Cardiovascular autonomic function assessed by autonomic function tests and serum autonomic neuropeptides in Egyptian children and adolescents with rheumatic diseases

Zeinab A. El-Sayed1, Gehan A. Mostafa1, Gamal S. Aly2, Ghada S. El-Shahed3, Manal M. Abd El-Aziz4 and Safaa M. El-Emam2

Objective. Cardiovascular autonomic neuropathy (CAN) in patients with rheumatic diseases may result in sudden death, possibly from arrhythmia and myocardial infarction due to its frequent association with microvascular disease. Autonomic dysfunction may contribute to initiation and perpetuation of rheumatic diseases. Thus, we aimed to assess cardiovascular autonomic function in lupus and juvenile idiopathic arthritis (JIA) patients.

Methods. Assessment of cardiovascular autonomic function was done in 20 lupus and 20 JIA patients, aged 9–16 years, by five non-invasive autonomic function tests (AFTs) and serum levels of neuropeptide Y (NPY) and vasoactive intestinal peptide (VIP), as indicators of sympathetic and parasympathetic functions, respectively, in comparison with 40 matched healthy control subjects.

Results. Clinical evidence of CAN was found in 65 and 40% of lupus and JIA patients, respectively, and in none of healthy controls. Lupus and JIA patients had significantly lower serum NPY and VIP than controls (P < 0.001). The five AFTs score had significant negative correlations to NPY and VIP (P < 0.001). Patients with CAN had significantly lower serum NPY and VIP than patients without (P < 0.001). Clinical evidence of CAN was found in 41.7 and 14.3% of asymptomatic lupus and JIA patients, respectively. There was significant positive association between CAN and important disease manifestations, including activity, in these patients.

Conclusions. CAN is common in lupus and JIA patients, even in absence of relevant symptoms. Thus, assessments of cardiovascular autonomic function, by AFTs and serum autonomic neuropeptides (NPY and VIP), and the therapeutic effects of NPY and VIP are recommended in these patients.

Key words: Autonomic function tests, Autonomic nervous system, Cardiovascular autonomic neuropathy, Juvenile idiopathic arthritis, Neuropeptide Y, Systemic lupus erythematosus, Vasoactive intestinal peptide.

Introduction

Cardiac automaticity is intrinsic. However, heart rate (HR) and rhythm are largely under the control of autonomic nervous system (ANS) [1]. Cardiovascular autonomic neuropathy (CAN) in patients with rheumatic diseases may result in sudden death possibly from arrhythmia and myocardial infarction due to its frequent association with microvascular disease with subsequent myocardial hyperperfusion [2]. Thus, assessment of cardiovascular autonomic function in these patients is mandatory [3].

Cardiac autonomic function could be evaluated clinically by several non-invasive cardiovascular reflex autonomic function tests (AFTs) based on measuring the reflex changes in HR in response to standardized stimuli [4]. The battery of AFTs helps in the detection of early changes of CAN [5].

A growing body of evidence points towards modulation of the immune responses and inflammatory activity of autoimmune diseases by ANS. The disturbance in the interaction between ANS and the immune system may contribute to the initiation and perpetuation of rheumatic diseases [6–8]. The autonomic neural control of cardiovascular and immune functions involves a number of autonomic neuropeptides such as neuropeptide Y (NPY) and vasoactive intestinal peptide (VIP), which have a role in the orchestration of many cytokines and exert modulatory effects on immune cells [9]. NPY, a 36 sympathetic amino acid neuropeptide [10], modulates various immunological functions and provides novel insights into the neuroimmunological basis of autoimmunity [11]. VIP, a 28 amino acid parasympathetic neuropeptide, is a potent anti-inflammatory factor that may be beneficial in treatment of Th1 type autoimmune diseases [12–14].

This study aimed at assessment of cardiac autonomic function in patients with SLE and juvenile idiopathic arthritis (JIA) by five non-invasive AFTs and serum levels of NPY and VIP, as indicators of sympathetic and parasympathetic functions, respectively. The relationship between cardiac autonomic function and important disease characteristics in terms of disease activity and important manifestations was also studied.

Methods

Study population

This cross-sectional, case–control study was conducted on 40 children and adolescents with rheumatic diseases (SLE and JIA), over a period of 1 year from the beginning of April 2007 to the end of March 2008. Patients were recruited from the Pediatric Allergy and Immunology Unit, Children’s Hospital of Ain Shams University, Cairo, Egypt. The local Ethical Committee of the Faculty of Medicine, Ain Shams University, approved this study. In addition, an informed written consent was signed by the parents or the caregivers of each studied subject before enrolment in the study. Children and adolescents also gave a verbal consent in the study.

SLE group. It comprised of 20 patients fulfilling the American Rheumatism Association Revised Criteria for diagnosis of SLE [15]. Their ages ranged between 8 and 16 years.

JIA group. It included 20 patients classified according to the ILAR into polyarticular (n = 12), oligoartritic (n = 5) and
systemic onset \((n=3)\) JIA patients [16]. Their ages ranged between 7 and 16 years. Eleven patients had an active disease.

Patients were excluded from the study if they had associated problems known to affect the results of cardiac autonomic function such as medical diseases (e.g. diabetes mellitus and heart failure), symptoms suggesting myocardial ischaemia and an evidence of cardiac arrhythmia documented by ECG recording.

Control group. It included 40 age- and sex-matched apparently healthy children and adolescents who had no clinical evidence of cardiac or systemic diseases. Control children were recruited from the General Outpatients Clinic, Children’s Hospital, Faculty of Medicine, Ain Shams University, Cairo, Egypt. They were the apparently healthy sibs of the children attending this clinic because of a minor illness (e.g. common cold, tonsillitis, acute bronchitis, etc.). Their ages ranged between 7 and 16 years.

Study measurements

Clinical evaluation of the patients. This was based on clinical history taking from caregivers, reviewing follow-up sheets and clinical examination. Special emphasis was done on:

- symptoms suggesting CAN which include: palpitation, exercise intolerance and symptoms suggesting orthostatic hypotension (e.g. postural weakness, faintness, dizziness, visual impairment and syncope) by using a standard questionnaire.
- Disease duration and current medications used by the patients. The cumulative dose of steroid therapy during the whole duration of the disease was calculated from the patient’s records. The dose of intravenous pulse steroids was also added. The average dose of steroids used per square meter body surface area was calculated.
- Disease activity was assessed by using SLEDAI [17], in lupus patients and the ACR paediatric score [18, 19] in JIA patients.
- Clinical assessment of cardiac autonomic function of all studied subjects, in an identical fashion, using five AFTs [20] which include:
  - Resting HR (RHR), which was determined after the patient was supine for 15 min. Rate in excess of 100 beats/min was considered abnormal.
  - HR variability (HRV) during inspiration and expiration: the patient was asked to sit quietly and then to breath deeply and evenly at 6 breaths/min. The ECG was recorded during the manoeuvre and HR was monitored. The difference between the highest HR recorded during deep inspiration and lowest HR recorded during deep expiration was measured per cycle.
  - HR response to Valsalva manoeuvre: the patient was asked to strain for 15 s. The ECG was continuously recorded during the manoeuvre and for the following 30 s. The Valsalva ratio was calculated as the ratio between longest RR interval after the manoeuvre to the shortest RR interval during straining. The test was carried out twice and the average value from the two results was calculated.
  - The 30:15 ratio of HR response to standing: the patient was kept in lying position for 5 min. Then, he was asked to stand up quickly within 3–4 s. ECG registration started immediately as the patient got up from lying position and continued over a period of 60 s. The 30:15 ratio (the ratio between the RR interval of 30th beat and the 15th beat) was estimated. This test was carried out twice and the average value was determined.
  - Blood pressure response to standing: blood pressure was measured after the patient was asked to relax for 1–2 min. It was measured immediately after the patient stood up and at 1, 2 and 3 min intervals thereafter. The average of the four readings was calculated. The difference between the systolic reading at rest and the average systolic reading after standing was calculated. The same was also done for diastolic readings. Values of systolic blood pressure greater than \(-20\text{mmHg}\) and diastolic blood pressure greater than \(-5\text{mmHg}\) were considered abnormal. The autonomic nervous function was evaluated and given points according to the results of the previous five clinical tests. Normal test was given 0 point, borderline test was given 1 point and abnormal test was given 2 points. Then, the degree of autonomic dysfunction was determined according to the overall 10-point scale. Clinical evidence of CAN is diagnosed when the overall 10-point scale (the score of the five AFTs) is 2 or more, whether the patient had symptoms suggesting CAN or not [20]. A standardized format was applied to all tests. Therefore, the study was performed when the patient had no acute illness for the preceding 48 h, unaccustomed vigorous exercise for 24 h, no intake of food and caffeine for 8 h. Moreover, the study was performed in the morning in a quiet relaxed atmosphere and the patient was not wearing compressive clothing and was not emotionally upset [5, 20].

Measurement of serum NPY by radioimmunoaassay. This was carried out using an antisera raised against synthetic NPY conjugated to bovine thyroglobulin with 100% specificity to NPY (Euro-Diagnostica, Medeon, Malmo, Sweden) [21].

Measurement of serum VIP by radioimmunooassay. This was carried out using rabbit antibodies to a VIP–albumin conjugate with 100% specificity to VIP (JBL-America, Minneapolis, MN, USA) [22]. Collection of blood samples for NPY and VIP assay was done in the morning. As data distribution was non-parametric, serum NPY and VIP were considered to be low if they were below the 5th percentile values of healthy controls (18 pmol/l for NPY and 16 pmol/l for VIP).

Routine investigations of SLE and JIA were conducted including ESR per first hour, routine microscopic urine analysis, 24 h urine proteins, serum creatinine, corrected creatinine clearance, serum C3, ANA and anti-dsDNA for lupus patients. ESR per first hour, classic IgM RF and radiographic evaluation of the joints for scoring of soft tissue swelling, joint space narrowing, osteoporosis and erosions according to modified Larsen scoring methods [23] to calculate a mean score of all joints for JIA patients.

Statistical analysis

The results were analysed by commercially available software package (Statview, Abacus Concepts, Berkeley, CA, USA). Data were presented as median and interquartile range (IQR), which is between the 25th and 75th percentiles. Mann–Whitney test was used for comparison between non-parametric data. Spearman’s correlation coefficient \(r\) was used to determine the relationship between different quantitative variables. Chi-square test was used for the comparison between qualitative variables. Logistic regression analysis was used to find the significant independent clinical predictors for the clinical evidence of CAN (i.e. the score of the five AFTs is 2 or more). The chosen independent clinical predictors for CAN were the important studied clinical parameters. They included the duration of illness, the disease activity scores in addition to lupus nephritis and neuropsychiatric lupus in SLE patients and disease-onset type in JIA patients. All were put forward independent of their univariate associations with CAN. An enter-method selection approach was used to arrive at the final model. For all tests, a probability \(P < 0.05\) was considered significant. ROC (receiver operating characteristic) curve was used to calculate the sensitivity and specificity of serum NPY and VIP for the clinical evidence of CAN at their calculated best cut-off values.
Results
The present study included 20 lupus patients (17 females and 3 males), their ages ranged between 8 and 16 years with a mean (s.d.) age of 13.45 (2.7) years, and 20 JIA patients (15 females and 5 males), their ages ranged between 7 and 16 years with a mean age (s.d.) of 13.9 (2.15) years. The duration of illness of lupus patients ranged between 2 and 9 years [median (IQR): 3.5 (3–6) years] and the duration of illness of JIA patients ranged between 2 and 11 years [median (IQR): 8 (5–9) years]. A control group of 40 children and adolescents (33 females and 7 males) were also included in the study, their ages ranged between 7 and 16 years with a mean age (s.d.) of 13.75 (2.69) years. All groups were matched as regards age and sex. All lupus patients were receiving oral steroids (0.5–2 mg/kg/day) either alone (n = 7) or in combination with cytotoxic therapy such as intravenous pulse cyclophosphamide or oral AZA (n = 13). The duration of cyclophosphamide or AZA intake ranged between 2 and 3 years with a mean duration (s.d.) of 2.3 (0.7) years. All JIA patients were receiving at least one of the NSAIDs either alone (n = 4) or in combination with steroids and/or MTX (n = 16). The cumulative dose of steroids ranged between 450 and 495 40 mg/m² [median (IQR): 4716.2 (4002.1–20263.4) mg/m²] in lupus patients and ranged between 120 and 9325 mg/m² [median (IQR): 3455 (1044.2–2600.2) mg/m²] in JIA patients. There was a significant positive correlation between the cumulative dose of steroids and the duration of illness in lupus patients (r = 0.56; P = 0.01) and in JIA patients (r = 0.6; P = 0.004). A significant positive correlation was also found between the duration of cytotoxic therapy and the duration of illness in lupus patients (r = 0.75; P < 0.001).

Clinical evidence of CAN (i.e., the score of the five AFTs is 2 or more) was found in 65% (13/20) of lupus patients (5% had mild dysfunction, 10% had definite dysfunction and 50% had severe dysfunction), in 40% (8/20) of JIA patients (10% had mild dysfunction, 10% had definite dysfunction and 50% had severe dysfunction), in 40% (8/20) of lupus patients (10% had mild, 15% had definite and 50% had severe dysfunctions) and in none of healthy controls. HRV during inspiration and expiration was affected in all lupus and JIA patients with clinical evidence of CAN (Table 1).

Table 1. Results of the five cardiovascular AFTs of the patients with rheumatic diseases and healthy controls

<table>
<thead>
<tr>
<th>AFTs</th>
<th>Lupus patients</th>
<th>JIA patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHR, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>12 (60)</td>
<td>17 (85)</td>
<td>40 (25)</td>
</tr>
<tr>
<td>Affected (≥100 beats/min)</td>
<td>8 (40)</td>
<td>3 (15)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>HR variability during inspiration and expiration, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (≥1.15 beats/min)</td>
<td>7 (35)</td>
<td>12 (60)</td>
<td>40 (25)</td>
</tr>
<tr>
<td>Affected (&lt;1.15 beats/min)</td>
<td>13 (65)</td>
<td>8 (40)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>HR response to Valsalva manoeuvre, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (≥1.3 ms)</td>
<td>8 (40)</td>
<td>17 (85)</td>
<td>40 (25)</td>
</tr>
<tr>
<td>Borderline (1.14–1.29 ms)</td>
<td>10 (50)</td>
<td>1 (5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Affected (&lt;1.15 ms)</td>
<td>2 (10)</td>
<td>2 (10)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>The 30:15 ratio of HR response to standing, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (≥1.07 ms)</td>
<td>6 (30)</td>
<td>10 (50)</td>
<td>34 (17)</td>
</tr>
<tr>
<td>Borderline (1.04–1.06 ms)</td>
<td>3 (15)</td>
<td>5 (25)</td>
<td>6 (15)</td>
</tr>
<tr>
<td>Affected (&lt;1.03 ms)</td>
<td>11 (55)</td>
<td>5 (25)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Blood pressure response to standing, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (SBP 10–20 mmHg, DBP &lt;5 mmHg)</td>
<td>10 (50)</td>
<td>16 (80)</td>
<td>36 (90)</td>
</tr>
<tr>
<td>Borderline (SBP 15–20 mmHg, DBP &lt;5 mmHg)</td>
<td>1 (5)</td>
<td>1 (5)</td>
<td>4 (10)</td>
</tr>
<tr>
<td>Affected (SBP &gt;20 mmHg, DBP &gt;5 mmHg)</td>
<td>9 (45)</td>
<td>3 (15)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Score of the five AFTs, median (IQR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal autonomic function (0–1), n (%)</td>
<td>6 (1–8)</td>
<td>1 (0–4.8)</td>
<td>0 (0–1)</td>
</tr>
<tr>
<td>Mild autonomic neuropathy (2–3), n (%)</td>
<td>7 (35)</td>
<td>12 (60)</td>
<td>40 (25)</td>
</tr>
<tr>
<td>Moderate autonomic neuropathy (4–6), n (%)</td>
<td>1 (5)</td>
<td>2 (10)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Definite autonomic neuropathy (≥7), n (%)</td>
<td>2 (10)</td>
<td>3 (15)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Severe autonomic neuropathy (≥10), n (%)</td>
<td>10 (50)</td>
<td>5 (25)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

DBP: diastolic blood pressure; SBP: systolic blood pressure.
Symptoms suggesting CAN (palpitation, exercise intolerance and symptoms suggesting orthostatic hypotension such as postural weakness, faintness, dizziness, visual impairment and syncope) were found in 40% of lupus patients, in 30% of JIA patients and in none of healthy controls. Interestingly, clinical evidence of CAN was found in 41.7% and 14.3% of lupus and JIA patients, respectively, who had no suggestive symptoms of CAN.

Although lupus patients had higher frequency of low serum VIP and NPY (80 and 65%, respectively) than JIA patients (60 and 50%, respectively), this difference did not reach statistical significance ($P = 0.16$ and $P = 0.93$, respectively).

Lupus and JIA patients with clinical evidence of CAN had significantly lower serum NPY and VIP than patients without (Table 2). The former groups had significantly higher frequency of low serum VIP (100% for both) than the latter groups (43 and 33.3%, respectively). Although the former groups had higher frequency of low serum NPY (77 and 62.5%, respectively) than the latter groups (43 and 41.7%, respectively), this difference did not reach statistical significance ($P = 0.013$ and $P = 0.36$, respectively). Serum VIP had higher sensitivity and specificity for CAN than serum NPY at their calculated best cut-off value using ROC curve (Table 3).

The five AFTs score had significant negative correlations to NPY and VIP in lupus ($r = 0.81$, $P < 0.001$ and $r = 0.8$, $P < 0.001$, respectively) and JIA patients ($r = 0.79$, $P < 0.001$ and $r = 0.95$, $P < 0.001$, respectively). In addition, there were significant positive correlations between NPY and VIP in lupus ($r = 0.82$, $P < 0.001$) and JIA patients ($r = 0.8$, $P < 0.001$).

Clinical evidence of lupus nephritis [24], neuropsychiatric manifestations [25] and cutaneous vasculitis were found in 60, 50 and 45%, respectively, of lupus patients. Patients with lupus nephritis had significantly higher five AFTs score than patients without [median (IQR): 8 (3–6.5) and 1 (1–5), respectively, $P = 0.008$]. The former group had significantly lower serum NPY and VIP [median (IQR): 10.5 (10–13.3) and 9 (9–10.8) pmol/l, respectively] than the latter group [median (IQR): 19.5 (10–19.8) and 16 (5–19) pmol/l, respectively], $P = 0.001$ and $P = 0.003$, respectively. On the other hand, patients with and without NPSLE had comparable five AFTs scores ($P = 0.09$). Patients with NPSLE had significantly lower serum NPY and VIP [median (IQR): 10 (3–7.8) and 9 (9.5–12) pmol/l, respectively] than patients without [median (IQR): 19 (5–18) and 14 (13.5–26.8) pmol/l, respectively], $P = 0.09$ and $P = 0.002$, respectively. In contrast, patients with and without cutaneous vasculitis had comparable results of the five AFTs score, serum NPY and VIP ($P = 0.25$, 0.33 and 0.38, respectively).

Ten lupus patients were receiving anti-hypertensive treatment (β-blockers, calcium channel blockers and renin angiotensin system inhibitors). Lupus patients who were receiving and those who were not receiving anti-hypertensive therapy had comparable results of the five AFTs score and serum NPY and VIP ($P = 0.45$, 0.56 and 0.68, respectively).

In lupus patients, SLEDAI, cumulative steroid dose and anti-dsDNA had significant positive correlations to the five AFTs score ($r = 0.96, P < 0.001$; $r = 0.5$, $P = 0.03$; and $r = 0.93$, $P < 0.001$, respectively) and significant negative correlations to NPY ($r = 0.85, P < 0.001$; $r = 0.66$, $P = 0.001$; and $r = 0.83$, $P < 0.001$, respectively) and VIP ($r = 0.88, P < 0.001$; $r = 0.55$, $P = 0.01$; and $r = 0.83$, $P < 0.001$, respectively). Disease duration correlated positively to the total score of the five AFTs ($r = 0.47, P = 0.03$) and negatively to VIP ($r = 0.55, P = 0.008$).

In JIA patients, ACR paediatric score, cumulative steroid dose and Larsen score correlated positively to the five AFTs score ($r = 0.76, P < 0.001$; $r = 0.62$, $P = 0.004$; and $r = 0.84$, $P < 0.001$, respectively) and negatively to NPY ($r = 0.8$, $P < 0.001$; $r = 0.77$, $P < 0.001$; and $r = 0.77$, $P < 0.001$, respectively) and VIP ($r = 0.88$, $P < 0.001$; $r = 0.57$, $P = 0.009$; and $r = 0.83$, $P < 0.001$, respectively). Disease duration did not correlate significantly to the five AFTs score, NPY or to VIP ($r = 0.38$, 0.64 and 0.36, respectively).

Logistic regression analysis showed that SLEDAI and disease duration were the only significant clinical predictors of CAN in lupus patients ($P < 0.001$ and $P = 0.03$, respectively). In contrast, no significant clinical predictors of CAN were found in JIA patients.

**Discussion**

The cross-talk between the brain and immune system in inflammatory arthritis is exerted mainly through the activation or down-regulation of the hypothalamic-ANS, hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes [7].

In the present study, CAN, diagnosed by abnormal results of the five AFTs, was found in 65 and 40% of lupus and JIA patients, respectively. Researches on cardiac autonomic function in patients with rheumatic diseases are limited. Abnormal results of cardiac AFTs were previously reported in 44.7% [26] and 48% [3] of adult lupus patients and in 20% of adult patients with RA [27]. We could not trace data in literature regarding cardiac autonomic function in paediatric patients with rheumatic diseases to compare our results.

In our series, the results of the five AFTs denoted that all patients with CAN had parasympathetic dysfunction as evidenced by abnormality in HRV during inspiration and expiration in all of them. On the other hand, some of these patients had also sympathetic dysfunction as evidenced by the abnormal results of the other four AFTs that are mediated by sympathetic nervous system either alone (postural blood pressure control) or with parasympathetic nervous system as well (RHR, HR response to Valsalva manoeuvre and the 30:15 ratio of HR response to standing).

These results could be explained by the fact that in CAN, both sympathetic and parasympathetic activities of the heart begin to decline [28]. However, the parasympathetic activity declines first and more rapidly resulting in an imbalance between the two arms of ANS. The relative increase in sympathetic activity would result in an increase in the HR. The subsequent progressive impairment of sympathetic nervous system gradually slows the HR. Finally, both sympathetic and parasympathetic functions are maximally impaired. Thus, CAN is not simply an all-or-none phenomenon and ranges from minor to severe [29].

---

**Table 2.** Comparison between serum levels of autonomic neuromodulators of lupus and JIA patients with and without clinical evidence of CAN

<table>
<thead>
<tr>
<th>Studied patients</th>
<th>Median NPY, pmol/l</th>
<th>Serum NPY, pmol/l</th>
<th>Median VIP, pmol/l</th>
<th>Serum VIP, pmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lupus patients with CAN</td>
<td>10 (10–15)</td>
<td>2.9</td>
<td>9 (8–10)</td>
<td>3.3</td>
</tr>
<tr>
<td>Lupus patients without CAN</td>
<td>20 (16–30)</td>
<td>(0.004)</td>
<td>18 (13–29)</td>
<td>(0.001)</td>
</tr>
<tr>
<td>JIA patients with CAN</td>
<td>9 (11.3–19)</td>
<td>2.1</td>
<td>12 (10.3–13)</td>
<td>3.5</td>
</tr>
<tr>
<td>JIA patients without CAN</td>
<td>18 (16.3–30.8)</td>
<td>(0.04)</td>
<td>27.5 (14–29.5)</td>
<td>(0.001)</td>
</tr>
</tbody>
</table>

**Table 3.** Sensitivity and specificity of serum NPY and VIP for the clinical evidence of CAN at their calculated best cut-off values using ROC curve

<table>
<thead>
<tr>
<th>The studied laboratory marker of CAN</th>
<th>AUC*</th>
<th>The best cut-off value, pmol/l</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum NPY</td>
<td>0.85</td>
<td>16.5</td>
<td>61.9</td>
<td>73.7</td>
</tr>
<tr>
<td>Serum VIP</td>
<td>0.95</td>
<td>13.5</td>
<td>85.7</td>
<td>84.2</td>
</tr>
</tbody>
</table>

*Area under the curve. AUC < 0.05 means that the studied marker is able to differentiate the presence from the absence of CAN. The closer this area to 1, the better its differentiating ability is. The best cut-off value is based on the highest sensitivity with the lowest false positive results.
In the present study, serum NPY, a marker of sympathetic function, and VIP, a marker of parasympathetic function, were significantly lower in lupus and JIA patients than controls. These findings, together with the results of the five AFTs, may denote hypofunction of both divisions of ANS in lupus and JIA patients. Other studies also demonstrated sympathetic hypofunction, as evidenced by low serum catecholamines, in lupus patients [3].

We are the first to study an autonomic neuropeptide (VIP) as an indicator of parasympathetic function in these patients.

In our series, serum levels of NPY and VIP were significantly lower in lupus and JIA patients with clinical evidence of CAN than patients without such evidence. These results together with our finding of significant negative correlations between the score of the five AFTs and both NPY and VIP might denote that serum NPY and VIP could be valuable laboratory markers of sympathetic and parasympathetic functions, respectively, in these patients.

The current study revealed that all lupus and JIA patients with clinical evidence of CAN had low serum VIP. On the other hand, 77 and 62.5% of lupus and JIA patients, respectively, had low serum NPY. Moreover, serum VIP had higher sensitivity and specificity for CAN (85.7 and 84.2%, respectively) than serum NPY (61.9 and 73.7%, respectively) at its calculated best cut-off value. The previous findings support the results of the five AFTs that also denoted a more evident parasympathetic than sympathetic dysfunction in patients with rheumatic diseases, with parasympathetic dysfunction in all and sympathetic dysfunction in many, but not all, patients with CAN. On the other hand, our finding of a significant positive correlation between serum NPY and VIP might denote the close association of both sympathetic and parasympathetic dysfunction in these patients.

It is worth mentioning that the absence of clinical evidence of autonomic dysfunction does not exclude the presence of CAN. This is evident from our finding of low serum levels of NPY and VIP in some lupus and JIA patients who did not have clinical evidence of CAN. Thus, assessment of serum levels of these autonomic neuropeptides is recommended in lupus and JIA patients even in the absence of clinical evidence of CAN.

The pathogenesis of ANS dysfunction in rheumatic diseases is poorly understood. The contribution of a direct immunological damage to components of neural pathways can be postulated. This assumption was supported by the demonstration of circulating complement fixating autoantibodies directed against sympathetic and parasympathetic nervous structures, represented by superior cervical ganglia and vagus nerve, respectively, in patients with SLE and RA. There was a significant positive association between AFTs score and the presence of these antibodies [30]. Furthermore, catalytic autoantibodies to VIP were demonstrated in patients with autoimmune diseases [31].

In the present study, 38 and 25% of lupus and JIA patients, respectively, with clinical evidence of CAN had no suggestive symptoms. Thus, subclinical involvement of ANS can occur without evident neuropathy. For this reason, evaluation of cardiac autonomic function is mandatory, even in the absence of suggestive symptoms, in these patients.

In this work, we tried to study lupus patients who were not receiving anti-hypertensive treatment, but we found only 10 non-hypertensive lupus patients during the study period. Thus, it was unavoidable that 10 lupus patients were receiving anti-hypertensive treatment (B-blockers, calcium channel blockers and renin angiotensin system inhibitors), which may influence the autonomic function. Lupus patients who were receiving and those patients who were not receiving anti-hypertensive therapy had comparable results of the five AFTs score and serum NPY and VIP (P > 0.05). Thus, fortunately, the use of anti-hypertensive therapy by 50% of lupus patients did not skew the results of the autonomic function.

In our series, AFTs score had significant positive correlations with the disease activity scores in lupus and JIA patients, with serum anti-dsDNA titre in lupus patients and with Larsen score in JIA patients. In addition, SLEDAI and disease duration were significant clinical predictors of CAN in lupus patients. These results may support the previous findings of the contribution of the disturbance in the interaction between the ANS and the immune system to the pathogenesis of rheumatic diseases [6-8].

A dysfunctional communication between the sympathetic nervous system and the immune system was reported in Th1-mediated autoimmune diseases [11]. Reduction of β-2 receptor densities on B lymphocytes, mirrored by an impaired intracellular cAMP generation in patients with chronic rheumatic diseases, indicate a decreased influence of ANS on B cells in these conditions. In activated macrophages, VIP inhibits the expression of pro-inflammatory cytokines through effects on de novo expression or nuclear translocation of some transcription factors. In addition, VIP promotes Th2-type and inhibits Th1-type responses, through several mechanisms, including preferential survival of Th2 effectors and subsequent generation of Th2 memory cells. Thus, VIP may be beneficial in the treatment of Th1-mediated autoimmune diseases [12, 13].

The concept of treating patients with asymptomatic CAN may not appeal to some physicians, particularly in light of the fact that there are no outcome data from clinical trials yet [32]. Anti-oxidants may ameliorate CAN. Similar to rheumatic diseases, type 2 diabetes mellitus is associated with an increase in oxidative stress and a decline in anti-oxidant defence. These diseases are also characterized by an imbalance in the ratio of cardiac sympathetic to parasympathetic tone. Anti-oxidants, vitamin E in particular, have been shown to improve the ratio of cardiac sympathetic to parasympathetic tone for persons with type 2 diabetes. Such an effect might be mediated by a decline in oxidative stress [33]. Endurance training is a non-pharmacological intervention of CAN that induces a high parasympathetic tone [34]. We could not trace data in literature concerning the treatment of patients with chronic rheumatic diseases who have CAN. In view of the previous data, we supplied symptomatic patients with CAN with oral vitamin E (100 mg once daily).

In addition, we advised them to do endurance training. The five AFTs will be repeated after 6 months for cooperative patients. Patients with resting tachycardia according to age standards were treated with B-blockers. Furthermore, we advised these patients to do the non-pharmacological measures to reduce the symptoms of orthostatic hypotension such as increasing the consumption of water [35] and wearing lower extremity stockings [36]. Asymptomatic patients will be closely followed up clinically and the AFTs will be repeated every 6 months for detection of progression.

Cardiovascular autonomic function testing may be an important component in the risk assessment of coronary artery disease [37] due to its frequent association with microvascular disease resulting in myocardial hypoperfusion. Furthermore, in rheumatic patients with CAN reduced parasympathetic function or increased sympathetic activity may provide the propensity for lethal arrhythmias and increased cardiovascular risk [2]. In view of these data, our rheumatic patients with CAN will be followed up clinically and instructed to report immediately when they have chest pain. In addition, frequent ECG and exercise ECG every 6 months will be done for these patients to detect myocardial hypoperfusion before the occurrence of myocardial infarction because painless myocardial ischaemia is one of the clinical manifestations of CAN. Frequent 24-h ECG monitoring will be also performed to detect arrhythmias with subsequent treatment of these arrhythmias.

In conclusion, CAN is a common occurrence in lupus and JIA patients, even in the absence of relevant symptoms. Thus, assessment of autonomic function in these patients, by AFTs
and serum autonomic neuropeptides (NPY and VIP), is recommended. CAN correlated positively to the disease activity and some of its important manifestations. Thus, CAN may play a role in the pathophysiology of these diseases. It is quite promising to follow this avenue in investigating ANS–immune interactions at various time points of rheumatic diseases, and to study the effect of VIP and NPY, as new therapeutic tools, on amelioration of the manifestations and severity of these diseases.

**Rheumatology key messages**

- CAN is common in lupus and JIA patients, even in the absence of relevant symptoms.
- The pressor role of cardiac autonomic function is mandatory in lupus and JIA patients.
- AFTs and serum autonomic neuropeptides can be helpful in this regard.

**Disclosure statement:** The authors have declared no conflicts of interest.

**References**

11. Bedoui S, Miyake S, Straub RH, von Horsten S, Yamamura T. More sympathy of the pressor role of cardiac autonomic function is mandatory in lupus and JIA patients. AFTs and serum autonomic neuropeptides can be helpful in this regard.