with albumin levels and with IL-6 levels (Fig. 1C and Table 6).

A modest correlation between Harvey–Bradshaw index score to IL-6 level was evident. Several additional correlations were identified between white blood cell, CRP, albumin levels, and IL-6 levels with various HRV parameters as outlined in Table 6.

DISCUSSION

This study aimed to investigate the link between autonomic dysfunction and disease severity, inflammation, and anxiety state. There is increasing evidence that stress and autonomic abnormalities may play a role in the pathophysiology of IBD. Stress is an important factor altering the ANS and can induce long-term modifications of the sympathovagal balance.[6–11] Autonomic nerve imbalance and vagal nerve dysfunction were studied and described previously as a feature of IBD.[11] Higher sympathetic tone at rest was demonstrated, using HRV measurements, among patient with chronic ulcerative colitis.[26] Lower levels of spectral activity in VLF and LF band were also found among adolescents

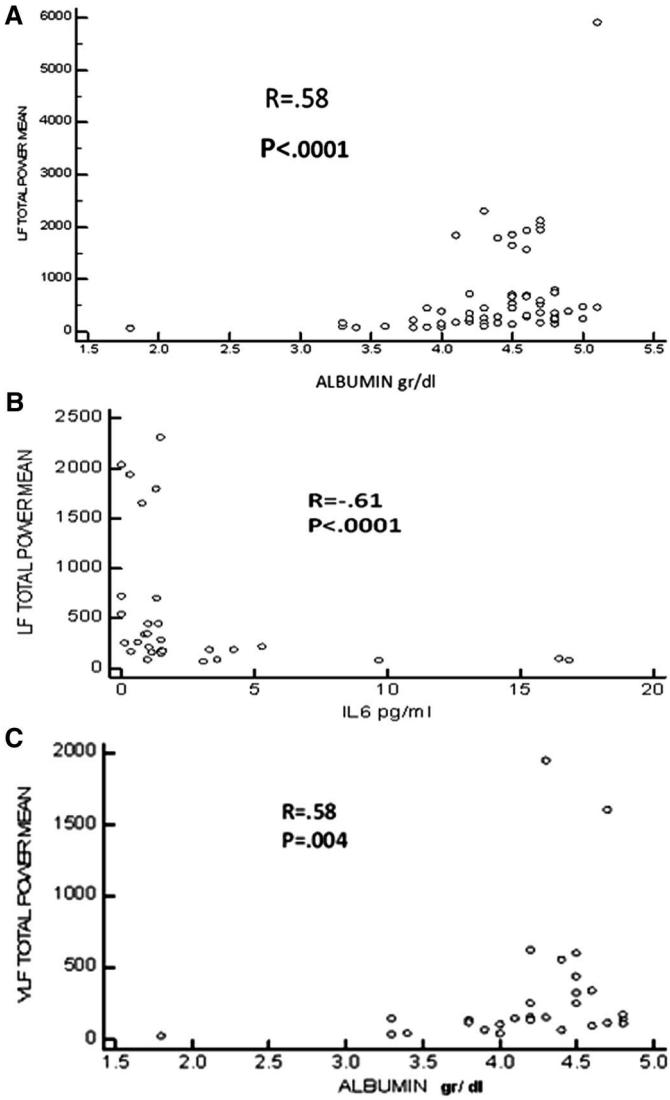

FIGURE 1. A, LF correlation to albumin level among patients with CD. B, LF correlation to IL-6 level among patients with CD. C, VLF HRV correlation to albumin level among patients with CD.

TABLE 4. Comparison Between CD Subjects in Remission to Active Disease

	Remission (HBI <5) (N = 16)	Active (HBI Score ≥5) (N = 14)	*P*
Age, mean ± SD, yr	32.06 ± 8.67	34.71 ± 14.26	0.884
Pulse, mean ± SD, beats per minute	72.94 ± 11.74	83.00 ± 11.334	0.026
SYS, mean ± SD, mm Hg	116.56 ± 11.69	108.35 ± 16.26	0.197
DIAS, mean ± SD, mm Hg	73.68 ± 7.33	69.85 ± 13.28	0.296
MAP, mean ± SD, mm Hg	87.97 ± 8.06	82.69 ± 13.5	0.205
BMI, mean ± SD, kg/m^2	23.11 ± 2.63	20.30 ± 5.98	0.010
WBC, mean ± SD, $10^3/mL$	6.90 ± 2.41	8.01 ± 2.57	0.253
RBC, mean ± SD, $10^6/mL$	4.63 ± 0.605	4.62 ± 0.480	0.803
HB, mean ± SD, g/dL	13.67 ± 1.42	12.68 ± 1.32	0.064
CRP, mean ± SD, mg/L	6 ± 7.57	20.6 ± 25.72	0.04
ALB, mean ± SD, g/dL	4.38 ± 0.38	3.91 ± 0.73	0.023
IL6, mean ± SD, pg/mL	1.64 ± 2.55	4.01 ± 5.45	0.005
State anxiety score, mean ± SD	34.94 ± 11.01	39.86 ± 14.12	0.252
Trait anxiety score, mean ± SD	42.50 ± 8.39	41.00 ± 9.364	0.667

CI, confidence interval; HBI, Harvey–Bradshaw index; WBC, white blood cell.

with IBD in a recent study.[27] Autonomic hyperreflexia was significantly associated with more severe inflammation and systemic disease in IBD. Moreover, autonomic imbalance was also demonstrated in patients in remission and was interpreted to suggest underlying abnormality in the autonomic neural system.[11,14–16]

Tracey et al described a nervous system-mediated mechanism for modulation of proinflammatory cytokine secretion, which was designated the cholinergic anti-inflammatory pathway. The evidence for such effects came from observations indicating that cholinergic agonists inhibit cytokine synthesis and protect against cytokine-mediated diseases and that stimulation of the vagus nerve results in lower serum and organ tumor necrosis factor and other proinflammatory cytokine and attenuation of inflammation.[13] Consequently, it was suggested that dysfunction of the cholinergic anti-inflammatory pathway may predispose individuals to excessive inflammatory responses.[10,13,28]

In this study, HRV indexes were found to be lower and anxiety score was higher among patients with CD, both in remission and during active disease, compared with healthy subjects. Furthermore, we found that HRV indexes were reduced among patients with CD with active disease compared with subjects in remission (Table 5), and this was associated with higher inflammatory markers (CRP, IL-6) among the active disease group. These findings point to a shift in the autonomic balance in patients with IBD, which is also modulated by disease inflammatory activity. Importantly, no correlation to anxiety score was evident, arguing against the hypothesis that autonomic imbalance may be merely an outcome of increased anxiety among chronically diseased patients or those with active disease.

In our study, patients with CD had a lower blood pressure than controls and their heart rate was accelerated, possibly as a compensatory reaction. In this context, it is known that both LF

TABLE 5. HRV Indexes–Mean Values Comparison Between CD Subjects in Remission to Active Disease

	Remission (HBI <5) (N = 16)	Active (HBI Score ≥5) (N = 14)	*P*
Heart rate, mean ± SD, beats per minute	76.92 ± 11.78	87.59 ± 12.31	0.025
SDRR, mean ± SD	121.91 ± 92.39	93.41 ± 92.40	0.114
RMSSD, mean ± SD	10.43 ± 3.73	8.95 ± 3.76	0.114
VLF total power, mean ± SD	348.45 ± 96.16	249.06 ± 132.425	0.018
LF total power, mean ± SD	907.82 ± 367.05	575.71 ± 367.05	0.034
HF total power, mean ± SD	982.71 ± 1975.55	547.10 ± 1405.66	0.339
LF/HF ratio, mean ± SD	3.19 ± 2.40	2.66 ± 1.83	0.693

CI, confidence interval; RMSSD, root mean squared square SD; SDNN (SDRR), SD of the N-N intervals (R-R).

TABLE 6. Correlations in the Entire Cohort

	CRP	IL6	Albumin
HR, mean			
Correlation coefficient	0.445	0.484	−0.321
Significance (2-tailed)	0.001	0.003	0.01
HBI			
Correlation coefficient	0.219	0.44	−0.469
Significance (2-tailed)	NS	0.004	0.008
SDRR			
Correlation coefficient	−0.369	0.49[a]	0.36
Significance (2-tailed)	0.045	0.006	0.004
RMSSD			
Correlation coefficient	−0.369	−0.252	0.36
Significance (2-tailed)	0.045	0.05	0.004
VLF total			
Correlation coefficient	−0.391	−0.35	0.35
		−0.618[a]	0.539[a]
Significance (2-tailed)	0.033	0.006	0.006
		0.000	0.004
LF total			
Correlation coefficient	−0.313	0.35	0.35
		−0.613	0.584[a]
Significance (2-tailed)	0.015	0.008	0.004
		0.000	0.000

[a]Among CD subjects only.
RMSSD, root mean squared square SD; SDNN (SDRR), SD of the N-N intervals (R-R).

and VLF are related to a parasympathetic dominance. LF is more related to the function of the baroreceptors and VLF to neuroregulation. Thus, these observations may suggest that although LF level is reduced, there is still a certain level of feedback and compensation with an acceleration of the pulse rate.[19–21]

Our study has several limitations. The study procedures were not performed in a blinded fashion. However, HRV readings were obtained independently of the operator. The size of the population tested was modest; so, it possible that the study may be underpowered to detect some more subtle differences in autonomic function between the 2 groups. Nonetheless, the fact that differences in biochemical markers of inflammation were picked up in this study indicates that canonical biophysiologic parameters of similar robustness would have been detected.

Exposure to cigarette smoke leads to changes in ANS, resulting in sympathetic predominance.[29] The fact that there were numerically more smokers in the patients' group could theoretically influence the results. However, a subanalysis, after excluding smokers from both groups, revealed similar results as the main analysis (data not shown).

In conclusion, compared with age- and sex-matched individuals, patients with CD have higher anxiety levels. However, regardless and independent of this anxiety state, active patients with CD exhibit depressed HRV that is correlated with degree of inflammatory markers. These data suggest an inherent imbalance of parasympathetic autonomic function, whereby depressed parasympathetic activity in patients with CD correlates with disease activity. These findings may support the hypothesis of a lower parasympathetic activity and a reduction in the cholinergic anti-inflammatory network driving the inflammation in patients with CD. However, future studies are needed to delineate the exact mechanism of interaction between inflammation and ANS function and to examine this cross-talk as a possible target for treatment.

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M. Beer-Gabel and S. Ben-Horin conceived the study, T. Engel acquired and analyzed the data and drafted the manuscript. S. Ben-Horin participated in designing the study, drafting of the manuscript, and in data acquisition and analysis and in critical revision of the manuscript.

REFERENCES

1. Cosnes J, Gowerrousseau C, Seksik P, et al. Epidemiology and natural history of inflammatory bowel diseases. *Gastroenterology*. 2011;140:1785–1794.
2. Hugot JP. Genetic origin of IBD. *Inflamm Bowel Dis.* 2004;10(suppl 1):S11–S15.
3. Shanahan F. Inflammatory bowel disease: immunodiagnostics, immunotherapeutics, and ecotherapeutics. *Gastroenterology*. 2001;120:622–635.
4. Sajadinejad MS, Asgari K, Molavi H, et al. Psychological issues in inflammatory bowel disease: an overview. *Gastroenterol Res Pract.* 2012;2012:106502.
5. Neuman MG. Immune dysfunction in inflammatory bowel disease. *Transl Res.* 2007;149:173–186.
6. Fiocchi C. Inflammatory bowel disease: etiology and pathogenesis. *Gastroenterology*. 1998;115:182–205.
7. Papadakis KA, Targan SR. Role of cytokines in the pathogenesis of inflammatory bowel disease. *Annu Rev Med.* 2000;51:289–298.
8. Sartor RB. Pathogenesis and immune mechanisms of chronic Inflammatory bowel diseases. *Am J Gastroenterol.* 1997;92:5S–11S.
9. Podolsky DK. Inflammatory bowel disease. *N Engl J Med.* 2002;347:417–429.
10. Ben-Horin S, Chowers Y. Neuroimmunology of the gut: physiology, pathology, and pharmacology. *Curr Opin Pharmacol.* 2008;8:490–495.
11. Straub RH, Antoniou E, Zeuner M, et al. Association of autonomic nervous hyperreflexia and systemic inflammation in patients with Crohn's disease and ulcerative colitis. *J Neuroimmunol.* 1997;80:149–157.
12. Mikocka-Walus AA, Turnbull D, Holtmann G, et al. An integrated model of care for inflammatory bowel disease sufferers in Australia: development and the effects of its implementation. *Inflamm Bowel Dis.* 2012;18:1573–1581.
13. Tracey KJ. Physiology and immunology of the cholinergic anti inflammatory pathway. *J Clin Invest.* 2007;117:289–296.
14. Lindgren S, Lilja B, Rosén I, et al. Disturbed autonomic nerve function in patients with Crohn's disease. *Scand J Gastroenterol.* 1991;26:361–366.
15. Mouzas IA, Pallis AG, Kochiadakis GE, et al. Autonomic imbalance during the day in patients with inflammatory bowel disease in remission. Evidence from spectral analysis of heart rate variability over 24 hours. *Dig Liver Dis.* 2002;34:775–780.
16. Lindgren S, Stewenius J, Sjölund K, et al. Autonomic vagal nerve dysfunction in patients with ulcerative colitis. *Scand J Gastroenterol.* 1993;28:638–642.
17. Santos J, Alonso C, Vicario M, et al. Neuropharmacology of stress-induced mucosal inflammation: implications for inflammatory bowel disease and irritable bowel syndrome. *Curr Mol Med.* 2008;8:258–273.

18. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation and clinical use. *Circulation*. 1996;93:1043–1065.
19. Kleiger RE, Stein PK, Bigger JT Jr. Heart rate variability: measurement and clinical utility. *Ann Noninvasive Electrocardiol.* 2005;10:88–101.
20. Hill LK, Siebenbrock A. Are all measures created equal? Heart rate variability and respiration—biomed 2009. *Biomed Sci Instrum.* 2009; 45:71–76.
21. Von Känel R, Carney RM, Zhao S, et al. Heart rate variability and biomarkers of systemic inflammation in patients with stable coronary heart disease: findings from the Heart and Soul Study. *Clin Res Cardiol.* 2011; 100:241–247.
22. Ghia JE, Blennerhassett P, Kumar-Ondiveeran H, et al. The vagus nerve: a tonic inhibitory influence associated with inflammatory bowel disease in a murine model. *Gastroenterology*. 2006;131: 1122–1130.
23. Tilton SR. *Review of the State-Trait Anxiety Inventory (STAI)*: News-Notes; 2008. Retrieved 2011-03-25.
24. Spielberger CD, Gorssuch RL, Lushene PR, et al. *Manual for the State-Trait Anxiety Inventory (Form Y)*. Palo Alto, CA: Consulting Psychologists Press, Inc; 1983.
25. Harvey R, Bradshaw JM. A simple index of Crohn's-disease activity. *Lancet*. 1980;8:514.
26. Maule S, Pierangeli G, Cevoli S, et al. Sympathetic hyperactivity in patients with ulcerative colitis. *Clin Auton Res.* 2007;17:217–220.
27. Jelenova D, Ociskova M, Prasko J, et al. Heart rate variability in children with inflammatory bowel disease. *Neuro Endocrinol Lett.* 2015;36:72–79.
28. Czura CJ, Tracey KJ. Autonomic neural regulation of immunity. *J Intern Med.* 2005;257:156–166.
29. Middlekauff HR, Park J, Moheimani RS. Adverse effects of cigarette and noncigarette smoke exposure on the autonomic nervous system: mechanisms and implications for cardiovascular risk. *J Am Coll Cardiol.* 2014; 64:1740–1750.