Cough Test to Assess Cardiovascular Autonomic Reflexes in Diabetes

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Coughing induces cardioacceleratory responses under cholinergic control. The Cough Test (CT), a standardized test that uses a series of coughs with electrocardiographic monitoring, was used to assess the functional integrity of cardiovascular autonomic nerves. In 224 control subjects and 235 diabetic patients, heart-rate (HR) responses were compared with four established tests: lying to standing (LS), standing to lying (SL), deep breathing (DB), and Valsalva maneuver (VM). In control subjects, HR responses declined significantly with age in a curvilinear pattern. Log-transformed indices were used to estimate percentiles. The CT-HR responses were reproducible and significantly associated with other HR-response tests. Sixty-nine (29%) diabetic patients had an abnormal value ≤1st percentile, whereas only 2 control subjects had such an abnormality. Among diabetic patients, age and duration of diabetes exerted a significant negative influence. With the use of the criteria of two abnormal or one abnormal and two borderline tests (among CT, LS, SL, DB, or VM) as a minimal criteria for cardiovascular autonomic neuropathy, CT and LS had the least overlap between the control and diabetic populations and were significantly better than SL or VM ($P < 0.005$ for CT, $P < 0.01$ for LS). They were not different from DB. We found the CT to be simple to perform, reproducible, and useful for the assessment of cardiovascular autonomic reflexes. *Diabetes Care* 13:719–24, 1990

Coughing induces a characteristic change in the heart rate (HR). Immediately after several coughs, the R-R interval shortens, reaching a peak at 2–3 s. Thereafter, the R-R interval slowly lengthens to resting values in ~12–14 s (1). In the Cough Test (CT), a series of coughs is used to evaluate the HR response. Cough-induced HR changes decline with age and are not affected by sex or systolic or diastolic arterial pressure (2).

Our demonstration of the reflex nature of the cough-induced cardioacceleratory responses, with a definite cholinergic efferent component, suggested a possible role of CT in the evaluation of cardiac parasympathetic integrity in diabetes mellitus (3). Already, there are several standardized tests of cardiovascular reflexes for the bedside assessment of cardiovascular autonomic neuropathy (CVAN; 4,5). Although their functional and pathological bases are only partially understood, they share favorable characteristics; i.e., dysfunction reflects impairment of the reflex pathway, and the test is easy to perform, quick, noninvasive, inexpensive, sensitive, specific, and meaningful. In this study, we define the cough-induced HR response in healthy subjects and demonstrate its reproducibility. Next, we show how the response in diabetic subjects differs from that in healthy subjects. Then, by comparing responses to those of other tests, we show that CT presents favorable features for the assessment of cardiovascular autonomic reflexes in diabetes.

RESEARCH DESIGN AND METHODS

Cough-induced HR response was assessed in 224 control subjects 20–82 yr old (mean ± SD 48 ± 17 yr). The same test was given to 235 diabetic patients 19–76 yr

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old (44 ± 14 yr). According to the criteria of the National Diabetes Data Group (1979), 145 had insulin-dependent diabetes mellitus, 85 had non-insulin-dependent diabetes mellitus, and 5 were secondary to pancreatopathy. To participate in the study, subjects gave informed consent.

Control subjects underwent a complete historical and physical examination, an electrocardiogram, and a blood glucose level determination. Diabetic patients were admitted to our department for a 6- to 12-day workup within 32 mo. In both groups, diseases known to interfere with cardiovascular reflexes were excluded (6). Ischemic heart disease, heart failure, valvular heart disease, and cardiac arrhythmias (except rare ventricular premature beats) were excluded. No one was involved in a physical exercise program for >2 times/wk. No patient was in overt metabolic decompensation (average daily blood glucose <12 mM). The list of medications is shown in Table 1.

All subjects and patients underwent a battery of five tests of cardiovascular reflex function 3 h after lunch: CT, lying to standing (LS), standing to lying (SL), deep breathing (DB), and Valsalva maneuver (VM).

Three minutes after assuming the supine position, the subject was instructed to give three vigorous (maximal) coughs evenly spaced over 3 s (1). The cough-induced HR response was expressed by the CT ratio, which is the ratio between the baseline R-R interval (mean of 10 beats just before coughing) and the shortest R-R interval in the first 12 s after coughing.

Three minutes after lying quietly, the subject was asked to rise quickly (≤5 s) and stand still (4). This response was expressed by the ratio of the longest R-R interval from beat 25 to beat 35 and the shortest R-R interval from beat 10 to beat 20 from onset of standing (30:15).

Three minutes after standing still, the subject was asked to lie down quickly (≤5 s) and quietly (7). SL was expressed by the SL1 ratio between the baseline R-R interval and the shortest R-R interval in the first 6 beats from lying.

TABLE 1
Control subjects and diabetic patients taking medications in last 6 mo before assessment of cardiovascular reflexes

Drug	Control	Diabetic
n	224	235
Diuretics	14	18
Methyldopa	0	3*
β-Blockers	0	2*
Angiotensin-converting enzyme inhibitors	0	2*
Calcium blockers	0	2*
Benzodiazepines	10	8
Aminophylline	2	1
Antacids	6	4

*Associated with diuretics for treatment of hypertension.

While seated, the subject was asked to inhale and exhale maximally for a minute at a rate of 6 breaths/min (8). DB was expressed by the expiration-inspiration ratio between the mean of the three longest R-R intervals in expiration and the mean of the three shortest R-R intervals in inspiration (9).

While seated, the subject was instructed to blow into a mouthpiece connected to a manometer keeping a pressure of 40 mmHg for 15 s (4). An air leak had been incorporated to ensure that the subject had to maintain a given pressure. VM was expressed by the Valsalva ratio between the longest R-R interval after the maneuver and the shortest R-R interval during the maneuver.

CT, LS, and SL were performed twice; VM three times. In analysis, mean results were used. The tests were explained to the subjects, and test trials were conducted to ensure adequate performance. For each test a continuous electrocardiographic recording at a paper speed of 25 mm/s was taken, starting 20 s before the test and for the duration of the test (30 s for CT and 60 s for LS, SL, DB, and VM).

Statistical analysis. Responses of healthy subjects were analyzed for the effect of age and sex with regression analysis and replication technique. To reduce heteroscedasticity, log transformations were used. The transformed data had to satisfy three criteria: linearity, a high determination coefficient (R^2), and normal plotted residuals. The association between indices were assessed with linear regressions. Among diabetic subjects, multiple regressions were used to analyze the interrelationship among CT ratio, age, and diabetes duration (10). Unless specified, data are expressed as means ± SD. A response was considered normal when it was ≥5th percentile, borderline when it was <5th percentile and >1st percentile, and abnormal when it was ≤1st percentile.

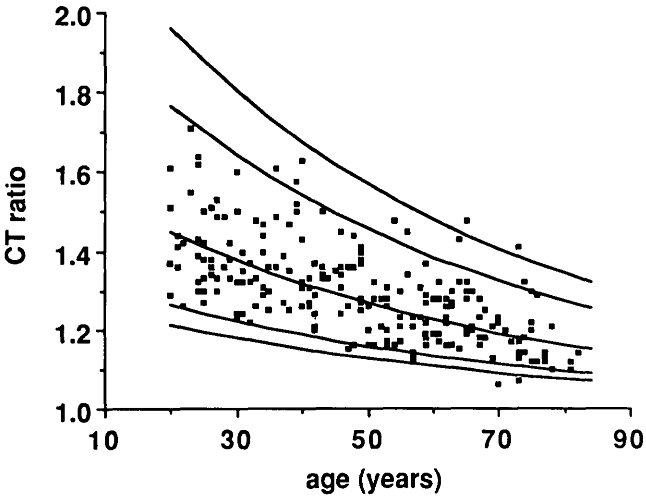

FIG. 1. Cough Test (CT) ratio in relation to age in 224 control subjects. *Top to bottom*, curvilinear *lines* represent 99th, 95th, 50th, 5th, and 1st nontransformed percentiles.

RESULTS

Response in health. The CT ratio plotted as a function of age in 224 control subjects is shown in Fig. 1. The transformation that satisfied the three required criteria was the natural logarithm of the ratio minus one. The identified equation was

$$\ln(\text{CT ratio} - 1) = -0.4543 - 0.0172 \times (\text{age in yr})$$

The regression in the control population was linear ($F = 1.30$, $P = 0.10$) with normally distributed residuals. Age exerted a significant influence on the CT ratio ($F_{(1,222)} = 191.8$, $P < 0.0001$, $R^2 = 0.46$). Percentiles were calculated with linear regression analysis. Nontransformed percentiles are shown in Fig. 1. Borderline and abnormal values for intervals of 5 yr are presented in Table 2. Six CT ratios (2.7%) were <5th percentile and two CT ratios (0.9%) were ≤1st percentile. Basal R-R interval was not significantly associated with the CT ratio ($P = 0.08$).

To test reproducibility of the CT ratio, the CT was performed twice 4 days apart in 12 healthy subjects (age 27 ± 3 yr). For each subject the difference of CT ratios was plotted against their mean. The mean difference (0.003) was not significantly different from zero ($t = 0.24$, $P > 0.40$), suggesting that learning was not involved in the response. The repeatability coefficient was 0.097 (11). The variation coefficients (%) of HR at rest (on which the CT ratio was calculated) and CT ratio were, respectively, 7.8 ± 4.1 and 4.3 ± 1.6. The reduced variability of the CT ratio was achieved by a change in the cough-induced R-R interval that appeared to be proportional to the basal R-R interval. To assess the reliability of the proposed normal ranges, we repeated the test in 42 control subjects (age 51 ± 12 yr) 3 yr later (Fig. 2). The second CT ratio was 1.25 ± 0.11 compared with 1.27 ± 0.11 from the first trial ($P > 0.05$, rank-sum test). The number of abnormal responses was not significantly different from that of the previous evaluation (χ^2-test).

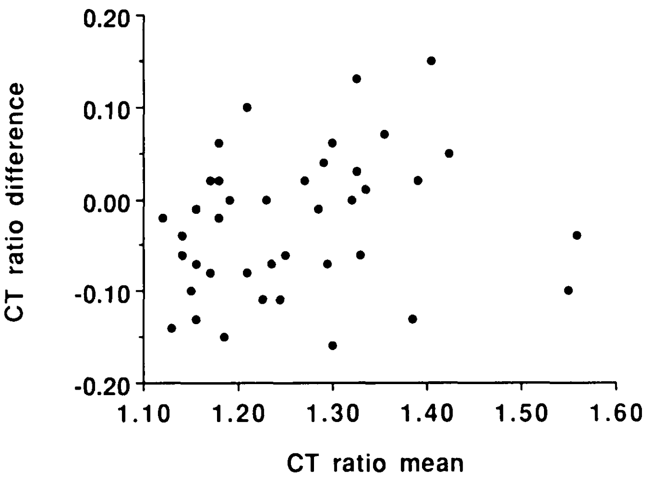

FIG. 2. Cough Test (CT) ratio assessed in 42 control subjects at beginning of study and after 3 yr. For each subject, difference of CT ratios is plotted against its mean.

Response in diabetes. The CT ratio in diabetic patients compared with the percentiles identified in the healthy group is shown in Fig. 3. Ninety ratios (38%) were <5th percentile and 69 ratios (29%) were ≤1st percentile. Analysis of the partial regression coefficients showed that age ($t = 6.45$, $P < 0.0001$) and diabetes duration ($t = 2.99$, $P < 0.005$) exerted a significant influence on the CT performance of diabetic subjects. The association between age and duration of diabetes was of low magnitude ($r = 0.165$).

TABLE 2
Borderline and abnormal values for Cough Test ratio determined in 224 control subjects for intervals of 5 yr

Age (yr)	Borderline	Abnormal
20–24	1.24–1.20	≤1.19
25–29	1.21–1.19	≤1.18
30–34	1.20–1.17	≤1.16
35–39	1.18–1.16	≤1.15
40–44	1.16–1.15	≤1.14
45–49	1.15–1.13	≤1.12
50–54	1.13–1.12	≤1.11
55–59	1.12–1.11	≤1.10
60–64	1.11–1.10	≤1.09
65–69	1.10–1.09	≤1.08
70–74	1.09	≤1.08
75–79	1.08	≤1.07
80–84	1.07	≤1.06

Percentiles determined by linear regression analysis: ≤1st percentile, abnormal values; <5th and >1st percentile, borderline values.

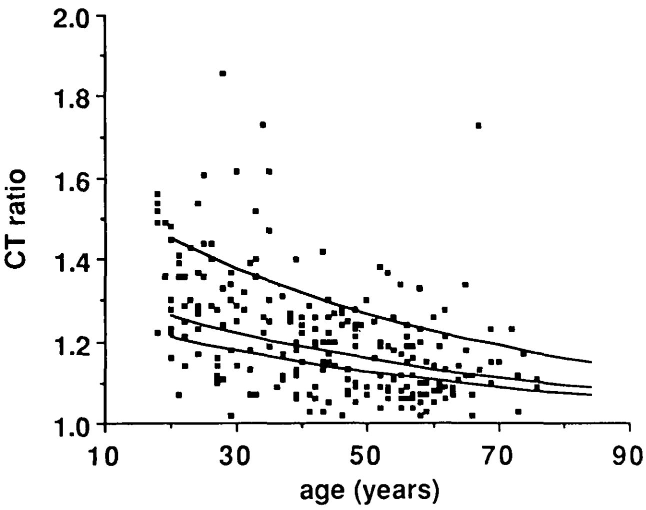

FIG. 3. Cough Test (CT) ratio in relation to age and normal percentiles in 235 diabetic patients. *Top to bottom*, curvilinear *lines* represent 50th, 5th, and 1st nontransformed percentiles of control population.

TABLE 3
Cardiovascular responses to 5 autonomic tests

	Control				Diabetic			
Test (index)	<5th percentile	%	≤1st percentile	%	<5th percentile	%	≤1st percentile	%
Cough Test (CT ratio)	6	2.7	2	0.9	90	38.3	69	29.4
Lying to standing (30:15)	12	5.4	3	1.3	96	40.9	69	29.4
Standing to lying (SL1 ratio)	8	3.6	4	1.8	55	23.4	39	16.6
Deep breathing (EI ratio)	8	3.6	0	0	79	33.6	56	23.8
Valsalva maneuver	6	2.7	0	0	62	26.4	42	17.9

Control subjects (n = 224) <5th and ≤1st percentile and diabetic patients (n = 235) <5th and ≤1st percentile determined in population of 224 control subjects.

Comparison to other tests. Indices of other cardiovascular reflex tests in the control subjects were analyzed to determine age-related percentiles. In Table 3 the number of indices, respectively, <5th and ≤1st percentile in both control and diabetic populations are shown. No significant differences among the indices were observed in the number of control subjects <5th percentile (χ^2 = 1.776, $P > 0.75$). In the diabetic population, the highest number of patients <5th percentile was observed with LS and CT; DB ranked third (Table 3). Because higher rates of abnormal responses could have been related to higher frequencies of false positives, we determined how the other indices responded when one test showed an abnormal HR response. For each test, the distribution of the abnormal responses in five categories of individual scores was determined (Table 4). The individual score was the sum of the points for all the indices, with 0 given to a normal HR response, 1 to a borderline response, and 2 to an abnormal response.

We assumed that when a score of 2–3 had been obtained (a single abnormal response), a diagnosis of CVAN would have been unlikely (false positive), and when a score of 4–10 had been obtained (at least 2 abnormal responses or 1 abnormal and 2 borderline responses) a definite cardiovascular reflex impairment would have been present. In the healthy population, 8 subjects (3.6%) scored 2–3, and 1 subject (0.4%) scored 4. In the diabetic population, 37 patients (16%) scored 2–3, and 75 patients (32%) scored 4–10. No differences among the five tests (when abnormal) existed in the number of diabetic patients with a score of 2–3 (χ^2 = 3.52, $P > 0.30$). The number of diabetic patients with a score of 4–10 was significantly more numerous when CT and LS rather than SL and VR showed an abnormal response (χ^2 = 9.47, $P < 0.005$ for CT and χ^2 = 7.09, $P < 0.01$ for LS). CT and LS were not significantly better than DB. CT ratio showed a good correlation with the other indices (Table 5). Five of 9 diabetic subjects in a two-drug regimen for hypertension scored ≥4 (Table 1).

The CT was performed satisfactorily by all subjects (even the older ones), provided the instructions were as plain as possible and the subject coughed and did not just clear his/her throat. Few patients required several trials. More than three coughs were not observed to modify the CT ratio (unpublished observations).

DISCUSSION

Confirming previous observations (2), our studies show that CT produces a consistent cardioacceleratory response that is reduced with age. A reduction in response has also been found when other maneuvers are used to test HR response (12,13). Although not yet understood, all components of the cardiovascular reflexes may be affected by increasing age. Whether the parasympathetic system reduces its competence with age has not been answered. For example, the reduced HR response to atropine in humans or to vagal stimulation in animals may suggest a decrease in parasympathetic function with age (12,14). But these alterations may be secondary to a modified sympathetic activity or ascribed to a less responsive heart.

To judge a response to CT as normal or abnormal, age-related normal values are needed. Ratios of peak

TABLE 4
Frequency distribution in diabetic patients of abnormal responses of each test in 5 categories of individual scores

Score	n	Cough Test	Lying to standing	Standing to lying	Deep breathing	Valsalva maneuver
2–3	37	7	11	4	8	7
4–5	25	13	14	6	9	3
6–7	15	14	11	7	8	4
8–9	24	24	22	11	20	17
10	11	11	11	11	11	11

Score is sum of points for all tests: 1, borderline response; 2, abnormal response.

TABLE 5
Correlation coefficients (*r*) between Cough Test ratio and indices of other cardiovascular reflex tests

Test (index)	Control	Diabetic
n	224	235
Lying to standing (30:15)	0.65*	0.67*
Standing to lying (SL1 ratio)	0.54*	0.71*
Deep breathing (EI ratio)	0.62*	0.62*
Valsalva maneuver	0.46*	0.45*

*$P < 0.001$.

responses to baseline instead of absolute differences from baseline were used because cough-induced responses appeared proportional to the basal R-R interval. The reproducibility of CT was good and the proposed normal values dependable when CT was measured 4 days apart and 3 yr apart.

CT responses are unequivocally different in control subjects and diabetic patients. In diabetic patients, age and duration of diabetes were also significantly associated with worsening of the CT response.

To introduce a new test for the diagnosis of diabetic CVAN, sensitivity and specificity should be determined and compared with that of established tests. But to determine sensitivity and specificity, an independent standard is necessary against which the new test can be judged. Independent standards are not available in the field of CVAN. For example, clinical assessment has shown that symptoms and signs of autonomic failure may follow cardiovascular reflex impairment (15,16). Electrophysiological and pathological studies are still limited to the experimental setting. The best solution appeared to be to judge the value of CT through a direct comparison with the other tests. Based on the HR responses of the control subjects, we drew for each test the percentile lines that defined borderline and abnormal ratios. These lines appeared to be homogeneous among the tests and were used to measure their ability to separate normal from abnormal. CT and LS appeared to separate the diabetic from the healthy group to a greater extent, followed by DB and then SL and VM.

Less overlap between normal and pathological states could mean a better test as well as a less accurate test identifying as abnormal a patient with an otherwise normal HR variability. To judge the power of these tests to give a correct answer, two points should be taken into account: *1*) the explored reflex pathways are only partially different among the tests and *2*) diffuse multisystem defects in reflex response are conceivably present in diabetic CVAN (16). In view of these considerations, it is reasonable to expect a homogeneous and coherent response within the performed tests. Consequently, we used the criteria that at least a score of 4 should have been obtained before considering a definite cardiovascular reflex impairment (17). Within the group with a score of 4–10, CT and LS still showed the least overlap between control and diabetic populations. When only one test is abnormal, it is less clear that cardiovascular reflex abnormality has been demonstrated. This situation (score of 2–3) appeared to be not so infrequent in our group of healthy subjects (3.6%). The use of a single test to detect an impairment of cardiovascular autonomic reflexes is probably inadequate (18). It has been suggested that the VM acts differently from the other tests because the sympathetic component during the strain (phase 2) can still be present when the parasympathetic component has already disappeared (19). Our data do not show that VM becomes abnormal after the other tests, and there are no prospective studies that support a preferential late involvement of VM in diabetic CVAN.

The assessment of cardiovascular autonomic reflexes in diabetes appears justified for several reasons: *1*) to provide diagnostic and prognostic information; *2*) to delineate prevalence, incidence, and natural history of their derangement; *3*) to study the association with other manifestations of peripheral neuropathy; and *4*) to identify the risk factors associated with their impairment and assess the effectiveness of therapy. The performance of several tests instead of a single one is required to reduce the chance of false positives, to confirm that an abnormality has been detected, and, possibly, to quantify a deficit more accurately. How often these tests show an abnormal response in the general population is still unknown. The CT is simple, does not require postural changes as in LS, and is probably not affected by modest volume depletion (5). It does not need difficult maneuvers as in VM and is reproducible and not more variable in control subjects than other tests. The CT was able to discriminate normal from abnormal to a greater or equal degree than other tests that were used. Furthermore, evoking a cholinergic reflex, it can give information on parasympathetic integrity. The CT appears to be a reliable test that can be selected from among those used for the assessment of CVAN in diabetes.

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