# Association of Autoimmunity to Autonomic Nervous Structures With Nerve Function in Patients With Type 1 Diabetes: A 16-Year Prospective Study

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## OBJECTIVE

**We prospectively evaluated the association between autoimmunity to autonomic nervous structures and autonomic neuropathy in type 1 diabetes in relation to clinical variables.**

## RESEARCH DESIGN AND METHODS

**A cohort of 112 patients with type 1 diabetes was prospectively followed from adolescence (T0) to approximately 4 (T4) and 16 (T16) years later. Standard cardiovascular (CV) tests and neurological examination were performed and related to the presence of circulating antibodies (Ab) to autonomic nervous structures detected at T0 and T4. Quality of life was assessed by a diabetes-specific questionnaire.**

## RESULTS

**Sixty-six patients (59% of the cohort) were reexamined at T16 (age 31.4 ± 2 years; disease duration 23.4 ± 3.7 years). Nineteen had circulating Ab to autonomic structures. Prevalence of abnormal tests and autonomic symptoms were higher in Ab-positive (68 and 26%, respectively) than Ab-negative (32 and 4%) patients ($P < 0.05$). Among Ab-positive patients, the relative risk (RR) of having at least one altered CV test was 5.77 (95% CI 1.56–21.33), and an altered deep breathing (DB) test (<15 bpm) was 14.65 (2.48–86.46). Previous glycemic control was the only other predictor (RR 1.06 [1.002–1.13]/mmol/mol $HbA_{1c}$ increase). Presence of Ab carried over a 68% probability of developing an altered CV test; absence of Ab carried a 91% probability of not having an altered DB test and an 89% probability of not having an altered Valsalva ratio. Autonomic neuropathy was independently associated with worse quality of life.**

## CONCLUSIONS

**Circulating Ab to autonomic structures are associated with the development of autonomic dysfunction in young diabetic patients independent of glycemic control.**

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Neuropathy is a chronic complication that includes a number of distinct syndromes and autonomic dysfunctions and contributes to increase morbidity and mortality in the diabetic population. In particular, cardiovascular autonomic neuropathy (CAN) is an independent risk factor for mortality in type 1 diabetes and is associated with poor prognosis and poor quality of life (1–3). Cardiovascular (CV) autonomic regulation rests upon a balance between sympathetic and parasympathetic innervation of the heart and blood vessels controlling heart rate and vascular dynamics. CAN encompasses several clinical manifestations, from resting tachycardia to fatal arrhythmia and silent myocardial infarction (4).

The mechanisms responsible for altered neural function in diabetes are not fully understood, and it is assumed that multiple mutually perpetuating pathogenic mechanisms may concur. These include dysmetabolic injury, neurovascular insufficiency, deficiency of neurotrophic growth factors and essential fatty acids, advanced glycosylation products (5,6), and autoimmune damage. Independent cross-sectional and prospective (7–13) studies identified circulating autoantibodies to autonomic nervous structures and hypothesized that immune determinants may be involved in autonomic nerve damage in type 1 diabetes. Such a concept is supported by evidence that other forms of dysautonomia, idiopathic and paraneoplastic, can be immune mediated (14,15). However, demonstration of a cause–effect relationship between antibodies (Ab) and diabetic autonomic neuropathy awaits confirmation.

We report on a 16-year follow-up study specifically designed to prospectively examine a cohort of patients with type 1 diabetes and aimed at assessing whether the presence of circulating Ab to autonomic nervous structures is associated with increased risk and predictive value of developing CAN. This, in turn, would be highly suggestive of the involvement of autoimmune mechanisms in the pathogenesis of this complication.

## RESEARCH DESIGN AND METHODS

### Subjects

In the original design, all patients with type 1 diabetes, older than 11 years and in their adolescence, attending the Pediatric Diabetic Clinic of the University of Turin between 1994 and 1997 were considered for participation in the study. Their clinical characteristics at recruitment (T0) and at first follow-up, approximately 4 years later (T4), were described previously (11,12). At this third follow-up visit, 16.5 $\pm$ 1.2 years later (T16), 66 (59%) patients out of the original cohort of 112 were available for restudy. One patient had died, 11 had moved, 27 could not be located, and 7 declined to participate. Their clinical characteristics are shown in Table 1. In total, 19 patients had circulating Ab against autonomous nerve structures, previously detected at T0 (11) and persisting at T4 (12): 8 (12%) patients for vagus nerve, 10 (15%) for cervical ganglia, and 1 patient for both. The Ab had been assessed by indirect immunofluorescent complement-fixation technique as described previously (8). Briefly, 5-$\mu$m cryostat sections of snap-frozen cervical ganglia and vagus nerve, obtained from adult New Zealand white rabbit, were allowed to air dry fixed in acetone and incubated with 50 $\mu$L of neat test serum for 30 min. Slides were then washed in PBS, and 50 $\mu$L of normal human serum, as a source of complement, was added at 1:5 dilution in PBS for 30 min at 37°C. After washing, 50 $\mu$L of 1:20 fluorescein isothiocyanate sheep antihuman C3c antiserum were added for a further 30 min at room temperature. The slides were washed, mounted in 90% glycerol in PBS, and examined by ultraviolet microscopy by two independent observers unaware of clinical details. Positive staining of nerve fibers or cytoplasmic staining of ganglion cell bodies were confirmed by repeated measurements using substrates from a different animal.

Informed consent was obtained, and the investigations were carried out in conformity with the Declaration of Helsinki.

$HbA_{1c}$ levels (high-performance liquid chromatography, International Federation of Clinical Chemistry standardized) of the previous year (mean of three values) and previous 5 years (mean of available data) were collected to assess long-term metabolic control.

Data on frequency of hypoglycemic episodes over the previous 5 years were collected by interview, severe hypoglycemia being defined as needing the assistance of another person. Further information was acquired on the level of physical activity, occupation, schooling level, smoking habit, and alcohol consumption.

### Assessment of Neuropathy

A structured questionnaire, designed according to Dyck (16) was used to identify symptoms related to autonomic, motor, and sensory function. Consumption of food and caffeine-containing beverages and smoking was restricted for 2 h before testing. Heart rate was measured after 10 min of supine resting. Autonomic neuropathy was assessed between 8:00 A.M. and noon by four standard CV tests on an automated, computer-integrated system consisting of heart rate variability upon six maximal breaths per minute (deep breathing [DB] test; abnormal $<$15 bpm, borderline 15–19 bpm), lying-to-standing heart rate change (30:15 ratio; abnormal $<$1.17, borderline 1.17–1.27), heart rate change during Valsalva maneuver (Valsalva ratio; abnormal $<$1.35, borderline 1.35–1.41), and postural systolic blood pressure decrease on standing (abnormal $>$20 mmHg) as previously described (17). Age-related index values (18) were used to establish abnormality of the tests.

Peripheral somatic neuropathy was assessed by clinical and neurological examination, including deep tendon knee and ankle reflexes and recording of vibratory perception threshold at the tip of the great toe using a biothesiometer (Bio-Medical Instruments Co., Newbury, OH). The mean of three readings was used.

### Quality of Life

Diabetes-related quality of life was measured in 56 patients by the diabetes quality-of-life (DQOL) questionnaire (19),a diabetes-specific tool designed by the Diabetes Control and Complications

**Table 1—Clinical characteristics of the diabetic patients at T16 screening**

| | Diabetic patients at T16 *n* = 66 | Nervous tissue Ab-positive patients *n* = 19 | Nervous tissue Ab-negative patients *n* = 47 |
|---|---|---|---|
| Age, years (range) | 31.4 ± 2 (28–36) | 31.3 ± 2 (28–36) | 31.5 ± 2 (28–35) |
| Male:female | 33:33 | 9:10 | 24:23 |
| Duration of diabetes, years (range) | 23.4 ± 3.7 (16–31) | 22.6 ± 3.2 (16–28) | 23.6 ± 3.8 (16–31) |
| Follow-up duration, years | 16.5 ± 1.2 | 16.2 ± 1.4 | 16.6 ± 1.1 |
| $HbA_{1c}$, % (mmol/mol)* | | | |
| Previous year | 7.81 ± 1.14 (61 ± 12) | 8 ± 0.8 (64 ± 9) | 7.7 ± 1.3 (61 ± 14) |
| Previous 5 years | 7.8 ± 1 (62 ± 11) | 7.9 ± 0.7 (63 ± 8) | 7.8 ± 1.2 (61 ± 13) |
| Insulin, units/kg | 0.73 ± 0.17 | 0.70 ± 0.15 | 0.74 ± 0.18 |
| Microalbuminuria | 7 (11%) | 3 (16%) | 4 (9%) |
| Background retinopathy | 37 (54%) | 12 (63%) | 25 (53%) |
| Laser treatment | 7 (11%) | 2 (11%) | 5 (11%) |
| Smoking habit | 21 (32%) | 4 (21%) | 17 (36%) |
| Ex-smokers | 5 (7%) | 1 (5%) | 4 (9%) |
| Hypothyroidism/hyperthyroidism | 8/1 | 5/0 | 3/1 |
| Celiac disease | 4 | 3 | 1 |
| Hypertension | 4 (6%) | 0 | 4 (9%) |
| Antivagus nerve Ab positive | 8 (12%) | 8 | — |
| Anticervical ganglia Ab positive | 10 (15%) | 10 | — |
| Both Ab positive | 1 | 1 | — |

Data are *n* (%) or means ± SD, unless otherwise indicated. *$HbA_{1c}$ levels refer to values of the previous year (mean of three values) and the previous 5 years (mean of available data).

Trial (DCCT) Research Group and consisting of a 46-item multiple-choice questionnaire exploring four primary subscales regarding satisfaction with life, impact, diabetes-related worries, and social/vocational worries. Responses are along a Likert scale ranging from 1 to 5 (1 = never, 5 = all the time for impact and social and diabetes-related worries subscales; 1 = very satisfied, 5 = very unsatisfied for all others). Hence the total score ranges between 46 (highest quality of life) and 230 (lowest quality). The questionnaire had been translated and validated in Italian (20).

### Statistical Analysis

Values for all CV tests and for the vibration threshold in the patient groups satisfied the hypothesis of normality, as assessed by the Kolmogorov-Smirnov goodness-of-fit test. Differences between T0, T4, and T16 data, within and between patient groups, were tested by means of *t* tests (paired and unpaired, respectively).

Correlations between neurological and clinical parameters were analyzed using the Pearson or Spearman correlation coefficient, depending on the variable distribution.

Association between presence of nervous tissue auto-Ab and clinical data of autonomic neuropathy (dichotomized as "presence of at least one altered CV test" versus "no altered CV tests") was assessed using Fisher exact test or $\chi^2$ test on a 2 × 2 table. A multivariate logistic regression analysis was used to test the independent effect of the Ab presence on autonomic neuropathy, adjusting the model for glycemic control, disease duration, exercise level, insulin dose, and value at T0 of the corresponding CV test.

The correlation between the DQOL scores, total and for its four different dimensions, and presence and severity of autonomic and somatic neuropathy was computed by means of a linear regression model where each of the different DQOL scores, taken one at a time, represented the dependent variable and presence and severity of retinopathy and presence and severity of autonomic and somatic neuropathy represented the independent ones; the model was adjusted for sex, diabetes duration, and frequency of hypoglycemic episodes.

Data were analyzed by the SPSS statistical package (SPSS, Chicago, IL). *P* values lower than 0.05 were considered significant.

## RESULTS

Ab-positive and Ab-negative patients did not differ for clinical characteristics or prevalence of diabetic microvascular and macrovascular complications at T16 (Table 1). The $HbA_{1c}$ levels of the year preceding T16 (mean of three values) correlated with the mean $HbA_{1c}$ of the 5 years preceding T16 ($r = 0.81$; $P < 0.01$).

Of the 56 patients who were administered the DQOL questionnaire, 10 did not report hypoglycemic episodes over the last 5 years, 11 reported less than 1 episode/month, 9 at least 1/month, 7 reported 1/week, and 19 reported more than 1/week. Severe hypoglycemia was experienced at least once by 15 (27%) patients.

### Assessment of Neuropathy at T16 and Relationship With Ab to Autonomic Nervous Structures

Clinical neurological variables are summarized in Table 2 and Fig. 1. Values for DB test, 30:15 ratio, and Valsalva ratio had decreased at T16 compared with recruitment at baseline (T0) in both study groups (Fig. 1*A*–*C*). On univariate analysis at T16, the Ab-positive patients

**Table 2—Abnormal CV tests, reflex examination, and somatic or autonomic symptoms according to the status of auto-Ab to autonomic nervous tissues**

| | Patients at T16 *n* = 66 | Nervous tissue Ab-positive patients *n* = 19 | Nervous tissue Ab-negative patients *n* = 47 |
|---|---|---|---|
| Resting heart rate, bpm | 77 ± 12 | 79 ± 11 | 76 ± 13 |
| Normal CV test, % | 38 | 6 | 32 |
| Abnormal/borderline CV tests | 28 (42%) | 13 (68%)* | 15 (32%) |
| One CV test | 16 | 5 | 11 |
| Two CV tests | 10 | 7 | 3 |
| Three CV tests | 1 | 1 | 0 |
| Four CV tests | 1 | 0 | 1 |
| Autonomic symptoms | 7 (11%) | 5 (26%)† | 2 (4%) |
| Postural pressure drop >20 mmHg | 2 | 1 | 1§ |
| Gastrointestinal symptoms | 2 | 1 | 1 |
| Genitourinary symptoms | 2 | 1 | 1 |
| Sudomotor symptoms | 3 | 2 | 1 |
| Absent or depressed reflexes | 19 (29%) | 5 (26%) | 14 (30%) |
| Persistently depressed since T4 | 4 | 1 | 3 |
| Somatic symptoms | 12 (18%) | 4 (21%) | 8 (17%) |
| Symptoms persistent since T4 | 4 | 1 | 3 |

Data are *n* (%) or means ± SD, unless otherwise indicated. *$P$ = 0.05 vs. Ab-negative patients, both for one and more than one abnormal test (DB test abnormal in 8/13, 30:15 ratio abnormal in 6/13, Valsalva ratio abnormal in 7/13). †$P$ = 0.05 vs. Ab-negative patients. §The same patient reported postural hypotension and gastrointestinal and genitourinary symptoms.

had lower DB test values compared with Ab-negative patients ($P < 0.05$), but the difference was no longer significant after adjusting for $HbA_{1c}$, diabetes duration, and baseline DB value by linear regression ($P = 0.081$) (Fig. 1*A*). Similarly, the 30:15 ratio and Valsalva ratio mean values were lower in the Ab-positive patients, although not significantly, compared with the Ab-negative patients. Using dichotomized data, Ab-positive patients had more abnormal tests (at least 1 abnormal test in 13/19 [68%]; >1 abnormal test in 8/19 [42%]) than Ab-negative patients (at least 1 abnormal test in 15/47 [32%]; >1 abnormal test in 4/47 [8%]; $P < 0.005$ and $P < 0.002$, respectively) (Table 2).

Seven (11%) patients reported autonomic symptoms at the CV, gastrointestinal, genitourinary or sudomotor level (Table 2). Postural systolic blood pressure drop >20 mmHg upon standing was detected in two patients, while another five reported occasional orthostatic symptoms (fainting feeling) with an average systolic blood pressure drop of −11 mmHg. Presence of autonomic symptoms was associated with positivity for either antivagus nerve Ab or anticervical ganglia Ab (5/19 [26%]) compared with Ab-negative patients (2/47 [4%]; $P < 0.05$) (Table 2). The patients who reported autonomic symptoms had higher $HbA_{1c}$ values during the previous 5 years (73.4 ± 16 mmol/mol) than asymptomatic ones (59.9 ± 9 mmol/mol; $P < 0.01$).

Symptoms of peripheral somatic neuropathy were reported by 12 (18%) patients at the feet or hands (Table 2). Carpal tunnel syndrome was diagnosed in four of them by electrophysiological assessment. One patient had developed multiple sclerosis. There was a trend to higher prevalence of symptoms among patients with depressed reflexes (5/19 [26%]) compared with those with evocable reflexes (7/47 [15%]). Vibratory perception threshold was higher in the patients with depressed reflexes (7.5 ± 3.2 vs. 5.2 ± 1.4 V; $P < 0.02$) and correlated with $HbA_{1c}$ levels over the previous 5 years ($r = 0.27$; $P < 0.05$). Vibratory perception threshold tended to be higher in the patients with (6.2 ± 1.9 V) than without (5.6 ± 1.6 V) somatic symptoms, but the difference was not significant.

Presence of somatic neuropathy did not differ according to Ab status and autonomic function. There was no association between neurological variables and presence of microvascular complications or smoking habit.

According to logistic regression analysis, the relative risk (RR) for Ab-positive patients of having at least one abnormal CV test at T16 was 5.77 (95% CI 1.56–21.33), and that of having an abnormal DB test was 14.65 (2.48–86.46). This effect was independent from $HbA_{1c}$, which was independently responsible for the increase of the RR for an altered CV test (RR 1.06 [95% CI 1.002–1.13]/mmol/mol $HbA_{1c}$ increase) (Table 3).

### Progression of Neuropathy

Prevalence of at least one abnormal/borderline CV test significantly increased from 5% at T0 to 12% (25%) at T4, without significant differences between Ab-positive and Ab-negative patients (11,12), to 42% at T16 ($P < 0.05$ vs. T0 and T4), with higher prevalence among the Ab-positive patients ($P < 0.005$ vs. Ab-negative patients) (Table 2). Prevalence of autonomic symptoms, which were not detected at T0 and T4, was also higher at T16 ($P < 0.05$). A postural pressure drop >20 mmHg consistently persisted at follow-up in two patients.

The presence of Ab to autonomic nervous structures at T0 carried over a

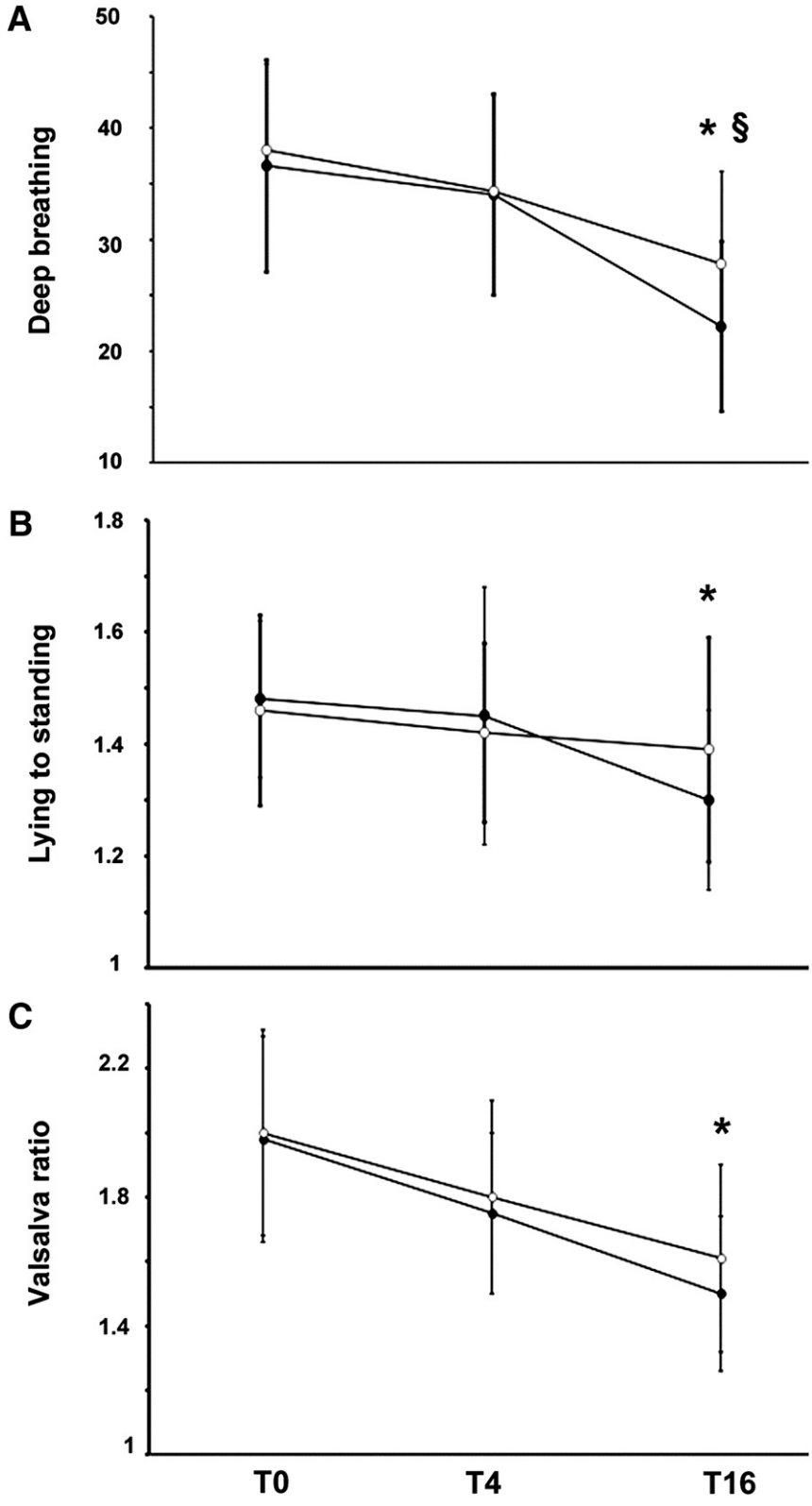


**Figure 1—**Results of CV tests. Mean ± SD value of (*A*) DB test, (*B*) 30:15 ratio, and (*C*) Valsalva ratio in Ab-negative (open circle) and Ab-positive (closed circle) patients. *$P < 0.05$ compared with values at T0 for both study groups; §$P < 0.05$ compared with values in Ab-negative patients.

**Table 3—Multivariate logistic regression analysis testing the independent effect of the Ab presence on altered CV tests adjusted for sex, disease duration, and $HbA_{1c}$ level**

| | β coefficient | RR (95% CI) | *P* value |
|---|---|---|---|
| At least one altered CV test at T16 | | | |
| Sex | −0.239 | 0.788 (0.227–2.729) | 0.707 |
| Disease duration | 0.132 | 1.141 (0.964–1.351) | 0.125 |
| 5-year $HbA_{1c}$ | 0.062 | 1.064 (1.002–1.13) | **0.04** |
| Ab presence | 1.752 | 5.77 (1.56–21.33) | **0.009** |
| Altered DB test at T16 | | | |
| Sex | −0.960 | 0.383 (0.072–2.033) | 0.26 |
| Disease duration | 0.199 | 1.22 (0.955–1.56) | 0.112 |
| 5-year $HbA_{1c}$ | 0.097 | 1.102 (1.021–1.19) | **0.013** |
| Ab presence | 2.685 | 14.65 (2.48–86.46) | **0.003** |
| DB at T0 | −0.098 | 0.907 (0.808–1.018) | 0.097 |

Boldface type indicates statistical significance.

68% probability for development of one altered CV test at T16, while the absence of Ab at T0 carried a 91% probability of not having an abnormal DB test and an 89% probability of not having an altered Valsalva ratio at T16.

The prevalence of somatic symptoms, not detected at T0, increased from 14% (13/92) at T4 to 18% (12/66) at T16, and the prevalence of impaired reflexes increased from 6% (5/85) at T0 to 16% (15/92) at T4 to 29% (19/66) at T16 ($P < 0.05$ vs. T0). Four patients consistently showed absent or depressed ankle reflexes, and four were still complaining of somatic symptoms since T4.

### Quality of Life

The total DQOL score was lower (better quality of life) in men (72.68 ± 12.2) than women (84.70 ± 20.1; $P < 0.001$). Presence of autonomic symptoms was associated with a higher (worse) score (94.3 ± 25.8) than in the patients without symptoms (75.4 ± 15.4; $P < 0.001$).

With reference to the four subscales explored, satisfaction did not significantly correlate with any of the explored clinical variables. Impact was better in men (29.6 ± 5.9) than women (34.1 ± 6.7; $P = 0.011$) and was associated with symptoms of autonomic neuropathy (36 ± 10.4 vs. 30.9 ± 5.8; $P = 0.008$). Social/vocational worries was also better in men (9.0 ± 2.1 vs. 11.4 ± 6.4; $P = 0.03$) and worse in patients with autonomic symptoms (14.8 ± 7.4 vs. 9.3 ± 3.5; $P = 0.01$). The score for diabetes-related worries was again better in men (6.3 ± 1.2 vs. 8.5 ± 3.3; $P < 0.001$) and worse in the presence of autonomic symptoms (9.3 ± 4.7 vs. 6.9 ± 2.1; $P = 0.023$).

A higher score for diabetes-related worries was associated with past occurrence of severe hypoglycemia (8.6 ± 4.3 vs. 6.8 ± 1.5; $P = 0.014$).

There were no correlations between the scores of the DQOL or any of its subscales, and the presence and severity of somatic neuropathy or retinopathy.

## CONCLUSIONS

The pathogenic mechanisms underlying diabetic peripheral polyneuropathies are multiple, interrelated, and, possibly, mutually perpetuating. Several lines of research indicate that immune determinants are likely to be involved in the events culminating in autonomic nerve damage within the constellation of autoimmune stigmata that characterize type 1 diabetes (7–10,21). The association, however, awaits confirmation. The present prospective study, conducted in young patients without established autonomic neuropathy at recruitment and followed for over 16 years until adulthood, strongly indicates that a cause–effect relationship may exist between auto-Ab to autonomic nervous tissues and development of diabetic autonomic neuropathy. Incipient or established CAN (22) reached a prevalence of 68% among the Ab-positive patients, significantly higher compared with the Ab-negative patients. The heart rate response to DB, mainly mediated by the parasympathetic nervous system, was the most impaired CV test in the time course analysis, possibly reflecting early parasympathetic damage. Logistic regression analysis indicates that auto-Ab carry an almost 15-fold increased RR of developing an abnormal DB test over 16 years and an almost sixfold increase of developing at least one abnormal CV test, independent of other variables.

Previous studies showed that, once present, auto-Ab to autonomic nervous structures persist (12,13) and were consistently detected in our patients at recruitment and 4 years later. The proportion of auto-Ab positives was similar to that reported in adult patients with established diabetic autonomic neuropathy (7–10). While in our previous reports on this cohort we could not detect a clear association with CAN, this longer follow-up study highlights a significant association. This suggests that autoimmune mechanisms targeting sympathetic and parasympathetic structures may play a primary etiologic role in the development and progression of autonomic dysfunction in type 1 diabetes in the long term. Indeed, positivity for auto-Ab had a high positive predictive value for the later development of autonomic neuropathy.

Our study is in line with the only other prospective study detecting an RR of 7.5 to develop at least one abnormal autonomic test among Ab-positive patients over a period of 13–14 years, unrelated to glycemic control (13). In addition, in line with others (6,23,24), our study indicates that autonomic abnormalities also have an independent relationship with glycemic control. In fact, multivariate regression analysis showed that previous long-term glycemic control was the only other determinant for increased RR of altered CV tests. In this series, diabetes duration appeared to be a minor predictor of CAN, in contrast to other reports (25).

A multistep process can be envisaged in which nerve damage is first initiated by vascular, metabolic, and/or autoimmune mechanisms and perpetuated/amplified by the same autoimmune mechanisms as neuronal autoantigens are released. Autoimmunity is evoked to explain

the pathogenesis of idiopathic and paraneoplastic dysautonomic syndromes, with levels of auto-Ab targeting ganglionic acetylcholine receptors correlating with the severity of autonomic dysfunction (14,26,27). Those studies indicate a potential therapeutic role for acetylcholinesterase inhibitors in the enhancement of autonomic function (15). Circulating auto-Ab to autonomic nervous structures and subclinical autonomic neuropathy are present in other autoimmune disorders such as rheumatoid arthritis and lupus (28). In type 1 diabetes, the target autoantigens within the vagus nerve and cervical ganglia have not been identified. They might include the ganglionic neurotransmission and the smooth muscle calcium channels (29). Intriguingly, borderline levels of ganglionic acetylcholine receptors Ab were detected in a patient with both celiac disease and type 1 diabetes and one patient with celiac disease and subclinical neuropathy (30). Furthermore, in the context of autoimmune diabetes, there is evidence for predominant active B-cell response in situ against pancreatic nervous system elements (31,32), suggesting a shared propensity for β-cell and neuronal tissue autoimmunity.

Diabetic autonomic neuropathy, possibly the least recognized and most overlooked of diabetes complications, has increasingly gained attention as an independent predictor of silent myocardial ischemia and mortality, as consistently indicated by several cross-sectional studies (2,3,33). The pooled prevalence rate risk for silent ischemia is estimated at 1.96 by meta-analysis studies (5). In this report, established CAN (22) was detected in nearly 20% of young adult patients with acceptable metabolic control, after over approximately 23 years of diabetes duration, against 12% of patients of the same cohort with subtle asymptomatic autonomic dysfunction (one abnormal CV test) a decade earlier, in line with other studies in type 1 diabetes (2,24). Approximately 30% of the patients developed signs of peripheral somatic neuropathy not associated with autonomic dysfunction. This discrepancy suggests the participation of pathogenic mechanisms different from metabolic control and a distinct clinical course, as indicated by the DCCT study, where hyperglycemia had a less robust relationship with autonomic than somatic neuropathy (6). Furthermore, symptoms at different levels of the autonomic system, which were previously absent, appeared in 10% of patients at T16. In line with the analysis of the risk determinants, autonomic symptoms were associated both with the presence of auto-Ab and a worse glycemic control. Orthostatic hypotension was the earliest documented symptom in the course of this longitudinal survey (12). However, it was only detected at follow-up in the two patients with altered CV tests and impaired quality of life. The data suggest that early detection of autonomic derangement might be susceptible to correction by appropriate intervention, at least in its functional if not organic component (34). Similar to what happened with the slowly evolving history of islet cell autoimmunity, identification of target antigens within nervous tissue will probably allow the setting of specific immunometric assays, overcoming the limits of immunofluorescence techniques. However, the assay used in this article is both feasible and reproducible (11–13), and it is hoped that demonstration of its predictive value will stimulate both the development of more specific techniques and more systematic search for autonomic neuropathy in the patients.

Furthermore, this study shows that autonomic neuropathy, together with female sex and the occurrence of severe hypoglycemia, is a major determinant for poor quality of life in patients with type 1 diabetes. This is in agreement with previous reports (35) and linked to such invalidating symptoms as orthostatic hypotension and chronic diarrhea. In fact, the subscales involved were impact of diabetes, social/vocational worries, and diabetes-related worries. In contrast, somatic neuropathy, not associated with impaired quality of life in this cohort, was mostly present as nonpainful paresthesias, whereas previous reports of impaired quality of life were in people with painful neuropathy or detected by the more specific NeuroQoL (36), which we did not use, because a validated Italian version is not available for this questionnaire. Previous reports had shown better quality of life among men than women, both in general and diabetic populations (37,38) and a relationship between quality of life and hypoglycemia was also reported by others (35,38). In this cohort, the impact of severe hypoglycemia was specifically related to the diabetes-related worries subscale.

In conclusion, the current study provides persuasive evidence for a primary pathogenic role of autoimmunity in the development of autonomic diabetic neuropathy. However, the mechanisms through which auto-Ab impair their target organ function, whether through classical complement action, proapoptotic effects of complement, enhanced antigen presentation, or channelopathy (26,39,40), remain to be elucidated.

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**Duality of Interest.** No potential conflicts of interest relevant to this article were reported.

**Author Contributions.** M.M.Z. was responsible for conception and design of the study, performed the study, analyzed data, and wrote the manuscript. A.R., E.C., M.Trev., and E.F. performed the study and collected data. M.Tren., F.C., M.P., and G.C. analyzed data and reviewed and finalized the manuscript. P.P. recruited patients. All the authors gave the final approval to the submission of the manuscripts. M.M.Z. and M.P. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

## References

1. O'Brien IA, McFadden JP, Corrall RJ. The influence of autonomic neuropathy on mortality in insulin-dependent diabetes. Q J Med 1991;79:495–502
2. Veglio M, Sivieri R, Chinaglia A, Scaglione L, Cavallo-Perin P. QT interval prolongation and mortality in type 1 diabetic patients: a 5-year cohort prospective study.

Neuropathy Study Group of the Italian Society of the Study of Diabetes, Piemonte Affiliate. Diabetes Care 2000;23:1381–1383

3. Mogensen UM, Jensen T, Køber L, et al. Cardiovascular autonomic neuropathy and subclinical cardiovascular disease in normoalbuminuric type 1 diabetic patients. Diabetes 2012;61:1822–1830
4. Vinik AI, Ziegler D. Diabetic cardiovascular autonomic neuropathy. Circulation 2007; 115:387–397
5. Vinik AI, Maser RE, Mitchell BD, Freeman R. Diabetic autonomic neuropathy. Diabetes Care 2003;26:1553–1579
6. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329: 977–986
7. Rabinowe SL, Brown FM, Watts M, Smith AM. Complement-fixing antibodies to sympathetic and parasympathetic tissues in IDDM. Autonomic brake index and heart-rate variation. Diabetes Care 1990;13: 1084–1088
8. Zanone MM, Peakman M, Purewal T, Watkins PJ, Vergani D. Autoantibodies to nervous tissue structures are associated with autonomic neuropathy in type 1 (insulin-dependent) diabetes mellitus. Diabetologia 1993;36:564–569
9. Muhr-Becker D, Ziegler AG, Druschky A, et al. Evidence for specific autoimmunity against sympathetic and parasympathetic nervous tissues in Type 1 diabetes mellitus and the relation to cardiac autonomic dysfunction. Diabet Med 1998;15:467–472
10. Ejskjaer N, Arif S, Dodds W, et al. Prevalence of autoantibodies to autonomic nervous tissue structures in Type 1 diabetes mellitus. Diabet Med 1999;16:544–549
11. Zanone MM, Burchio S, Quadri R, et al. Autonomic function and autoantibodies to autonomic nervous structures, glutamic acid decarboxylase and islet tyrosine phosphatase in adolescent patients with IDDM. J Neuroimmunol 1998;87:1–10
12. Zanone MM, Favaro E, Catalfamo E, et al. Autonomic function and autoantibodies to autonomic nervous tissues at follow-up in a cohort of young patients with type 1 diabetes. Effects of serum from diabetic patients on human adrenergic cells. J Neuroimmunol 2003;139:66–75
13. Granberg V, Ejskjaer N, Peakman M, Sundkvist G. Autoantibodies to autonomic nerves associated with cardiac and peripheral autonomic neuropathy. Diabetes Care 2005;28:1959–1964
14. Vernino S, Low PA, Fealey RD, Stewart JD, Farrugia G, Lennon VA. Autoantibodies to ganglionic acetylcholine receptors in autoimmune autonomic neuropathies. N Engl J Med 2000;343:847–855
15. Vernino S, Sandroni P, Singer W, Low PA. Invited article: autonomic ganglia: target and novel therapeutic tool. Neurology 2008;70:1926–1932
16. Dyck PJ. Detection, characterization, and staging of polyneuropathy: assessed in diabetics. Muscle Nerve 1988;11:21–32
17. Ewing DJ, Clarke BF. Diagnosis and management of diabetic autonomic neuropathy. Br Med J (Clin Res Ed) 1982; 285:916–918
18. O'Brien IA, O'Hare P, Corrall RJ. Heart rate variability in healthy subjects: effect of age and the derivation of normal ranges for tests of autonomic function. Br Heart J 1986;55:348–354
19. The DCCT Research Group. Reliability and validity of a diabetes quality-of-life measure for the diabetes control and complications trial (DCCT). Diabetes Care 1988;11:725–732
20. Mannucci E, Mezzani B, Conti A, Rotella CM. Valutazione della qualità della vita nei pazienti adulti diabetici tipo 1. Il Diabete 1994;6:223–228
21. Ejskjaer NT, Zanone MM, Peakman M. Autoimmunity in diabetic autonomic neuropathy: does the immune system get on your nerves? Diabet Med 1998;15:723–729
22. Ziegler D, Dannehl K, Mühlen H, Spüler M, Gries FA. Prevalence of cardiovascular autonomic dysfunction assessed by spectral analysis, vector analysis, and standard tests of heart rate variation and blood pressure responses at various stages of diabetic neuropathy. Diabet Med 1992;9: 806–814
23. Stella P, Ellis D, Maser RE, Orchard TJ. Cardiovascular autonomic neuropathy (expiration and inspiration ratio) in type 1 diabetes. Incidence and predictors. J Diabetes Complications 2000;14:1–6
24. Witte DR, Tesfaye S, Chaturvedi N, Eaton SE, Kempler P, Fuller JH; EURODIAB Prospective Complications Study Group. Risk factors for cardiac autonomic neuropathy in type 1 diabetes mellitus. Diabetologia 2005;48:164–171
25. Orchard TJ, Lloyd CE, Maser RE, Kuller LH. Why does diabetic autonomic neuropathy predict IDDM mortality? An analysis from the Pittsburgh Epidemiology of Diabetes Complications Study. Diabetes Res Clin Pract 1996;34(Suppl.):S165–S171
26. Lennon VA, Ermilov LG, Szurszewski JH, Vernino S. Immunization with neuronal nicotinic acetylcholine receptor induces neurological autoimmune disease. J Clin Invest 2003;111:907–913
27. Sandroni P, Vernino S, Klein CM, et al. Idiopathic autonomic neuropathy: comparison of cases seropositive and seronegative for ganglionic acetylcholine receptor antibody. Arch Neurol 2004;61: 44–48
28. Maule S, Quadri R, Mirante D, et al. Autonomic nervous dysfunction in systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA): possible pathogenic role of autoantibodies to autonomic nervous structures. Clin Exp Immunol 1997;110: 423–427
29. Vinik AI, Anandacoomaraswamy D, Ullal J. Antibodies to neuronal structures: innocent bystanders or neurotoxins? Diabetes Care 2005;28:2067–2072
30. Briani C, Doria A, Ruggero S, et al. Antibodies to muscle and ganglionic acetylcholine receptors (AchR) in celiac disease. Autoimmunity 2008;41:100–104
31. Winer S, Tsui H, Lau A, et al. Autoimmune islet destruction in spontaneous type 1 diabetes is not beta-cell exclusive. Nat Med 2003;9:198–205
32. Carrillo J, Puertas MC, Alba A, et al. Islet-infiltrating B-cells in nonobese diabetic mice predominantly target nervous system elements. Diabetes 2005;54:69–77
33. Prince CT, Secrest AM, Mackey RH, Arena VC, Kingsley LA, Orchard TJ. Cardiovascular autonomic neuropathy, HDL cholesterol, and smoking correlate with arterial stiffness markers determined 18 years later in type 1 diabetes. Diabetes Care 2010;33: 652–657
34. Rosengård-Bärlund M, Bernardi L, Fagerudd J, et al.; FinnDiane Study Group. Early autonomic dysfunction in type 1 diabetes: a reversible disorder? Diabetologia 2009;52:1164–1172
35. Solli O, Stavem K, Kristiansen IS. Health-related quality of life in diabetes: the associations of complications with EQ-5D scores. Health Qual Life Outcomes 2010;8: 18
36. Vileikyte L, Peyrot M, Bundy C, et al. The development and validation of a neuropathy- and foot ulcer-specific quality of life instrument. Diabetes Care 2003;26: 2549–2555
37. Hibbard JH. Sex differences in health and illness orientation. Int Q Community Health Educ 1983;4:95–104
38. Lloyd CE, Orchard TJ. Physical and psychological well-being in adults with type 1 diabetes. Diabetes Res Clin Pract 1999;44: 9–19
39. Fishelson Z, Attali G, Mevorach D. Complement and apoptosis. Mol Immunol 2001;38:207–219
40. Benitez-Ribas D, Adema GJ, Winkels G, et al. Plasmacytoid dendritic cells of melanoma patients present exogenous proteins to CD4+ T cells after Fc gamma RII-mediated uptake. J Exp Med 2006;203:1629–1635