LIMITS OF CLINICAL TESTS TO SCREEN AUTONOMIC FUNCTION IN DIABETES TYPE 1

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SUMMARY - Objectives: A precocious detection of cardiac autonomic dysfunction is of major clinical interest that could lead to a more intensive supervision of diabetic patients. However, classical clinical exploration of cardiac autonomic function is not easy to undertake in a reproducible way. Thus, respective interests of autonomic nervous parameters provided by both clinical tests and computerized analysis of resting blood pressure were checked in type 1 diabetic patients without orthostatic hypotension and microalbuminuria.

Material and methods: Thirteen diabetic subjects matched for age and gender to thirteen healthy subjects volunteered to participate in the study. From clinical tests (standing up, deep breathing, Valsalva maneuver, handgrip test), autonomic function was scored according to Ewing’s methodology. Analysis of resting beat to beat blood pressure provided autonomic indices of the cardiac function (spectral analysis or Z analysis).

Results: 5 of the 13 diabetic patients exhibited a pathological score (more than one pathological response) suggesting the presence of cardiovascular autonomic dysfunction. The most discriminative test was the deep breathing test. However, spectral indices of BP recordings and baroreflex sensitivity (BRS) of these 5 subjects were similar to those of healthy subjects and of remaining diabetic subjects.

Conclusion: Alteration in Ewing’s score given by clinical tests may not reflect an alteration of cardiac autonomic function in asymptomatic type 1 diabetic patients, because spectral indices of sympathetic and parasympathetic (including BRS) function were within normal range. Our results strongly suggest to confront results provided by both methodologies before concluding to an autonomic cardiac impairment in asymptomatic diabetic patients.

Key-words: autonomic failure, diabetes, Ewing’s test, microalbuminuria, cardiac baroreflex.

RESUME - Limitations dans les tests cliniques de dépistage de la dysautonomie chez le diabétique de type 1.


Matériel et méthodes : Treize patients diabétiques ont été appariés pour l’âge et le sexe à treize volontaires sains. Lors des tests cliniques (lever actif, respiration ample, manœuvre de Valsalva, handgrip), un score de dysautonomie a été obtenu selon la méthodologie décrite par Ewing. L’analyse de la variabilité tensionelle instantanée (analyse spectrale, analyse Z) a fourni des indices de fonction autonome cardiaque.

Résultats : Un score pathologique (plus d’un test pathologique) a été ob- serve chez cinq des 13 patients diabétiques suggérant une atteinte auto- nomique cardiaque. Le test le plus souvent pathologique a été le test de respiration ample. À l’inverse, les indices spectraux de la variabilité de la pression artérielle et le baroréflexe cardiaque spontané de ces 5 sujets n’étaient pas différents ni de ceux des autres patients diabétique, ni de ceux des volontaires sains.

Conclusion : Un score d’Ewing pathologique obtenu lors des tests clini- ques peut ne pas refléter une dysautonomie cardiaque chez les patients diabétiques insulino-dépendant asymptomatiques dont les indices sympa-thétiques et parasymphétique issus de l’analyse de leur variabilité ten- sionnelle sont normaux. Avant de conclure à une atteinte débutante et intraclinique du système nerveux autonome, il est souhaitable de confronter les résultats obtenus à partir des 2 méthodologies.

Mots-clés : dysautonomie, test d’Ewing, microalbuminurie, baro- reflexe cardiaque.

Cardiac autonomic nervous system dysfunction [1-3] have been reported to alter the prognosis of diabetes mellitus. Recently, we reported [4] that cardiovascular diabetic neuropathy could be reliably assessed during a beat-to-beat blood pressure (BP) recording in resting and dynamic conditions. In our study [4], well-established tests stimulating the cardiovascular autonomic system (scored according to Ewing methodology) [5, 6] were correlated with mathematical methods used to measure the baroreflex sensitivity (BRS) in order to quantify cardiac dysautonomia in established neuropathy. Among them, spectral and Z analyses were the most sensitive methods. The present study aimed to evaluate the respective contribution of clinical tests and computed beat to beat analysis to describe cardiac autonomic function in type 1 diabetics without any orthostatic hypotension. Such an extensive methodology can distinguish sympathetic and parasympathetic pathways impairment. Results obtained in diabetic subjects having low risk for autonomic neuropathy were compared to those obtained in healthy volunteers matched for age and gender.

# Research Design and Methods

## Subjects

Thirteen (6 women and 7 men, age 21-53 years) lean (BMI < 23 kg/m²) normotensive patients with type 1 diabetes (age 20-55 years) evolving for 10.8 ± 1.6 years were selected on:

1. orthostatic systolic BP (oscillometric method) decrease less than 15 mm Hg,
2. albuminuria below 20 mg/l on two consecutive urine samples.

All patients were on a normocaloric diet and intensive insulin therapy. None of diabetic patient had a retinopathy and none received any medication known to interfere with BP control. Thirteen healthy volunteers matched for age (within 2 years) and gender were included if their BP was below 140/90 mmHg and if their biological parameters (serum glucose, complete blood and platelet counts, liver enzymes, serum creatinine and dipstick urinalysis) were within normal limits.

Healthy subjects were recruited by advertisement in a local journal and none of them was involved in medical fields. The protocol was approved by the Lyons Ethics Committee and written informed consent was obtained from each subject.

## BP and HR Measurements

At the selection visit, BP was measured three times after 5 min resting in a recline position using an oscillometric method (dynamap®, Criticon, USA). The average of the last two (of three) BP measurements was considered as lying BP. Orthostatic BP variation was the difference between lying BP and the lowest of 3 BP determinations at 1, 3, 5 min after an active standing up.

Cardiac autonomic function was evaluated 2 hours after a light breakfast before which the usual morning insulin dose was injected. During tests, BP was recorded using a Finapres® device (model 2300, Ohmeda, Englewood, CO) with the cuffed finger held in the mid-axillary position at heart level throughout the procedure. After ten minutes of familiarization, the automatic calibration was switched off, and BP was recorded for 1 h.

The accuracy of a 1-h BP Finapres recording at rest has been assessed in comparison to an intra-arterial recording [7]. Respiration rate was controlled at 0.3 Hz using a metronome. Before each test, the Finapres was calibrated to obtain a satisfactory BP level (less than 5 mmHg difference compared to a sphygmomanometer determination). Signal acquisition and data processing were previously described [8].

## Clinical Tests

The five conventional autonomic function tests (standing, Valsalva maneuver, deep breathing, handgrip) were performed as described by Ewing et al. [6] and adapted to the use of the Finapres device. Normal values were not adjusted for age as recommended by Low et al. [9] because authors did not give normative values for 15/30 ratio and for diastolic blood pressure (dBP) increase during the handgrip test. Therefore, we used normative values provided by Ewing in his original paper [6] to assign scores as follows: 0 for a normal test, 0.5 for a borderline and 1 for an abnormal value (Table I). The sum of the 5 scores was defined as the Ewing’s score. The threshold for a pathological Ewing’s score was 1.5.

<table>
<thead>
<tr>
<th>Table I. Main characteristics of the two groups.</th>
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<tbody>
<tr>
<td>Healthy</td>
</tr>
<tr>
<td>Age (Years)</td>
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<tr>
<td>Diabetes duration (years)</td>
</tr>
<tr>
<td>Haemoglobin A1c (%)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>sBP (mm Hg)</td>
</tr>
<tr>
<td>dBP (mm Hg)</td>
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<tr>
<td>HR (beats/min)</td>
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</table>

Data are means ± SEM.
Spectral analysis

Spectral analysis was performed according to our previously described technique adapted to humans [8]. Spectra were computed using a fast Fourier transform analysis on 50 percent overlapping stationary segments of 512 points re-sampled every 0.7 seconds. Spectral power was computed within each band defined as follows: a low frequency band or Mayer’s band (LF: 0.07 to 0.14 Hz) and a high frequency band (HF: 0.25 to 0.35 Hz).

The strength of the linear coupling between sBP and HR in the LF band was expressed by mean of the coherence function. The average modulus of the transfer functions were taken as indices of BRS if > 0.5.

Z analysis

Statistical methods are powerful tools to examine relationships between two variables. Our statistical approach allows the computation of a coefficient of dependence between two events which we call the “Z coefficient” [10]. The strength of the dependence between sBP and HR is calculated as follows:

\[ Z(sBP, HR) = \frac{P(sBP, HR) - P(sBP) \times P(HR)}{\min(P(sBP) \times P(HR))} \]

where:

- \( P(sBP) \) and \( P(HR) \) are the probabilities of observing sBP and HR respectively. Probabilities of events are assessed by the calculation of the ratio between their frequencies to the total number of their observations,
- \( P(sBP, HR) \) is the joint probability of observing both sBP and HR together,
- \( \min(P(sBP) \times P(HR)) \) is the smallest value of probability calculated for either sBP or HR.

If observing sBP is always observed when HR is observed (sBP is totally dependent upon HR), \( P(sBP, HR) = P(sBP) \) (in that case, \( P(sBP) \) is necessarily less than \( P(HR) \)). Then, in case of total dependence of sBP event to HR event, the Z coefficient is equal to 1.

If sBP and HR events are independent, \( P(sBP) \times P(HR) \) is equal to \( P(sBP, HR) \) and the Z coefficient is equal to zero, because numerator is equal to zero.

The stronger the dependence between sBP and HR is, the more closely the Z coefficient approximates 1.

As previously described [10], the data were pooled in intervals of 3 mm Hg for sBP and of 2 beats/min for HR. Only (sBP, HR) couples with occurrence greater than 10 were taken into account. The modal class of the bivariate (sBP, HR) distribution was determined and (sBP, HR) couples were compared to the modal class to reflect the baroreflex activity.

The slope of the linear regression between these low sBPs and high HRs (or vice versa) was reported to a reliable index of the BRS if the regression coefficient was > 0.5.

Statistical analysis

All averaged data are expressed as mean ± SE. Because normal distribution cannot be reliably checked for 13 values, results in healthy and diabetic subjects were compared using the non parametric Mann and Withney’s test. Correlations between parameters used a Spearman’s rank test. Results were considered as significant when \( P < 0.05 \).

RESULTS

Main characteristics of the two groups were similar as shown in Table I.

Clinical tests

None of the healthy subjects exhibited a pathological score. However, in healthy subjects, borderline or pathological responses were observed during deep-breathing (3 and 2 respectively) and handgrip test (zero and 1 respectively). In diabetic patients, Ewing’s scores ranged from 0 to 3. Five diabetic patients had a pathological Ewing’s score ≥ 1.5. Average values of indices of the autonomic activity and number of pathological response of each bedside test are given in Table II.

Analyses of sBP and HR variabilities

Spectral powers in LF-sBP (1.84 ± 0.23 vs 1.63 ± 0.23 mm Hg²), LF-HR (2.03 ± 0.32 vs 1.27 ± 0.30 bpm²), HF-sBP (0.82 ± 0.24 vs 0.54 ± 0.10 mm Hg²), and HF-HR (1.64 ± 0.34 vs 1.56 ± 0.66 bpm²), were not different between diabetic and healthy subjects respectively. The BRS did not differ between diabetics and healthy subjects when determined either by the spectral method (15.3 ± 1.5 vs 12.6 ± 0.9 ms/mm Hg respectively) or by the Z analysis (11.2 ± 1.9 vs 9.7 ± 1.5 ms/mm Hg respectively).

Indices of BP variabilities and BRS were not different between the 5 diabetic patients with pathological Ewing’s scores compared to the 8 remaining diabetic patients and to healthy subjects (Fig. 1).

DISCUSSION

In our study, clinical autonomic tests are in discrepancies with indices of BP variability. Clinical tests found that 5 diabetic patients had a pathological Ewing’s score while their indices of BP variability and BRS were similar to the one observed in matched healthy volunteers. It is usually accepted that physiologic responses resulting in an increase in BP (handgrip) and in HR (up-right posture) are related to pe-
The BRS is also mainly determined by the parasympathetic activity [18]. Since the methods used to determine the BRS have already been reported to be sensitive enough to detect small impairments in BRS [19] and to be reproducible in humans [8] if used in standardized and controlled conditions [20], the preserved BRS in diabetics suggests that the cardiac parasympathetic component of the autonomic nervous system is not altered in our subjects [21]. The concordant results obtained in BRS determination by the two methods (spectral and Z analysis) reinforced the confidence in our results. Bed-side tests which depend on individual cooperation should be interpreted with caution before concluding to an alteration of cardiac autonomic function. For instance, although not recorded, a reduced breathing amplitude could explain the lower HR variations in diabetics during deep breathing which frequency was paced by a metronome. Furthermore, during the Valsalva maneuver, that has been reported to be the most reproducible test, HR responses were strictly equivalent in diabetics compared to healthy volunteer (1.47 ± 0.07 vs 1.49 ± 0.06 respectively).

Handgrip diastolic BP increase was also impaired in 3 diabetic patients. The handgrip test has long been used to investigate sympathetic pathways in diabetic patients [11]. However, factors involved in the hemodynamic response to handgrip have not been thoroughly established. A lower increase in DBP during handgrip in diabetics (18 ± 3 mmHg in healthy subjects) could suggest a sympathetic alteration. However, since none of our diabetic patients exhibited an orthostatic hypotension and since sympa-

<table>
<thead>
<tr>
<th></th>
<th>Normal Values</th>
<th>Borderline Values</th>
<th>Healthy (n = 13)</th>
<th>Diabetics (n = 13)</th>
</tr>
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<tbody>
<tr>
<td>Standing up</td>
<td></td>
<td></td>
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<tr>
<td>– Δ sBP</td>
<td>≤ 10</td>
<td>11-29</td>
<td>– 5.2 ± 1.8</td>
<td>– 3.8 ± 1.3</td>
</tr>
<tr>
<td>– pathological response</td>
<td></td>
<td></td>
<td>n = 0</td>
<td>n = 0</td>
</tr>
<tr>
<td>– 15/30 ratio</td>
<td>≥ 1.04</td>
<td>1.01-1.03</td>
<td>1.31 ± 0.0</td>
<td>1.34 ± 0.07</td>
</tr>
<tr>
<td>– pathological response</td>
<td></td>
<td></td>
<td>n = 0</td>
<td>n = 2</td>
</tr>
<tr>
<td>Deep breathing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>– HR min-max</td>
<td>≥ 15</td>
<td>11-14</td>
<td>15.3 ± 1.4</td>
<td>10.9 ± 1.8*</td>
</tr>
<tr>
<td>– pathological response</td>
<td></td>
<td></td>
<td>n = 2</td>
<td>n = 6</td>
</tr>
<tr>
<td>Valsalva maneuver</td>
<td></td>
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<tr>
<td>– HR min/max</td>
<td>≥ 1.21</td>
<td>1.11-1.20</td>
<td>1.49 ± 0.06</td>
<td>1.47 ± 0.07</td>
</tr>
<tr>
<td>– pathological response</td>
<td></td>
<td></td>
<td>n = 0</td>
<td>n = 0</td>
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<tr>
<td>Handgrip</td>
<td></td>
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<td></td>
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<tr>
<td>– Δ dBP</td>
<td>≥ 16</td>
<td>11-15</td>
<td>26.9 ± 4.3</td>
<td>17.8 ± 2.9*</td>
</tr>
<tr>
<td>– pathological response</td>
<td></td>
<td></td>
<td>n = 1</td>
<td>n = 3</td>
</tr>
<tr>
<td>Ewing’s score</td>
<td>0.35 ± 0.12</td>
<td></td>
<td>1.04 ± 0.29</td>
<td></td>
</tr>
<tr>
<td>Pathological response</td>
<td>n = 0</td>
<td></td>
<td>n = 0</td>
<td>n = 5</td>
</tr>
</tbody>
</table>

* p < 0.05 vs healthy subjects.

Data are means ± SEM.

Among clinical tests, the most differentiating one was the deep breathing test which found that 6 patients exhibited a pathological response. Abnormal HR variations during deep breathing in our patients could suggest a parasympathetic impairment in accordance with previously reported studies [11-16]. The HF spectral power (parasympathetically determined) [16, 17] that was included in the range determined in healthy subjects did not favor an alteration in parasympathetic pathways.

Table II. Ewing’s scores in healthy and diabetic subjects.

thetic fibers innervating lower limbs, that are longer should be altered at first [22], sympathetic pathways were probably preserved.

A primary alteration in afferent sensitive fibers may explain the reduced response to the handgrip test in our patients. Although not significant, the reduced maximum grip strength in diabetics (0.73 ± 0.05 vs 1.0 ± 0.09 bars in healthy volunteers) has to be taken into account. The strength-adjusted increase in DBP during handgrip that remains significant between groups favors the hypothesis of a peripheral sensitive alteration. The between groups similar Mayer’s band spectral power, that are in part sympathetically determined [17], conﬁrms the integrity of the sympathetic drive in our diabetics.

Finally, Ewing’s score clearly isolated (Fig. 1), 5 patients who exhibited a pathological response, all of them having a deep breathing pathological response. Although not matched, these 5 patients were compared to the remaining diabetic subjects and to healthy volunteers. The lack of difference in BP variability indices (LF and HF spectral power) and in BRS favors the integrity of both sympathetic and parasympathetic pathways in our diabetic patients.

In conclusion, alteration in autonomic clinical tests in asymptomatic type 1 diabetic patients may mislead to an alteration in cardiac autonomic function. Autonomic tests results may depend on patients’ cooperation and/or on peripheral sensitive neuropathy. If clinical tests are reliable to confirm and score a patent cardiac autonomic impairment, their lack of speciﬁcity may falsely evoke an emergent cardiac neuropathy. Since spectral indices of BP variability and BRS determination have been reported to be reliable and reproducible, our results strongly suggest that these indices should be an alternative to clinical tests to monitor cardiac autonomic function in diabetic subjects. At least, results provided by both methodologies.

Fig 1. Box plots representation of Ewing’s scores and indices of variabilities and baroreﬂexe sensitivity in healthy subjects (Control, n = 13), diabetics with (IDDM + ; n = 5) or without (IDDM; n = 8) pathological Ewing’s score.
Systolic blood pressure (SBP); Heart rate (HR); Spectral baroreﬂexe sensitivity (BRS); Z coefﬁcient (Z coef); Standart deviation (SD); Low frequency spectral power (LF); High frequency spectral power (HF).
should be confronted before concluding to an autonomic cardiac impairment in asymptomatic diabetic patients.

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