Autonomic imbalance during the day in patients with inflammatory bowel disease in remission. Evidence from spectral analysis of heart rate variability over 24 hours

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Background. Autonomic function in inflammatory bowel disease has not yet been studied by means of analysis of 24-hour heart rate variability.

Aim. To measure heart rate variability in inflammatory bowel disease patients in remission.

Patients and methods. Study population comprised 27 patients with inflammatory bowel disease in remission and 28 healthy, sex- and age-matched controls. Two frequency ranges were analysed: low frequency (0.06-0.15 Hz) and high frequency (0.15-0.40 Hz).

Results. Mean values of low frequency and low frequency/high frequency ratio were lower in patients than in controls (p<0.001). High frequency in patients tended to be higher than in controls (p=0.09). The only factor that had a marginal effect on heart rate variability indexes was age. In high frequency, there was a significant time effect (p=0.001) for both groups. There was also a significant time effect in low frequency/high frequency ratio in both groups (p<0.001). During daytime, the mean values in low frequency/high frequency ratio were lower in patients than in controls (p<0.001).

Conclusions. There is a shift in the autonomic balance in patients with inflammatory bowel disease in remission towards a condition of relative parasympathetic predominance, which, in the first place, reflects a sympathetic pullback. This imbalance has a circadian rhythm and it is more pronounced during the day.

Introduction

Inflammatory bowel diseases (IBD, i.e., ulcerative colitis (UC), Crohn’s disease (CD) and indeterminate colitis) are chronic inflammatory disorders of unknown aetiology. UC and CD are frequently associated with extraintestinal manifestations.

Impaired autonomic nerve function has been found in patients with UC and CD using non-invasive tests based on the heart reactions to deep breathing and tilting as well as on pupillary standardized autonomic nervous function tests. These methods of assessment, as well as others (Valsalva manoeuvre, cold stress), require active cooperation on the patient’s part and present difficulties in standardization. On the contrary, analysis of 24-hour heart rate variability is a reliable method with very good reproducibility, since changes, for most variables, in two consecutive measurements, do not exceed the 10% limit.
The measured heart rate is the product of interaction between the two main constituents of the autonomic nervous system, the sympathetic and parasympathetic nervous system. While the first increases (e.g. during physical exercise), the latter slows down (e.g. after meals or in resting conditions) the heart rate. In general, the heart rate, in humans, reflects the equilibrium between the two main components of the autonomic system during different time periods of the day. The interval between the consecutive heart beats oscillates in healthy subjects and this is commonly defined as heart rate variability (HRV). The assessment of HRV is based on measurements in the time and the frequency domain. In the time domain analysis, the indexes reflect the level of autonomic tone without determining to which of the two branches, vagal or sympathetic, the resulting autonomic tone is due. In the frequency domain analysis, the variance in the high frequency (HF) band reflects vagal activity whereas the variance in the low frequency (LF) band correlates with both sympathetic and parasympathetic control. HRV measurements depend upon sex, age and heart rate. Spectral analysis of the 24-hour HRV is a powerful tool for non-invasive monitoring of central autonomic nervous system dynamics. It has been applied in research on various conditions where the balance between the sympathetic and parasympathetic nervous system, as well as the vagal tone per se, are of importance.

However, there are no data on 24-hour recordings of heart rate variability in patients with IBD. The purpose of this study is to describe and compare parameters of HRV 24-hour monitoring, both in the time and frequency domain, in patients with IBD in remission.

Patients and methods

Patients

A total of 27 patients with IBD, 16 with UC and 11 patients with CD (mean age 52.4 years) attending the outpatient clinic of a University Hospital were invited to take part in the study. The diagnosis of UC or CD was made according to standard criteria. A detailed clinical examination by a cardiologist (M.M.) was performed in all patients. Patients were excluded if they had diabetes mellitus, abnormal thyroid function, any known cardiac arrhythmia that could interfere with the HRV assessment, cardiac failure, coronary heart disease, or if they were under any β-receptor agonist or antagonist treatment. All patients had regular physical activity; no heavily exercising patients were included. Patients who had undergone intestinal resection were excluded. All patients had been in clinical and endoscopic remission for the last 10-12 weeks (according to Harvey-Bradshaw Index for CD patients and Colitis Activity Index for UC patients). They had been receiving oral mesalamine medication as maintenance treatment, for at least two months.

During the week preceding the Holter recording, patients and controls were requested to take no anticholinergic drugs for abdominal cramps or diarrhoea. During the 24 hours prior to initiating HRV assessment, patients took no caffeinated beverages, and did not smoke. Patients were requested to undertake their usual daily activities during the 24 hours of HRV assessment. There are no published data demonstrating any impact of mesalamine on HRV, patients, therefore, continued the mesalamine treatment during the day of testing.

A group of 28 age- and sex-matched healthy volunteers were used as controls. Patients and controls gave informed consent before entering the study. Demographic data of patients and controls, as well as clinical data of patients, are summarized in Table 1. Demographic characteristics did not differ significantly between IBD patients and healthy controls.

### Table 1. Demographic characteristics of patient and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ulcerative</td>
<td>Crohn's</td>
</tr>
<tr>
<td></td>
<td>colitis</td>
<td>disease</td>
</tr>
<tr>
<td>Total number of patients</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td>Female patients</td>
<td>3 (19%)</td>
<td>3 (27%)</td>
</tr>
<tr>
<td>Duration of IBD, years (mean)</td>
<td>7.3</td>
<td>6</td>
</tr>
<tr>
<td>Bowel involvement:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proctitis</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Left side</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Pancolitis</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Small bowel</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Large bowel</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Small &amp; large bowel</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Intestinal resection</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hospitalized</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Age median (range) years</td>
<td>55 (34-75)</td>
<td>49 (23-54)</td>
</tr>
<tr>
<td>Patients &gt;50 years</td>
<td>11 (69%)</td>
<td>3 (27%)</td>
</tr>
<tr>
<td>Smokers</td>
<td>3 (19%)</td>
<td>5 (45%)</td>
</tr>
<tr>
<td>Therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients on mesalamine</td>
<td>16 (100%)</td>
<td>11 (100%)</td>
</tr>
<tr>
<td>Patients on corticosteroids</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Analysis of HRV

Electrodes were placed for a 24-hour electrocardiograph Holter recording. The three-channel Holter ECG recordings were obtained with Marquette 8500 series
recorders and were automatically processed on the Marquette laser Holter scanner (Marquette Electronics Inc., Milwaukee, WS, USA) with software version 5.8 to detect and label each QRS complex. This procedure was followed by careful manual review to limit any potential artifacts. Five time domain indexes were derived: the SD of all normal R-R intervals, the mean of all 5-minute SDS of R-R intervals, the SD of the 5-minute means of normal R-R intervals, the proportion of adjacent normal R-R intervals differing by >50 msec, and the mean square root of the difference between successive normal intervals. The first three of these indexes reflect total HRV, whereas the last two provide an indication of the vagal tone.

Spectral indexes of HRV were computed by fast Fourier transform on each 2-minute segment of the recording, with application of a Hanning window to minimize spectral leakage. The spectral power was quantitatively evaluated and expressed in absolute units (ms²) after measurement of the areas of two frequency ranges: low frequency (LF) (0.06 to 0.15 Hz), which gives mainly a measure of sympathetic activity with some influence from the parasympathetic nervous system, and high frequency (HF) (0.15 to 0.40 Hz), which reflects only parasympathetic activity. To channel out the influence of the parasympathetic activity on the LF spectral power, the ratio of the LF/HF band was then calculated. This ratio provides a measure of the sympathovagal balance where an increase in the LF/HF ratio reflects a predominance of sympathetic over parasympathetic activity.

All indexes of HRV were evaluated for the entire 24-hour period of monitoring. Spectral indexes (LF and HF) were also calculated separately for each hour. Test-retest reliability was assessed in the control group, and baseline values of all HRV indexes were similar to those at the follow-up visit.

Due to the highly skewed distribution of spectral HRV measures, the natural logarithm (ln) of the values was taken in order to use parametric methods of analysis which are more powerful than the non-parametric methods in detecting group differences.12

Statistical analysis
Data are expressed as mean ± standard deviation. Separate sub analyses were carried out to assess differences between the controls and IBD patients. Spectral indexes of HRV were compared for controls and IBD patients using a two-sided t-test; one way ANOVA (analysis of variance) was utilized to compare mean values.

In order to compare the time course of LF, HF and LF/HF between the groups, repeated measures with multivariate ANOVA with one grouping factor was used. This comparison was performed in 3 stages: after initially assessing differences over the whole 24-hour period, we then examined differences during the daytime (07:00 to 22:00) and at night (22:00 to 07:00). Normality and homogeneity assumptions were tested by the Kolmogorov-Smirnov and Cochran C test, respectively. Pillai's multivariate test was used when assumptions held; otherwise, Greenhouse-Geisser ε-adjusted p values were used. A p value <5% was the criterion of significance in all tests.

Results
Mean 24 hr HRV indexes
Tapes from all patients were of sufficiently good quality for analysis. Table II shows the means and standard deviations of all HRV indexes over the 24-hr period in the control group and the patients. Mean values of LF and LF/HF were lower in patients than controls over 24 hours (p<0.001). HF in patients tended to be higher than in healthy controls but the difference did not reach significance levels (p=0.090).

<table>
<thead>
<tr>
<th></th>
<th>Patients (mean±SD)</th>
<th>Controls (mean±SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF</td>
<td>6.39±0.79</td>
<td>6.01±0.82</td>
<td>0.090</td>
</tr>
<tr>
<td>LF</td>
<td>5.11±0.95</td>
<td>7.09±0.65</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LF/HF</td>
<td>0.78±0.08</td>
<td>1.19±0.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SDANN</td>
<td>121.51±27.22</td>
<td>129.46±38.75</td>
<td>0.385</td>
</tr>
<tr>
<td>SD</td>
<td>62.85±28.09</td>
<td>66.46±21.89</td>
<td>0.596</td>
</tr>
<tr>
<td>rMSSD</td>
<td>33.48±16.01</td>
<td>37.00±17.22</td>
<td>0.437</td>
</tr>
<tr>
<td>pNN50</td>
<td>11.27±10.25</td>
<td>13.52±10.97</td>
<td>0.434</td>
</tr>
<tr>
<td>MeanNN</td>
<td>804.31±194.38</td>
<td>806.97±97.24</td>
<td>0.916</td>
</tr>
<tr>
<td>SDNN</td>
<td>137.70±30.65</td>
<td>144.60±38.26</td>
<td>0.465</td>
</tr>
</tbody>
</table>

Abbreviations: see list.

As far as concerns sex, age, disease type (UC or CD), disease duration and smoking, the only factor that had a marginal effect on HRV indexes over 24 hours in patients was age. More specifically, patients aged <50 years had LF=5.45±0.9 vs 4.7±0.9 for patients >50 years (p=0.06). With regard to the IIF and LF/IIF ratio for the above age groups (less and more than 50 years) the values were 6.67±0.12 vs 6.21±0.11 and 0.82±0.08 vs 0.76±0.09, respectively (p=0.048 and p=0.090). Age seemed to have an effect on HRV indexes over 24 hours also in controls. Concerning smoking, patients who smoke had LF=4.69±0.73 (mean±SD), and non-smokers 5.28±1.01 (mean±SD), a non-significant difference (p=0.105). LF/HF ratio was 0.77±0.08 for smokers and 0.81±0.09 for non-smokers (p=0.262).
Autonomic dysfunction in IBD

Time course of spectral indexes of HRV
Figures 1A, 1B and 1C show the pattern of changes in HRV parameters over 24 hours in patients and controls. In LF, there was no time effect (p=0.07) in either
group but there was a marginal group effect (p=0.066). Mean values of LF were lower in patients than controls over the 24-hour period. In HF, there was a significant time effect (p=0.001) in both groups, but with no group effect (p=0.172). Mean values of HF at night were significantly higher than those during the day in both groups. In the LF/HF ratio, there was a significant time effect in both groups (p<0.001). Group effects failed to attain statistical significance for the entire 24-hour period (p=0.704). During the day, mean values of the LF/HF ratio were significantly lower in patients than in controls (p<0.001).

Discussion
In this study, we have shown that an autonomic imbalance exists in patients with IBD in remission. This disturbed autonomic function consists mainly in a shift of the autonomic balance towards a relative parasympathetic predominance during the day, and is the result of a lower sympathetic tone in patients with IBD compared to healthy controls. The fact that this autonomic imbalance occurs during disease remission suggests an underlying abnormality in the autonomic nervous system in IBD patients independent of disease activity. HRV has been used to assess the autonomic neural function in patients with acute myocardial infarction,
heart failure, and diabetes mellitus, in studies both of the short (2-5 min) as well as the long (24-hr) ECG recording periods\textsuperscript{13-15}. Spectral analysis of HRV provided important new insights into the conventional concept of sympathovagal balance. Results obtained in normal subjects, with spectral analysis of HRV, confirm that the sympathetic tone prevails during daytime, particularly during the first hours after awakening, while, during night-time, the vagal tone becomes predominant\textsuperscript{16}.

Motor activity of the gastrointestinal system is regulated by the enteric nervous system. Through communications with the central nervous system, the enteric nervous system interacts with the heart autonomic nervous system. When autonomic function is being measured by spectral analysis of HRV, it is the cardiac and not the abdominal autonomic nervous function that is being assessed. However, in studies on diabetic autonomic neuropathy, a close agreement has been shown between cardiovascular and abdominal vagal dysfunction\textsuperscript{17}. It is feasible to suggest, together with others, that abdominal vagal dysfunction can be inferred by cardiovascular dysfunction\textsuperscript{15,18}.

It has been observed that autonomic parameters may be abnormal in patients with gastro-oesophageal reflux disease, irritable bowel syndrome, coeliac disease, and chronic liver disease, implying that autonomic neuropathy may coexist or contribute in various gastrointestinal disorders\textsuperscript{19,20}. There are several pieces of evidence in favour of an interaction between the autonomic nervous system and intestinal symptoms in patients with IBD; abnormalities in the peptide-containing nerve fibres in the gut of CD patients\textsuperscript{21}, increased plasma levels of gastrointestinal peptide hormones in IBD patients\textsuperscript{22}, increased immunoreactivity of substance P and wide expression of its binding sites in IBD patients\textsuperscript{23}, substantial reduction of the severity of IBD patients\textsuperscript{24}, increased immunoreactivity of sub-

In IBD patients, using deep breathing and orthostatic tests, evidence has been found for an autonomic imbalance for almost half of the patients with CD and one third of those with UC. The presence of autonomic nerve dysfunction was not found to be associated with severe inflammation or disease duration\textsuperscript{12}. In contrast to these findings, in another study on IBD patients, investigated by means of several cardiovascular and pupillary autonomic nervous function tests, cardiovascular autonomic imbalance was very rare (<5%), and pupillary autonomic disturbance was more frequent, but still not common (20%). Pupillary autonomic hyperreflexia was found more often, in one third to one fourth of the patients, and was significantly associated with more severe inflammation\textsuperscript{4}. These conflicting results may be related to differences in methods for assessing autonomic function or to different selection criteria in the patients. In our study, the average LF as well as LF/HF ratio for patients with IBD were significantly lower during daytime than in healthy controls, implying a relative parasympathetic predominance. This condition could be the result of either increased vagal activity, reduced sympathetic activity or both. However, the fact that LF band variance is reduced while HF is essentially kept stable indicates that this reduction is due to low sympathetic tone. Time course analysis of spectral indexes of HRV leads to the conclusion that low sympathetic tone occurs during the daytime and is more pronounced in the afternoon. This disturbance of the autonomic balance in IBD patients who are in remission implies a contribution of the autonomic nervous system in the pathogenesis of IBD symptoms during active disease. Our data may have some implications regarding the symptomatic treatment of IBD as changes in sympathetic tone may be related to changes in intestinal motility.

Smoking is known to have a significant effect on cardiac vagal tone\textsuperscript{26}. However, this smoking effect could not be shown in IBD patients. No significant differences were found in any of the HRV indexes, between patients who were smokers and non-smoking patients. Most of the cardiovascular indices reflecting autonomically mediated heart rate responses decrease with advancing age\textsuperscript{27}. These observations were also confirmed in our study; we observed higher LF and LF/HF ratio in patients aged <50 years. It seems, therefore, that advancing age has a more pronounced effect on cardiac vagal tone, at least in IBD patients.

In conclusion, our data are compatible with the assumption that, in IBD patients, a shift exists in the balance between the sympathetic and parasympathetic autonomic nervous system. This shift is expressed as a condition of relative parasympathetic predominance, which reflects more a sympathetic pullback than an excess vagal arousal. There is a circadian cycle in this shift of autonomic balance, as it is more pronounced during the day. Further studies are needed for evaluation of sympathetic and vagal activity by means of spectral analysis of HRV in the frequency and time domain, conducted selectively on subgroups of IBD patients, such as patients in remission and regression, CD patients with different phenotypes, and UC patients with different extension of colitis. Alternatively, studies in healthy subjects could be carried out in order to establish whether there is a parasympathetic predominance when inducing an exogenous proinflammatory situation. In this way, more light would possibly be shed upon the interaction of the autonomic nervous system with the diseased intestine and the symptoms in IBD.
List of abbreviations

ANOVA: analysis of variance; CD: Crohn’s disease; HF: high frequency; HRV: heart rate variability; IBD: inflammatory bowel disease; LF: low frequency; In: logarithm; MeanNN: mean of all R-R intervals; RMSD: root mean square of differences between successive normal intervals; SDANN: standard deviation of 5-minute averages of all normal R-R intervals; SDN: standard deviation of all normal R-R intervals; UC: ulcerative colitis.

References