Reliability and Validity of Cardiovascular and Vasomotor Autonomic Function Tests

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OBJECTIVE — To determine the reliability and validity of autonomic function tests (AFTs) as clinical tools for diagnosing diabetic autonomic dysfunction.

RESEARCH DESIGN AND METHODS — Twenty-one healthy control subjects and 21 insulin-dependent diabetes mellitus (IDDM) patients (11 with no symptomatology and 10 with symptomatic diabetic autonomic neuropathy [DAN]) were matched for age, and administered three standard cardiovascular tests and two new vasomotor tests of autonomic function. Each of the cardiovascular tests (change in heart rate [Δbpm], Valsalva ratio [VR], change in systolic blood pressure [ΔsBP]) and vasomotor tests (total pulse amplitude [TPA] and percent vasoconstriction [%VC]) were repeated within 1 week. Infrared photoplethysmography measured sympathetic-mediated vasomotor function. Reliability was determined by intraclass correlation coefficients. Validity was determined by analysis of variance procedures to test for differences between known groups and by computing sensitivity, specificity, and positive and negative predictive values.

RESULTS — All AFTs were reliable, with %VC having highest reproducibility (r = 0.90). AFT scores were not different from time 1 to time 2. After controlling for age, two cardiovascular tests had significantly different values for control subjects and asymptomatic diabetic patients. AFTs, except ΔsBP, were significantly different between symptomatic diabetic patients and asymptomatic diabetic patients after controlling for age and duration of disease simultaneously. Sensitivity, specificity, and predictive values for %VC were comparable to the values for Δbpm and VR. TPA indexes were lower but clinically acceptable.

CONCLUSIONS — AFTs were found to be reliable and valid tests for detecting DAN. TPA and %VC are important because they measure an aspect of sympathetic function not assessed by standard cardiovascular AFTs, and they do not depend on the patient's cooperation or ability to exert effort.

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DAN, diabetic autonomic neuropathy; AFT, autonomic function test; VC, vasoconstriction; IDDM, insulin-dependent diabetes mellitus; VFT, vasomotor function test; ECG, electrocardiogram; VR, Valsalva ratio; sBP, systolic blood pressure; bpm, beats per minute; TPA, total pulse amplitude.

The most common diagnostic tests of diabetic autonomic neuropathy (DAN) are a battery of noninvasive autonomic function tests (AFTs) based on cardiovascular responses to positional and respiratory maneuvers. Although cardiovascular AFTs are currently used as the standard for determining impaired autonomic nervous system function (1–9), they have two major limitations. First, cardiovascular AFTs do not correlate well with early diabetic changes in autonomic nerves (3), and once abnormal, they do not readily revert to normal in response to diabetic therapies (10,11). Second, the tests assess proximal sympathetic function, but not distal sympathetic function (8,11), which determines vasomotor tone, vasoconstriction (VC), responses in the peripheral arterioles, and arteriovenous shunt flow in the skin (12–16). Impairment of distal sympathetic function is an important factor in the development of diabetic skin changes in the extremities, which contribute to some of the more severe and costly secondary complications of insulin-dependent diabetes mellitus (IDDM). Early detection of impaired distal sympathetic function may provide the opportunity for interventions that would diminish the consequences of these complications.

We have introduced infrared photoplethysmography as an efficient, noninvasive method for evaluating sympathetic-controlled vasomotor function (17). Our results with patients following pancreas transplant suggest that abnormal vasomotor responses may be reversible markers of DAN (18). Even in patients with long-standing diabetes, we have observed improvement in vasomotor function and subjective improvement in gastric function as reported by patients on the Total Gastric Symptom Score (19). We also have demonstrated that impaired vasomotor function correlates well with evidence of delayed gastric emptying in patients with early symptoms of diabetic gastroparesis (20). The initial standardization and validation of vasomotor func-

tion tests (VFTs) was accomplished in our laboratory through testing >300 healthy control subjects and diabetic patients with and without symptoms of diabetic neuropathy (21). The next step in refining the methodology was to determine reproducibility of the VFTs and their diagnostic potential in relation to accepted standards for diagnosing DAN. Thus, the purposes of this study were *1*) to compare reliability of the VFTs to reliability of the widely used cardiovascular AFTs and *2*) to determine the sensitivity, specificity, and positive and negative predictive values of the VFTs in diagnosing DAN.

RESEARCH DESIGN AND METHODS

— The sample consisted of 21 healthy control subjects and 21 IDDM patients. Subjects were included if they were between the ages of 20 and 60, were able to understand and follow verbal instructions during the tests, and gave informed consent to participate in the study. Women who were not in natural or surgical menopause were tested at least 1 week before the start of, or following the end of, their menstrual cycle. Only patients having IDDM for at least 1 year were included in the study. On the day of each test session, patients were instructed to not smoke, not ingest caffeine, and not eat for at least 4 h before the tests. Patients were excluded from the study if they were unable to comply with these restrictions, perform any of the AFTs, or if they had taken cardiac or hypotensive medications within 12 h of the tests. Each patient's blood glucose was tested immediately before the test using a Glucometer, which was calibrated weekly according to the manufacturer's directions. AFTs were performed only if the blood glucose was <200 mg/dl immediately preceding the test session.

The patients were divided into two groups according to whether they were asymptomatic or symptomatic for diabetic neuropathy. Symptomatic neuropathy was determined by the subject's report of two or more of the following symptoms, unexplained by other causes: diarrhea (nocturnal or without awareness), vomiting, nausea, fullness in the presence of a positive gastric emptying test or gastrogram, orthostatic hypotension, impotence, and bladder paresis (22–24). The control subjects were students in a health care center who volunteered to participate. They included 6 men and 15 women (81% Caucasian; age 22–43 years, mean ± SD of 29.5 ± 6.7 years). The diabetic subjects were patients referred to the laboratory for routine evaluation or for pre-pancreas transplant evaluation. The asymptomatic diabetic subjects included four men and seven women (73% Caucasian; age 20–52 years, mean 29 ± 13.6 years) who had been diagnosed with diabetes 1–37 years ago (mean 14.5 ± 9.5 years). The symptomatic diabetic subjects included six men and four women (90% Caucasian; age 20–54 years, mean 41 ± 10 years) who had been diagnosed with diabetes within 16–40 years ago (mean 26 ± 6.9 years). The symptomatic diabetic subjects were older than the control subjects ($P < 0.001$) and the asymptomatic diabetic subjects ($P < 0.035$). Duration of diabetes also was significantly greater for symptomatic than for asymptomatic diabetic patients ($P < 0.005$).

Preparation of technicians

Skills and training required to perform the tests are similar to those required of electrocardiogram (ECG) technicians. The technician must have the ability to apply limb leads, run ECG and plethysmograph tracings on the designated instrumentation channels, and reset the polygraph speed appropriately, depending upon whether ECG or pulse amplitude tracings are being obtained. Additional cost for the vasomotor tests is minimal, beyond the initial cost of the polygraph instrument. About three times the amount of graph paper is required for the vasomotor test as for the cardiovascular tests.

Protocol

Data were obtained from each subject at the same time of day on 2 separate days within 1 week of each other. Each test session, including both cardiovascular and vasomotor tests, took ~90 min. The vasomotor tests alone took ~45 min. All tests were performed in the laboratory's temperature-controlled 15 × 15 foot testing room, which is maintained at 25–27°C. Three electronic thermometers were used to record room, hand, forehead, and foot temperatures. To minimize sympathetic arousal in the patient during the test sessions, conversation within the laboratory was limited to the technician's giving necessary instructions to the patient.

Cardiovascular tests

Cardiovascular responses were assessed by three standard tests of heart rate and blood pressure responses (2,25). These tests were Valsalva ratio (VR), change in systolic blood pressure (ΔsBP) on standing, and change in R-R interval with respirations (change in beats per minute [Δbpm]).

Vasomotor tests

The vasomotor tests measure vascular responses in the left middle digit to ice water immersion of the contralateral hand via an intact reflex arc (26–28). Details of instrumentation and method of validating skin depth detected by the infrared sensor have been reported by Giltvedt et al. (29).

The vasomotor tests are performed with the patient seated in a reclining chair, the left hand resting fully extended, palm upward, on a tilt board at a 45° angle from the chest. An infrared sensor (Medasonics) is placed on the third finger of the left hand and is lightly secured to the pad of the distal phalanx with nonallergenic tape to maintain good skin contact and prevent interposition of ambient light between the skin and the sensor. On the middle finger of the right hand, a temperature probe is taped to the pad of the distal phalanx for continuous measurement of skin temperature.

Table 1—*Reference values for AFTs*

Test	Normal	Borderline	Abnormal
ΔsBP	≤10	11–29	>30
Δbpm	≥15	11–14	≤10
VR	≥1.21	1.11–1.20	≤1.10
%VC	≥70	—	<70
TPA	3,876–15,000	—	<3,876; >15,000

An 8-channel, 7 and 78 series polygraph (Grass Medical Instruments) converts signals from the infrared sensor into simple waveforms, each of which represents one pulsation and consists of an upstroke and a downstroke segment. Height of the waveforms can be altered by adjusting the polygraph instrument's sensitivity setting. Because individual pulsation waveforms vary in amplitude from beat to beat, the polygraph's integrator channel estimates the amplitude of each beat and integrates them into uniform composite waveforms 40 mm in height. These waveforms, called *epics*, are used to calculate total pulse amplitude (TPA) and %VC.

TPA, a measure of dermal blood flow, is calculated from the total number of epics generated by the polygraph integrator in 1 min. Ten minutes of baseline tracings are obtained at the start of the testing session. Epics within the 1-min segment, which contains the most even pattern and highest amplitude of individual pulsations, are to compute the TPA. The following formula is used:

[40 (number of full upstrokes) + mm of partial upstrokes] × sensitivity

Percent VC is based on ice water immersion of the hand. Before the test, a finger cot is placed over the temperature probe on the right middle finger to prevent wetting the finger and probe, thereby limiting cold exposure time to the 1-min immersion period. Four quarts of crushed ice are mixed with sufficient cold tap water in a calibrated basin to create an ice water bath at a temperature of 4°C. One minute after the ice water is placed at the subject's side, the subject is instructed to place his/her right hand in the basin up to the level of the lateral condyle of the wrist. At the end of 1 min, the subject removes his/her hand from the ice water. The examiner removes the finger cot and pats the subject's hand dry, taking care not to rub or touch the subject's skin with the examiner's warmer hands.

Percent VC measures the pulse amplitude reduction in the middle digit of the hand in response to ice water immersion of the contralateral hand for 1 min. Percent VC is determined by the following formula:

(TPA before ice − TPA during ice water) ÷ TPA before ice

Reliability of manual computations

Normal, borderline, and abnormal values for AFTs performed in our laboratory (Table 1) were based on results from testing >100 volunteers in our laboratory and from published criteria (2). Computations of heart rate responses, pulse amplitude, and VC were done manually by two laboratory technicians. To determine interscorer reliability, the principal investigator retrieved 10 records and computed the scores for each test without reference to the previously recorded scores. There was 100% agreement between the two sets of scores.

Statistical analysis

Univariate analysis was used to compute means, SDs, and SEs of AFT scores for each group. Where there was sufficient dispersion of data points, estimates of test-retest reliability were obtained by computation of intraclass correlation coefficients. Because computation of correlation coefficients assumes a linear relationship between variables, an alternate test of reliability was performed if the plotted test scores revealed a dispersion of data points insufficient to demonstrate a linear relationship between repeated scores. The alternate test was based on univariate analysis of difference scores (score at time 1 minus score at time 2). Whether the difference scores were significantly >0 was determined by Student's *t* tests for normally distributed data and Wilcoxon's sign-rank tests for skewed data (30). The a priori significance level for all comparative analyses was 0.05. Difference scores with $P > 0.05$ indicated that there was no difference between time 1 and time 2; higher *P* values therefore signified greater test-retest reliability.

Validity was determined by the "known groups" method (control subjects and diabetic patients with and without DAN symptoms). Because validity is concerned with "true" scores of each subject, the average of time 1 and time 2 scores was used as each subject's test score. Least-squares means (analysis of variance, using the SAS statistical software package) were computed to determine whether the AFTs distinguished *1*) control subjects from all diabetic patients, and 2) control subjects from people with diabetes, with and without DAN symptoms.

A final test of validity was to compare the sensitivity, specificity, and positive and negative predictive values of the vasomotor tests with values for the established cardiovascular tests. Because the purpose was to compare the diagnostic potential of the vasomotor tests to the older cardiovascular tests, only the results of each subject's first test were used to approximate the judgments that would be made in actual clinical practice. The vasomotor tests, %VC and TPA, were compared against two different standards: *1*) abnormal cardiovascular tests: a test was considered positive for DAN if both VR and Δbpm were abnormal or if one

Table 2—*Difference scores: test results at time 1 minus results at time 2 for total sample, control group, and combined diabetic group*

		ΔsBP		Δbpm		VR		%VC		TPA	
	n	Mean	*P*	Mean	*P*	Mean	*P*	Mean	*P*	Mean	*P*
Control group	21	−3*	0.24†	−0.81	0.63	0.102	0.11	0.004	0.67	−378.33	0.47
Combined diabetic group	21	1.56	0.70	2.11	0.13	0.009	0.77	−3.24	0.39	−123.5	0.75
Total sample	42	−0.19	0.94	0.69	0.52	0.05	0.32	−0.15	0.79	−318.93	0.24

* Mean difference between time 1 and time 2 tests. †*P* value is for Student's *t* test of null hypothesis that difference score = 0.

was abnormal and one was borderline (see reference values in Table 1); 2) abnormal cardiovascular tests or the presence of two or more DAN symptoms: VR and Δbpm were compared with the standard of two or more DAN symptoms. Values were computed with and without the inclusion of borderline values for VR and Δbpm.

RESULTS—

Reliability

Plots of the AFT scores (value at time 1 vs. value at time 2) were constructed for control subjects, diabetic subjects, and for the total sample. In the control group, plotted test scores formed a cluster of points with insufficient dispersion for establishment of a linear relationship between time 1 and time 2. Therefore, meaningful correlation coefficients could not be computed, and Student's *t* tests of difference scores were used as the test of reliability. Each AFT was reliable, as indicated by difference scores with $P > 0.05$ (Table 2). The vasomotor tests had greater reliability than two of the cardiovascular tests, ΔsBP and VR, as indicated by their higher *P* values.

Similar results were obtained from analysis of difference scores for the diabetic group and total sample. All AFTs had acceptable test-retest reliability in the diabetic group and the total sample.

Because plots of each AFT for the diabetic group ($n = 21$) and the total sample ($n = 42$) displayed sufficient dispersion for establishment of a linear relationship, intraclass correlation coefficients were selected as the reliability test for these two groups. In the diabetic group, %VC had the highest reliability of the vasomotor tests ($r = 0.89$; $P < 0.0001$) and was comparable in reproducibility to two of the standard cardiovascular tests: Δbpm ($r = 0.90$; $P < 0.0001$) and VR ($r = 0.95$; $P < 0.0001$). All AFTs had high test-retest reliability for the diabetic group (Table 3). Reliability of ΔsBP ($r = 0.59$) was lower than that of the other cardiovascular tests, but the correlation was statistically significant ($P < 0.01$).

For the total sample, all AFTs demonstrated high test-retest reliability, with *P* values ranging from 0.001 to 0.0001. Percent VC had the highest reliability ($r = 0.90$; $P < 0.0001$), exceeding that of ΔsBP ($r = 0.68$; $P < 0.0001$), Δbpm ($r = 0.89$; $P < 0.0001$), and VR ($r = 0.84$; $P < 0.0001$).

Validity

Results of least-squares means analysis are shown in Table 4. For the control and combined diabetic groups, the difference between the mean values of all AFTs were large and statistically significant ($P = 0.007$–0.0001). Despite the statistically significant differences between the two groups, the mean test scores for the combined diabetic group as well as for the control subjects were within the predefined normal range for ΔsBP, Δbpm, VR, and TPA. In contrast, the mean %VC score for the combined diabetic group was just below the normal cutoff score, while the mean score for control subjects was ~22 percentage points higher.

Diabetic patients were then divided into asymptomatic and symptomatic groups for comparison with the control group (Table 4). Because duration of

Table 3—*Test-retest reliability (intraclass correlation coefficient) of cardiovascular and vasomotor AFTs in combined diabetic and total sample groups*

		ΔsBP		Δbpm		VR		%VC		TPA	
	n	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Combined diabetic group	21	0.59	0.01	0.90	0.0001	0.95	0.0001	0.89	0.0001	0.78	0.0001
Total sample	42	0.68	0.0001	0.89	0.0001	0.84	0.0001	0.90	0.0001	0.60	0.0001

P value in ΔsBP is of the correlation coefficient.

Table 4—*Least-squares means and P values of AFTs comparing control, asymptomatic, and symptomatic patient groups after controlling for disease duration of groups with diabetes*

		ΔsBP		Δbpm		VR		%VC		TPA	
	n	Mean	*P*	Mean	*P*	Mean	*P*	Mean	*P*	Mean	*P*
Control group	21	−11.17		32.80		1.79		91.47		7,664.88	
			0.0001		0.0001		0.0003		0.0002		0.007
Combined diabetic group	21	8.39		15.21		1.39		69.51		5,513.57	
Control group	21	−11.17		32.80		1.79		91.47		7,664.89	
			0.02		0.008		0.30		0.11		0.49
Asymptomatic diabetic group	11	−0.20		23.44		1.69		82.51		7,105.45	
Control group	21	−11.17		32.80		1.79		91.47		7,664.89	
			0.0001		0.0001		0.0001		0.0001		0.0001
Symptomatic diabetic group	10	17.50		6.98		1.12		55.21		3,762.50	
Asymptomatic diabetic group	11	−1.80		23.35		1.69		80.63		6,753.25	
			0.06		0.001		0.0002		0.0002		0.0008
Symptomatic diabetic group	10	10.92		6.70		1.15		48.46		2,502.28	

The mean for each subject was the average of the score at time 1 and time 2.

disease was significantly greater for subjects with asymptomatic and symptomatic diabetes, those two groups were compared after controlling for disease duration. ΔsBP and Δbpm were both significantly different between control subjects and subjects with asymptomatic diabetes, though mean values of both were within the normal range. All tests were significantly different between control subjects and symptomatic diabetic subjects ($P < 0.0001$). ΔsBP was not different between asymptomatic diabetic patients and symptomatic diabetic patients ($P > 0.06$), though mean scores were in the expected direction. All of the other AFTs were significantly different between asymptomatic and symptomatic diabetic subjects ($P = 0.001$–0.0002). Δbpm, %VC, and TPA scores were normal vs. abnormal for asymptomatic and symptomatic diabetic subjects, respectively. VR scores were normal and borderline for the same groups.

Age was a second factor that was significantly different between groups: subjects with symptomatic diabetes were significantly older than control subjects ($P < 0.001$) and also significantly older than subjects with asymptomatic diabetes

Table 5—*Least-squares means and P values of AFTs comparing control, asymptomatic, and symptomatic patient groups after controlling for age (all groups) and disease duration (subjects with symptomatic and asymptomatic diabetes mellitus)*

		ΔsBP		Δbpm		VR		%VC		TPA	
	n	Mean	*P*	Mean	*P*	Mean	*P*	Mean	*P*	Mean	*P*
Control group	21	−11.19		31.64		1.78		91.16		7,580.73	
			0.02		0.002		0.31		0.11		0.50
Asymptomatic diabetic group	11	0.17		21.88		1.68		82.24		7,030.04	
Control group	21	−11.19		31.64		1.78		91.16		7,580.73	
			0.0001		0.0001		0.0001		0.0001		0.0005
Symptomatic diabetic group	10	17.58		10.86		1.15		56.14		4,022.18	
Asymptomatic diabetic group	11	2.35		21.50		1.63		79.66		7,513.81	
			0.17		0.02		0.007		0.004		0.0005
Symptomatic diabetic group	10	15.11		8.92		1.17		43.22		3,313.31	

The mean for each subject was the average of the score at time 1 and time 2.

Table 6—*Sensitivity, specificity, and predictive values of AFTs in detecting DAN*

AFT (Standard for determining DAN)	Sensitivity	Specificity	PV^+	PV
%VC (CV standard)	87.5	77.7	77.7	87.5
%VC (CV + two symptoms standard)	90.0	78.0	81.8	87.5
TPA (CV standard)	75.0	66.7	66.7	75.0
TPA (CV + two symptoms standard)	70.0	66.7	70.0	66.7
VR (Abnormal/borderline vs. normal by two symptoms standard)	90.0	100.0	100.0	91.7
VR (Abnormal vs. normal by two symptoms standard)	83.0	100.0	100.0	91.7
Δbpm (Abnormal/borderline vs. normal by two symptoms standard)	90.0	81.8	81.8	90.0
Δbpm (Abnormal vs. normal by two symptoms standard)	88.9	81.8	80.0	90.0

PV^+, predictive value positive; PV^-, predictive value negative.

($P < 0.035$). Analysis of covariances for differences between these groups showed that age alone had no effect on any of the tests when comparing control subjects and subjects with asymptomatic diabetes (Table 5). After adjusting for age simultaneously with disease duration, ΔsBP was even less significantly different between groups with asymptomatic and symptomatic diabetes ($P > 0.17$). Adjustment for age, or age and duration simultaneously, did not change the statistical significance of the difference between any of the groups for Δbpm, VR, or the two vasomotor tests.

Table 6 shows the sensitivities, specificities, and positive and negative predictive values of each vasomotor and cardiovascular test in diagnosing DAN, using the standards outlined in METHODS. The cardiovascular standard selected for evaluating the vasomotor tests was either two abnormal or one abnormal and one borderline cardiovascular test, because clinically, the concurrence of an abnormal and a borderline cardiovascular test is likely to be considered diagnostic of DAN. The ΔsBP test was below normal only when VR and Δbpm together were diagnostic of DAN; therefore, ΔsBP did not affect the numbers of subjects diagnosed as having DAN.

Only five subjects with diabetes had abnormal values for both VR and Δbpm, and all five had abnormal test results for %VC and TPA. Because the number of symptomatic subjects was so small, symptoms alone were not used as the DAN standard for evaluating the vasomotor test. Two symptoms and two cardiovascular tests were used as an alternative DAN criterion for testing the vasomotor tests' predictive values. The %VC test had very high sensitivity and PV negative scores, comparable to those for the standard cardiovascular tests. TPA had lower diagnostic and predictive values, with more false-positives and false-negatives than %VC.

CONCLUSIONS— AFTs are performed at intervals to assess disease progression and patients' responses to therapies, making it important to ensure that the tests are reliable. This clinical study provided strong support for the reliability and clinical validity of AFTs measured by infrared photoplethysmography. A limitation of the study is that there were relatively small numbers of subjects in the individual diabetic groups. The small numbers may reduce the ability to generalize and may have prevented finding significant differences between groups, as with the VR and %VC scores between control subjects and asymptomatic diabetic subjects ($P < 0.11$ and 0.31, Table 5). Despite the relatively small sample size, both standard cardiovascular tests and vasomotor tests were reliable, with the most reliable measures being %VC, Δbpm, and VR. This study also demonstrated that the two vasomotor tests, TPA and %VC, show significant differences between subjects with symptomatic diabetes and the other two groups, just as the cardiovascular tests, VR and Δbpm, do. In addition, like VR and Δbpm, TPA and %VC differences between groups remain after controlling for age and disease duration. For the Δbpm, %VC, and TPA tests, the scores that were significantly different between the groups were in the expected normal and abnormal categories. In contrast, the mean VR scores for the symptomatic subjects were borderline, though the scores were significantly lower than for the control subjects and asymptomatic subjects. Therefore Δbpm, VR, %VC, and TPA were shown to be important AFT markers for DAN, though VR reaches the abnormal range later than the others.

Pulse amplitude fluctuations reflect the phenomenon of beat-to-beat small vessel autoregulation and are associated with numerous stimuli, such as mental stress, worry, and anxiety. For example, a mental arithmetic exercise of silently counting backward from 100 was associated with immediate, profound decreases in TPA for most subjects in our study. Though these mental stresses are difficult to control, even in test situations, the TPA of diabetic subjects had good test-retest reliability, with a correlation coefficient of 0.78 ($P < 0.0001$). Its overall diagnostic and predictive scores, however, were lower than those for %VC (Table 6), though one would expect lower specificity and PV positive scores for tests that become abnormal early in the course of a disease. Percent VC, which is derived

from the TPA, was one of the most reliable AFTs ($r = 0.90$ for total sample; $r = 0.89$ for diabetic subjects) (Table 2). This suggests that, under standardized test conditions, %VC is a highly reliable measure of vasomotor function regardless of baseline TPA. In addition, it has high sensitivity and predictive values when tested against standard cardiovascular tests and the presence of DAN symptoms. Its lower specificity (78% using either standard) is understandable if vasomotor impairment is an early marker of DAN, and as such, precedes both symptoms and abnormal cardiovascular tests.

Vasomotor tests examine sympathetic-mediated skin blood flow, adding an important dimension to autonomic function testing that is not assessed in the standard cardiovascular tests. Specifically, the tests measure sympathetic-mediated vasomotor activity, which is responsible for thermoregulation (27) and tissue blood flow (13,16,31). Gilmore, Allen, and Hayes (14) found that vasomotor tests were more sensitive indicators of sympathetic dysfunction than fall in blood pressure on standing and recommended that when autonomic function is being assessed, tests of both cardiovascular autonomic responses and sympathetic precapillary vasomotor function should be used. Such testing is particularly important for diabetic patients, because alteration in precapillary vasomotor function may be important in development of foot ulcers (13). Widespread clinical use of these vasomotor tests should be undertaken to demonstrate their clinical utility and to determine their ability to identify patients at risk for neuropathic foot ulcers. Based on our findings and the work of others (13,14), TPA and %VC should be considered valid and reliable tests for assessing sympathetic neuropathy and should be included in screening tests for DAN.

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