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Longitudinal Autonomic Nervous System Measures Correlate With Stress and Ulcerative Colitis Disease Activity and Predict Flare

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Background: Differences in autonomic nervous system function, measured by heart rate variability (HRV), have been observed between patients with inflammatory bowel disease and healthy control patients and have been associated in cross-sectional studies with systemic inflammation. High HRV has been associated with low stress.

Methods: Patients with ulcerative colitis (UC) were followed for 9 months. Their HRV was measured every 4 weeks using the VitalPatch, and blood was collected at baseline and every 12 weeks assessing cortisol, adrenocorticotropin hormone, interleukin-1β, interleukin-6, tumor necrosis factor-α, and C-reactive protein (CRP). Stool was collected at enrollment and every 6 weeks for fecal calprotectin. Surveys assessing symptoms, stress, resilience, quality of life, anxiety, and depression were longitudinally collected.

Results: Longitudinally evaluated perceived stress was significantly associated with systemic inflammation (CRP, $P = 0.03$) and UC symptoms ($P = 0.02$). There was a significant association between HRV and stress (low-frequency to high-frequency power [LFHF], $P = 0.04$; root mean square of successive differences [RMSSD], $P = 0.04$). The HRV was associated with UC symptoms (LFHF, $P = 0.03$), CRP (high frequency, $P < 0.001$; low frequency, $P < 0.001$; RMSSD, $P < 0.001$), and fecal calprotectin (high frequency, $P < 0.001$; low frequency, $P < 0.001$; RMSSD, $P < 0.001$; LFHF, $P < 0.001$). Significant changes in HRV indices from baseline developed before the identification of a symptomatic or inflammatory flare ($P < 0.001$).

Conclusions: Longitudinally evaluated HRV was associated with UC symptoms, inflammation, and perceived and physiological measures of stress. Significant changes in HRV were observed before the development of symptomatic or inflammatory flare.

Key Words: heart rate variability, autonomic nervous system, ulcerative colitis, stress, wearable device

INTRODUCTION

The inflammatory bowel diseases (IBD), comprising Crohn disease and ulcerative colitis (UC), are chronic inflammatory conditions of the gastrointestinal (GI) tract.[1] The brain-gut-microbiome axis, the primary pathway through which the central nervous system (brain) and enteric nervous system (gut) communicate, has been implicated in both the pathogenesis and the phenotypical expression of UC through several pathways, including through the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal axis (HPA axis). The ANS, comprising the sympathetic nervous system (SNS) and the parasympathetic nervous system (PSN), and the HPA axis are the main stress response systems. Psychological stress affects the brain-gut axis with several proposed mechanisms linking prolonged stress and UC symptoms, including increased intestinal permeability, altered secretion of glucocorticoids, and vagal nerve-mediated stimulation of catecholamine secretion. Stress has downstream proinflammatory GI effects through mast cell and macrophage stimulation and cytokine release.[2,3] Stress

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may also have a deleterious effect on GI motility, mucosal immunity, and alterations in the intestinal microbiome.[4] Unfortunately, studies that have sought to characterize the link between stress, gut inflammation, and IBD symptoms have been largely inconclusive because of the lack of objective, longitudinal, real-time assessment of the stress response on GI symptoms in well characterized, stable patients with UC.

Advances in wearable device technology allow for simple, longitudinal assessment of heart rate variability (HRV), a reliable measure of cardioautonomic function and a known indicator of central ANS activity[5] that has relevance to the enteric nervous system in the GI tract.[6] Research has shown that HRV is a calculation of the difference in beat-to-beat intervals on the electrocardiogram (ECG), with high HRV being associated with lower stress and reduced inflammation.[7] Cross-sectional studies evaluating HRV in IBD have shown differences in baseline HRV parameters in patients with IBD compared to healthy control patients and have established HRV associations with markers of systemic inflammation;[8,9] however, they have not longitudinally evaluated the link between HRV, psychological stress, and UC flares. We undertook a pilot study to test the methodological feasibility and estimated effect sizes for a simple, real-time, longitudinal approach to evaluate the impact of perceived stress on UC symptoms.

MATERIALS AND METHODS

Study Design

Patients with UC were enrolled in a prospective observational cohort study at a single center (The Susan and Leonard Feinstein Inflammatory Bowel Disease Clinical Center at Mount Sinai Hospital, New York, NY). Eligible patients were ages 18 to 65 years, had no IBD-related medications initiated in the 12 weeks before enrollment, and were on stable doses of medications prescribed for UC for at least 4 weeks. Patients were excluded if they were pregnant; had serious medical comorbidities, including malignancy or diabetes; used any corticosteroid formulation in the 4 weeks before enrollment; or had a pacemaker or defibrillator. The use of medications that interfere with ANS function or the cardiovascular system, including beta blockers, calcium channel blockers, and benzodiazepines, was exclusionary. Clinical remission was defined as a Simple Clinical Colitis Activity Index (SCCAI) score of ≤2 and a symptomatic flare was defined as a SCCAI score of >2. Inflammatory flare was classified as a C-reactive protein (CRP) level >5 or a fecal calprotectin (FC) level >150 mcg/g, independent of symptomatology. This study was approved by the institutional review board at Mount Sinai Hospital.

Study Survey

Surveys were administered throughout the study period to assess UC clinical activity, psychological parameters, and overall well-being. A brief description of included surveys is described as follows.

The SCCAI measures clinical symptoms of UC activity and quantifies symptom severity. It has 5 questions that are scored from 0 to 19, with higher scores related to increasing disease activity and remission considered to be a score ≤2. Questions concern quantification of bowel frequency during the day and night, fecal urgency, the presence of blood in the stool, general well-being, and the presence of extracolonic manifestations.[10]

The Perceived Stress Scale (PSS-4) measures perceived stress. It has 4 questions and is scored from 0 to 16, with higher scores correlating with increased self-reported stress. Questions in this scale grade a patient's sense of control over important things in life, confidence in handling problems, how often things are felt to be going the way of the participant, and the degree to which difficulties are felt to be piling up.[11]

The Connor-Davidson Resilience Scale (CD-RISC) measures multiple aspects of resilience. It has 10 questions and a maximum score of 40. Higher scores correlate with higher resilience.[12] The CD-RISC includes questions about adaptation to change, facing whatever comes the way of the participant, coping, the ability to bounce back, and whether a participant becomes discouraged.

The Patient-Reported Outcomes Measurement Information System (PROMIS) emotional distress-anxiety and emotional distress-depression scales (short forms) measure aspects of anxiety and depression, respectively.[13] They are each composed of 4 questions. Raw scores from each scale are converted into T-scores for standardization and analysis. Population norms correspond to a T-score of 50 with a standard deviation of 10. The anxiety scale asks patients to grade on a scale of never to always whether they feel fearful, have trouble focusing, are overwhelmed with worry, or feel uneasy. The depression scale asks participants to grade on a scale of never to always whether they feel worthless, helpless, depressed, and hopeless.

The Short Inflammatory Bowel Disease Questionnaire (SIBDQ) measures several domains of quality of life.[14] It has 10 questions and is scored from 10 to 70, with higher scores associated with better health-related quality of life. It includes questions grading aspects of fatigue; impact of bowel symptoms in general, on socialization, and on leisure activities; and how GI symptoms have made the patient feel.

Study Procedures

Baseline demographic information, past medical history, UC history, and medication history were collected at enrollment. Patients completed baseline assessments of clinical UC activity, perceived stress and resilience, emotional

distress, and quality of life. Baseline laboratory assessment included random cortisol, adrenocorticotropin hormone (ACTH), interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, and CRP. Stool was collected at enrollment to assess FC. Patients were prospectively followed for up to 9 months. They completed the PSS-4 questionnaire every 4 weeks, the SCCAI every 6 weeks, and the CD-RISC and PROMIS questionnaires every 12 weeks. Laboratory studies (cortisol, ACTH, IL-1β, IL-6, TNF-α, CRP) were repeated every 12 weeks, and FC was obtained every 6 weeks. Cortisol and ACTH were collected by participants at various times during the day (Fig. 1). If any baseline laboratory values were missing, then the first available specimen was used to classify individual disease activity status at study entry.

Wearable Monitoring Device

Patients' HRV was measured via the VitalPatch (VitalConnect, San Jose, CA), which is U.S. Food & Drug Administration–cleared wearable biosensor that adheres to the left upper chest and links via Bluetooth to an Android smartphone. At the time the study was conducted, the sensor was a disposable patch with a battery life of 72 hours. The device had 2 electrodes that generate a single-lead ECG with a sampling time of 125 Hz. The sensor allows the collection of an ECG waveform, temperature, activity, accelerometry in 3-axes, and respiratory rate. The device has internal storage capabilities for data collection when outside of Bluetooth range with the linked smartphone. Versions of this device have been used in other wearable device studies, including a large multicenter study in heart failure, highlighting the quality of ECG assessment.[15]

ANS Assessment

Patients wore the VitalPatch for 72 hours every 4 weeks for the duration of follow-up. They were recommended to carry out their daily activities as normal during this period. Data collected via the VitalPatch were stored, reviewed, and filtered for ectopic beats, artifact, and periods of exercise. The R peaks extracted from the ECG were used to create the RR time-series. A second round of processing was performed to remove possible high peaks in the time-series because of a disconnection between the VitalPatch and the participant's skin. After the filtering process, the RR intervals became NN intervals, which provided the input for different HRV techniques. Each NN time-series was divided into 3 different sub-time-series for analysis: (1) 24 hours in length, (2) from 10:00 PM to 6:00 AM (night), and (3) from 6:00 AM to 10:00 PM (day).

Patients' HRV was calculated in time and frequency domains. The time domain analysis performed was the root mean square of successive differences (RMSSD) between normal heartbeats. This measurement was calculated by determining the time differences between each heartbeat in milliseconds. Before the square root total was calculated, the values were squared and averaged.[16] The RMSSD predominantly reflects vagal change.[17] Frequency domains were calculated through the Fast Fourier Transform algorithm to obtain the power spectral density of the time-series. The HRV was separated into low-frequency (LF) bands, high-frequency (HF) bands, and LF to HF power (LFHF) for analysis in this study. The LF band may be produced by the PSN alone or may be influenced by both components of the PSN and of the SNS depending upon the conditions during measurement.[18,19] The HF band reflects parasympathetic function, although it may not fully reflect vagal tone and may have other contributing factors and inputs based upon the time of day when measurement occurs.[20] Traditionally, LFHF is thought to reflect sympathetic to parasympathetic balance; however, this notion has been challenged because LF is not derived fully from SNS function and the relationship between parasympathetic and sympathetic interactions is complex.[16,21]

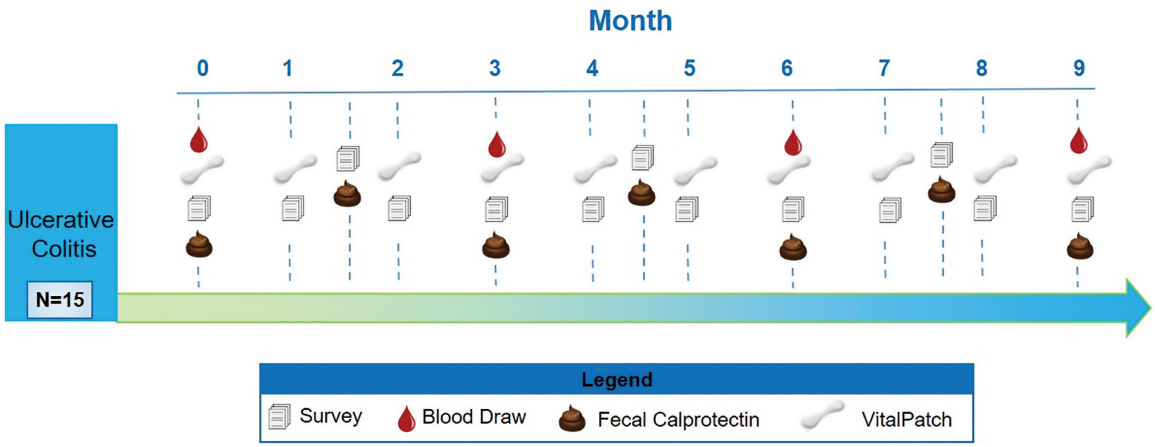

FIGURE 1. Patients were prospectively followed for up to 9 months. Patients wore the VitalPatch every 4 weeks, completed surveys every 4 to 12 weeks, provided stool for fecal calprotectin every 6 weeks, and had blood drawn every 12 weeks.

Statistical Analysis

In all analyses, a 14-day window was used when comparing variables to assure a reasonable and clinically relevant time period between associated metrics.

HRV indices

A linear mixed-effects model (LMEM) was used to evaluate the correlation between HRV metrics acquired from the 24-hour time period, night, and day time-series, incorporating repeated measures. The 24-hour time period was used for all subsequent HRV analyses because it best reflected continual longitudinal monitoring. We used the LMEM to estimate the association between the longitudinal HRV metrics and the clinical/physiological biomarkers. In general, a LMEM characterizes and compares changes in response over time and models the covariance among repeated measures in a parsimonious way (eg, as a linear trend), which is beneficial for small data. We used HRV as the dependent variable and FC, CRP, the SCCAI, cortisol, ACTH, IL-1β, IL-6, TNFα, the PSS-4, and the CD-RISC as the fixed effects. Each independent variable was included in the model because of its potential impact on the dependent and other independent variables. This framework was based on prior published works evaluating the relationship between HRV, the stress response, inflammation/cytokines, and IBD symptoms.[4,7,9] We included a random intercept to account for variability among the repeated measures of different individuals. The LMEM was implemented in R 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria) using the lme4 package.[22] Statistical significance was calculated using the lmerTest package, which applies the Satterthwaite method to estimate degrees of freedom and generate *P* values for mixed models.[23,24] Age, sex, and longitudinal measures of anxiety and depression (per the PROMIS questionnaires) were included as covariates in HRV analyses to control for their impact on ANS function.[25,26] To determine the directionality of the raw associations between variables of interest, univariate analysis was performed controlling for the above covariates used in the LMEM.

Psychological metrics

To evaluate the correlation between perceived stress and clinical predictors, we fitted an LMEM with PSS-4 scores as the dependent variable and used the SCCAI, the SIBDQ, the CD-RISC, FC, CRP, anxiety, and depression as the independent variables. Each independent variable was included in the model because of its potential impact on the dependent and other independent variables based on the relationship between stress, quality of life, inflammation, anxiety, and depression.[27,28] In addition, anxiety and depression were controlled for because of their possible confounding of the relationship between stress and the other outcomes of interest. A random intercept was used to allow patients to have their own patient-specific mean response trajectories over time.

We also fitted a LMEM with a random intercept to assess the association between resilience and clinical predictors. We included the CD-RISC as the dependent variable and added the SCCAI, the SIBDQ, FC, CRP, anxiety, and depression as the fixed-effect terms. Statistical significance was calculated using the lmerTest package, which applies the Satterthwaite method to estimate degrees of freedom and generate *P* values for mixed models.[24] Longitudinal measures of anxiety and depression were included as covariates to control for their impact on measures of stress and resilience. Univariate analysis was performed to determine the directionality of the associations, controlling for covariates.

Prediction of flare

We used 2-sample Wilcoxon tests to compare baseline HRV indices between patients who did and patients who did not have a symptomatic or inflammatory flare during the follow-up period. The generalized estimating equation was used to investigate how HRV metrics changed over time before the development of a flare event, with an identity link for continuous outcomes.[29] We used robust standard errors for inference. The generalized estimating equation was implemented in R version 3.6.2 using the geepack package.[30] Two cutoffs for the prediction of symptomatic flare (SCCAI >2 and SCCAI ≥5) and inflammatory flare based on stool markers of inflammation (FC >150 mcg/g and FC >250 mcg/g) were assessed to discriminate the HRV predictive value. Based on blood markers of inflammation, a cutoff of CRP >5 was used. This analysis was exploratory, so *P* values were not corrected for multiple comparisons.

RESULTS

Patient Population

Fifteen patients were enrolled (Table 1). The median age at enrollment was 33 years, and 60% were women. The median duration of UC was 8 years at the time of inclusion. All patients identified their ethnicity and race as non-Hispanic White. Eleven patients had a history of extensive UC, and 66.7% were on a biologic agent at enrollment. Patients had few comorbid conditions, which were well-controlled during follow-up, as seen in Table 1. At first assessment, 14 of the 15 patients were in clinical remission based on SCCAI scores and 11 of 15 and 7 of 15 were in remission based on FC and CRP, respectively. The median PSS-4 and CD-RISC scores at enrollment were 5 (range, 5-11) and 33 (range, 13-40). The median PROMIS-Depression T-score at enrollment was 41, and the median PROMIS-Anxiety T-score was 48. The mean duration of follow-up was 246.9 (SD, 59.3) days.

TABLE 1. Baseline Demographics of Patients at Enrollment

	Cohort, n = 15 (%)
Age, y, median	33
Female sex	9 (60)
Race	
White	15 (100)
BMI, median	26
Smoking history	
Current	0 (0)
Former	2 (13.3)
Never	13 (86.7)
Age at UC diagnosis, y, median	22
Duration of UC, y, median	8
Disease extent	
E1	2 (13.3)
E2	2 (13.3)
E3	11 (73.3)
Medical history	
Hypertension	1 (6.7)
Anxiety	1 (6.7)
Gastroesophageal reflux disease	1 (6.7)
Attention-deficit hyperactivity disorder	1 (6.7)
Hypothyroidism	1 (6.7)
Hyperlipemia	1 (6.7)
Melanoma	1 (6.7)
Herpes simplex	1 (6.7)
Uterine fibroids	1 (6.7)
Polycystic ovarian syndrome	1 (6.7)
Erythema nodosum	2 (13.3)
Pyoderma gangrenosum	1 (6.7)
Asthma	2 (13.3)
Lichen sclerosis	1 (6.7)
Surgical history	
Appendectomy	1 (6.7)
Cholecystectomy	3 (20)
Tonsillectomy	1 (6.7)
Medications	
Mesalamine	7 (46.7)
Azathioprine, mercaptopurine, or methotrexate	7 (46.7)
TNF-α antagonist	6 (40)
Vedolizumab	4 (26.7)
Baseline laboratory values at enrollment	
Cortisol, mean ± SD	8.7 ± 2.9
ACTH, mean ± SD	14.7 ± 8.3
CRP, mean ± SD	11.3 ± 15.6
IL-1β, mean ± SD	1.28 ± 1.5
IL-6, mean ± SD	2.0 ± 1.3
TNF-α, mean ± SD	3.3 ± 1.1
FC, mean ± SD	279.1 ± 678.3
Baseline questionnaire scores at enrollment	
SCCAI, median (range)	0 (0-3)
PSS-4, median (range)	5 (5-11)
SIBDQ, median (range)	57 (39-70)
CD-RISC, median (range)	33 (13-40)
PROMIS-Depression (T-score), median (range)	41 (41-62.2)
PROMIS-Anxiety (T-score), median (range)	48 (40.3-67.3)

HRV Indices

Each HRV index (HF, LF, LFHF, RMSSD) showed a significant positive correlation ($P < 0.001$) with itself when calculated over 24 hours, at night, or during the day (Table 2). Evaluation of the 24-hour time-series showed significant associations between longitudinal HRV indices with prospectively collected clinical and physiological biomarkers (Table 3). Clinical disease activity (SCCAI scores) showed a significant association with LFHF ($P = 0.03$), which on univariate analysis revealed a positive association. In addition, systemic and intestinal inflammatory markers were associated with HRV indices over time. We found that FC had a significant negative association with HF ($P < 0.001$) and LF ($P < 0.001$) and a significant positive association with LFHF ($P < 0.001$) and RMSSD ($P < 0.001$). Furthermore, CRP had a negative association with HF ($P < 0.001$) and LF ($P < 0.001$) and a positive association with RMSSD ($P < 0.001$). Further associations between cytokine levels with HRV metrics were evident. TNF-α levels showed a negative association with HF ($P < 0.001$); IL-6 levels were negatively associated with HF ($P < 0.001$), LF ($P = 0.001$), and RMSSD ($P < 0.001$), and IL-1 was negatively associated with HF ($P = 0.01$) and LF ($P = 0.005$) and positively associated with LFHF ($P = 0.006$) and RMSSD ($P = < 0.001$).

Significant relationships between HRV and stress and resilience were found. Changes in the PSS-4 scores were negatively associated with LFHF ($P = 0.04$) and RMSSD ($P = 0.04$) measurements over time. The CD-RISC scores had a significant negative association with HF ($P < 0.001$) and LF ($P < 0.001$) and a positive association with RMSSD ($P < 0.001$). Furthermore, significant associations were noted between cortisol, ACTH, and ANS function, with cortisol being positively associated with HF ($P = 0.02$), LF ($P = 0.01$), and RMSSD ($P < 0.001$) and negatively associated with LFHF ($P = 0.005$). The ACTH level was positively associated with HF ($P < 0.001$), LF ($P < 0.001$), and RMSSD ($P < 0.001$).

Psychological Metrics

There were significant associations between longitudinal perceived stress, clinical GI symptoms, resilience, and inflammatory markers. When controlling for longitudinal measures of anxiety

and depression, we found that longitudinal PSS-4 scores were significantly and positively associated with prospectively collected CRP ($P = 0.03$), though not with FC ($P = 0.25$). A significant inverse relationship between PSS-4 scores and CD-RISC scores ($P = < 0.001$) was found, with no significant relationship observed between longitudinal perceived stress and quality of life ($P = 0.32$), anxiety ($P = 0.65$), or depression ($P = 0.59$). A significant positive relationship was also observed between prospectively collected perceived stress and UC-related symptoms (SCCAI; $P = 0.02$). Resilience was not found to be significantly associated with CRP, FC, quality of life scores, or clinical UC activity. Evaluation of the relationship between symptoms and inflammation showed a significant positive association over time between SCCAI scores and CRP ($P = < 0.001$) but not with FC ($P = 0.14$). Our findings showed significant associations of higher perceived stress, worse clinical symptoms, increased inflammatory measures, and lower resilience in patients with UC.

Prediction of Flare

Patients in symptomatic remission (n = 14), inflammatory remission based on CRP <5 (n = 7), and inflammatory remission based on FC <150 mcg/g (n = 11) at first recorded survey or biomarker were evaluated. During follow-up, 6 of the 14

TABLE 2. Correlation Between HRV Indices Based on Time-Series Analysis

HRV Time-Series	Effect Estimate	Standard Error	Statistic	Degrees of Freedom	*P*
24-h vs day					
LFHF	1.13	0.03	43.62	116.59	< 0.001
LF	1.01	0.06	17.03	44.11	< 0.001
HF	0.98	0.07	14.86	28.66	< 0.001
RMSSD	1.00	0.04	25.74	54.79	< 0.001
Day vs night					
LFHF	0.28	0.03	8.13	457.69	< 0.001
LF	0.09	0.03	2.81	455.60	0.005
HF	0.06	0.03	2.02	452.45	0.04
RMSSD	0.27	0.04	6.53	461.10	< 0.001
24-h vs night					
LFHF	0.74	0.03	21.79	314.95	< 0.001
LF	1.06	0.05	22.06	263.73	< 0.001
HF	1.08	0.05	22.79	291.31	< 0.001
RMSSD	1.08	0.04	25.87	244.00	< 0.001

TABLE 3. Association Between Clinical and Physiological Biomarkers With HRV Indices

	HF		LF		LFHF		RMSSD	
	Estimate*	*P*	Estimate*	*P*	Estimate*	*P*	Estimate*	*P*
Symptoms								
SCCAI	4.22E-05	0.44	6.87E-05	0.69	0.13	0.03	0.002	0.36
Inflammation								
FC	–0.001	< 0.001	–0.014	< 0.001	66.89	< 0.001	0.37	< 0.001
CRP	–1.43E-04	< 0.001	–1.20E-04	< 0.001	1.33	0.09	0.008	< 0.001
Cytokines	–4.10E-05	< 0.001	–7.11E-05	0.15	0.09	0.06	–0.002	0.17
TNF								
IL-6	–2.56E-05	< 0.001	–4.36E-05	0.001	0.08	0.07	–0.004	< 0.001
IL-1	–2.72E-05	0.01	–3.48E-05	0.005	0.07	0.006	–0.003	< 0.001
Stress	–40.40	< 0.001	–21.49	< 0.001	–0.02	0.10	0.05	< 0.001
CD-RISC								
PSS-4	–104.52	0.11	–81.46	0.48	–0.02	0.04	–0.89	0.04
Cortisol	5.56E-04	0.02	8.11E-04	0.01	–0.09	0.005	0.05	< 0.001
ACTH	6.72E-04	< 0.001	9.90E-04	< 0.001	0.28	0.52	0.04	< 0.001

*Estimates were derived from univariate analyses to determine and highlight the directionality of associations.

patients in symptomatic remission met the criteria for a symptomatic flare (SCCAI >2; median, 194.5 days), 4 of the 7 patients in inflammatory remission based on CRP developed an inflammatory flare (CRP >5; median, 142.5 days), and 7 of the 11 patients in remission based on FC developed an inflammatory flare (FC >150; median, 78 days). There was no difference in baseline HRV indices (HF, LF, LFHF, RMSSD) between patients who did and did not have a flare as defined above.

We found statistically significant changes in HRV indices compared to baseline before the development of an SCCAI score >2, CRP >5, and FC >150 in patients who started in remission at enrollment, in each respective category. Specifically, LFHF decreased (estimate, –0.003; $P < 0.001$), LF increased (estimate, 0.003; $P < 0.001$), HF increased (estimate, 0.004; $P < 0.001$), and RMSSD increased (estimate, 0.001; $P < 0.001$) significantly from baseline, a mean of 108.2 days, before the development of an SCCAI score >2. In addition, LFHF decreased (estimate, –6.81E-04; $P < 0.001$), LF decreased (estimate, –6.36E-05; $P < 0.001$), HF increased (estimate, 0.002; $P < 0.001$), and RMSSD decreased (estimate, –5.93E-04; $P < 0.001$) significantly from baseline, a mean of 68.0 days, before the development of CRP >5. The LFHF decreased (estimate, –0.006; $P < 0.001$), LF increased (estimate, 0.006; $P < 0.001$), HF increased (estimate, 0.005; $P < 0.001$), and RMSSD increased (estimate, 0.003; $P < 0.001$) from baseline, a mean of 66.7 days, before the development of FC >150.

When flare was defined as an SCCAI score ≥5 or a FC >250 mcg/g, there were 15 patients in symptomatic remission and 12 patients in inflammatory remission based on FC at the first recorded survey or biomarker. During follow-up, 4 of the 15 patients in symptomatic remission met the criteria for a symptomatic flare (SCCAI ≥5), whereas 7 of the 12 patients in inflammatory remission based on FC met the criteria for inflammatory flare (FC >250 mcg/g). There were no differences in baseline HRV indices (HF, LF, LFHF, RMSSD) between patients who did and patients who did not have a flare as defined above. Again, there were statistically significant changes in HRV indices compared to baseline before the development of a flare at these cutoffs. Specifically, LFHF decreased (estimate, –0.003; $P < 0.001$), LF increased (estimate, 0.003; $P < 0.001$), HF increased (estimate, 0.003; $P < 0.001$), and RMSSD increased (estimate, 0.002; $P < 0.001$) significantly from baseline, a mean of 108.6 days, before the development of an SCCAI score ≥5. Finally, LFHF decreased (estimate, –0.006; $P < 0.001$), LF increased (estimate, 0.009; $P < 0.001$), HF increased (estimate, 0.009; $P < 0.001$), and RMSSD increased (estimate, 0.004; $P < 0.001$) from baseline, a mean of 80.5 days, before the development of FC >250 mcg/g.

DISCUSSION

In this prospective study, longitudinally evaluated HRV was found to be associated with UC symptoms, inflammation, and perceived and physiological measures of stress. This is the first study to show such an association and find that significant changes in HRV metrics precede the development of a symptomatic or inflammatory flare. Taken together, this pilot study identifies a possible novel, modifiable precursor of UC disease activity and physiological stress in UC. It also shows the feasibility of using the VitalPatch device to measure HRV repeatedly for up to 9 months in patients with UC.

Several studies have evaluated the relationship between stress and IBD clinical flare, although with conflicting results.[28,31,32] Our study supports a relationship between stress and UC flare, finding a significant positive association between perceived stress and symptom activity. There has been a limited evaluation of the association between stress and inflammatory flare in prior studies, which have failed to show a relationship between the 2 factors. However, these studies have relied predominantly on FC.[27,28] We similarly found no relationship between perceived stress and FC, although we did show a positive and significant relationship between perceived stress and systemic inflammation (ie, CRP levels). Although we did not determine causality, this association warrants further evaluation in larger studies to better define the direction of this relationship and to assess whether worsening stress precedes the development of symptoms and/or inflammation. It is unclear why a relationship between stress with systemic inflammation was found, but not with intestinal inflammation. Further evaluation is needed; however, this association may be because perceived stress has a closer relationship with systemic stress rather than with intestinal inflammation, or that the degree of mucosal inflammation was not significant enough.

Our group undertook a comprehensive assessment of perceived stress, its relationship with HRV, and subsequent markers related to UC activity. Research has suggested that ANS dysfunction predisposes individuals with IBD to inflammation. Homeostatic regulation of the ANS couples a high vagal tone with low cortisol levels. In chronic inflammatory conditions, this coupling is altered and the HPA axis becomes hyporeactive, resulting in autonomic imbalance and the maintenance of inflammation through effects on cytokines such as TNF-α and IL-6.[33,34] Therefore, imbalances in the ANS may play an important role in the pathophysiology of UC. Patients with IBD have altered ANS function compared to healthy control patients, with autonomic hyperreflexia associated with disease severity in UC and shifts toward either relative parasympathetic or sympathetic predominance.[35-39] Interestingly, we found an inverse longitudinal association between RMSSD, which predominantly assesses parasympathetic function, and perceived stress measures. Increased stress is associated with increased noradrenergic activation, which supports the inverse relationship between stress and vagal tone. Although this finding requires further assessment in larger prospective studies, it shows a longitudinal relationship between perceived stress and physiological metrics of the ANS. Further delineation of whether changes in perceived stress precede ANS alterations is needed because a bidirectional relationship between the 2 exists.

The relationship we observed between longitudinally collected HRV metrics and the clinical, inflammatory, and immune

features of UC suggests the identification of a unique noninvasive marker of disease activity that warrants further large-scale exploration. Gunterberg et al[8] previously evaluated the relationship between baseline ANS function in 51 patients with UC at the time of first disease flare and IBD-related measures over a 3-year period. They found that sympathovagal balance was associated with systemic inflammatory markers, including CRP and TNF-α, at disease onset, and that PNS activity predicted erythrocyte sedimentation rate during follow-up. Although those authors revealed a clinically relevant association between HRV metrics and clinical measures, their study was limited by a single time point of HRV measurement, could not incorporate longitudinal fluctuations in ANS function over time, and could not incorporate real-time assessment of disease activity in an actionable fashion. Leveraging the advancement of wearable device technology, we were able to assess HRV for 72 hours every 4 weeks, thereby incorporating HRV fluctuations over time and allowing for a more robust assessment of this dynamic system. We showed that as symptoms, inflammation, and cytokine levels increased, HRV markers largely reflecting sympathetic tone (LFHF) increased whereas those generally reflective of parasympathetic tone (RMSSD, LF, HF) decreased. However, the directionality of the relationship between RMSSD to FC and CRP was positive, which was the opposite expected direction. This may reflect increased vagal reactivity in response to the development of inflammation. Vagal tone has an anti-inflammatory and anti-nociceptive effect.[40] Further longitudinal evaluation is needed in larger studies to show the reproducibility of this finding.

Strengthening the potential role for HRV to serve as a noninvasive marker of disease activity is our observation that significant changes of HRV indices from baseline were noted before the development of clinical and inflammatory flare. This condition is analogous to the changes seen in other biomarkers, such as FC, before presentation with symptomatic flare. Furthermore, we were able to show that these changes occurred a significant amount of time before the observed elevation of the clinical or inflammatory marker. This lead time possibly allows for early identification of future flares and underscores the ability of modalities such as wearable devices to identify incipient flares at the earliest most actionable time.

Although larger studies are needed to verify our results and better delineate the predictive ability of changes in HRV, this pilot study identifies HRV as a possible biomarker warranting further exploration. In addition, the relationship between the vagal nerve and IBD activity warrants further evaluation as a therapeutic target. Supportive of this potential are small studies showing the possible efficacy of vagal nerve stimulators in improving IBD.[41] Behavioral interventions targeting stress have been successfully employed in IBD.[42-44] However, although specific behavioral interventions aimed at modifying HRV and stress have shown efficacy in multiple conditions ranging from blood pressure management to posttraumatic stress disorder, their potential impact modifying IBD has yet to be explored.[45,46]

There are several limitations of our study. This was an exploratory pilot study exploring various longitudinal relationships. In the future, with larger sample sizes, investigation of the described associations can be performed for more robust conclusions. Although we collected detailed information from each participant, our sample size was limited to 15. This small size could result in the undue influence of outliers on our analysis. Furthermore, the small sample size limited the statistical modeling that could be performed. An additional limitation is that this was a single-center study and the recruited population lacked racial and ethnic diversity. Furthermore, we did not specifically prohibit or instruct participants to limit the intake of caffeine or alcohol, which could impact HRV during collection periods. Baseline data on characteristics including caffeine, alcohol, and recreational drug use was also not captured. A further limitation is that endoscopic evaluation was not performed in the study, with FC used as a surrogate marker for intestinal inflammation, allowing longitudinal assessment. In addition, cytokines were only measured in the serum and not in tissues. Finally, we measured HRV indices only for 3 days every 4 weeks. The lack of continual measurement throughout the study period can result in missed data points, which could influence the associations described above.

The strengths of our study include its prospective design, long follow-up period, and multimodal assessment of patients. In addition, it is the first study in IBD to incorporate frequent longitudinal measurement of ANS metrics, allowing assessment of a dynamic index.

CONCLUSIONS

We have shown a relationship between longitudinally collected UC disease markers, perceived stress, and ANS function and observed that significant changes in HRV precede the development of flare. This pilot study supports the further evaluation of these metrics as a potential biomarker of disease activity and physiological stress in UC, which may offer the opportunity to monitor IBD in a noninvasive fashion through the course of daily life. It may provide the ability to detect flare sooner and an opportunity for early optimization and intervention to improve disease outcomes.

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