Troponin T Parallels Structural Nerve Damage in Type 2 Diabetes: A Cross-sectional Study Using Magnetic Resonance Neurography

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Clinical studies have suggested that changes in peripheral nerve microcirculation may contribute to nerve damage in diabetic polyneuropathy (DN). High-sensitivity troponin T (hsTNT) assays have been recently shown to provide predictive values for both cardiac and peripheral microangiopathy in type 2 diabetes (T2D). This study investigated the association of sciatic nerve structural damage in 3 Tesla (3T) magnetic resonance neurography (MRN) with hsTNT and N-terminal pro-brain natriuretic peptide serum levels in patients with T2D. MRN at 3T was performed in 51 patients with T2D (23 without DN, 28 with DN) and 10 control subjects without diabetes. The sciatic nerve's fractional anisotropy (FA), a marker of structural nerve integrity, was correlated with clinical, electrophysiological, and serological data. In patients with T2D, hsTNT showed a negative correlation with the sciatic nerve's FA ($r = -0.52$, $P < 0.001$), with a closer correlation in DN patients ($r = -0.66$, $P < 0.001$). hsTNT further correlated positively with the neuropathy disability score ($r = 0.39$, $P = 0.005$). Negative correlations were found with sural nerve conduction velocities (NCVs) ($r = -0.65$, $P < 0.001$) and tibial NCVs ($r = -0.44$, $P = 0.002$) and amplitudes ($r = -0.53$, $P < 0.001$). This study is the first to show that hsTNT is a potential indicator for structural nerve damage in T2D. Our results indirectly support the hypothesis that microangiopathy contributes to structural nerve damage in T2D.

Diabetic polyneuropathy (DN) is one of the most frequent and most severe complications of diabetes, causing high morbidity, a reduction in affected patients' quality of life, and enormous health care costs (1,2). Although several risk factors and pathophysiological pathways associated with DN, such as inflammatory processes, hyperglycemia-induced deposition of advanced glycation end products in the extracellular matrix of Schwann cells, a decrease in renal function, and effects of dyslipidemia, have been identified and investigated in in vivo and in vitro studies, the overall pathology of DN remains poorly understood (3,4). Previous studies on potential serological markers that allow for an early quantification and prediction of nerve damage in DN focused on glycated hemoglobin (HbA_{1c}) levels, serum cholesterol levels, inflammatory markers levels, or parameters of renal function. These studies yielded controversial results, and to date, it has not been possible to identify a reliable serological marker for nerve damage in DN (4–6). Several clinical and in vitro studies have suggested that microvascular disease and consecutive nerve ischemia may contribute to nerve damage in DN (7,8). Furthermore, recent studies have shown that high-sensitivity troponin T (hsTNT) assays and N-terminal pro-brain natriuretic peptide (proBNP) provide predictive values for both cardiac and peripheral microangiopathy in type 2 diabetes (T2D) (9–12).

Studies on in vivo high-resolution magnetic resonance neurography (MRN) at 3 Tesla (3T) in DN have found that MRN allows a precise localization and quantification of nerve lesions at early stages of DN with a higher accuracy than clinical and electrophysiological examinations alone,

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that different patterns of nerve damage can be attributed to different risk factors (e.g., hyperglycemia and dyslipidemia), and that diffusion tensor imaging (DTI) allows for a very accurate assessment of the structural integrity of affected nerves (13–15).

This study combined hsTNT and proBNP assays with results derived from automated nerve fiber segmentation after 3T DTI MRN, as well as clinical, electrophysiological, and serological testing, to investigate whether hsTNT and proBNP may serve as potential markers for nerve damage in DN.

RESEARCH DESIGN AND METHODS

Study Design and Participants

The local ethics committee approved this study (Heidelberg Study on Diabetes and Complications [HEIST-DiC], local ethics number S-383/2016, ClinicalTrials.gov identifier NCT03022721), and all study participants gave informed, written consent. There were 51 patients (17 women, 34 men; mean age 66.7 ± 9.7 years; age range 41–86 years) with T2D (23 without DN, 28 with DN) and 10 control subjects (7 women, 3 men; mean age 55.9 ± 12.6 years; age range 31–69 years) who took part in this study between June 2015 and February 2019. Overall exclusion criteria were age <18, pregnancy, any history of lumbar surgery or disc protrusion, any contraindications for MRI, any other risk factors for neuropathy, such as alcoholism, hypovitaminosis, malignant or infectious diseases, any previous or ongoing exposure to neurotoxic agents, monoclonal gammopathy, and any chronic neurological diseases such as Parkinson disease, multiple sclerosis, or restless leg syndrome. Additional exclusion criteria for control subjects were any signs of neuropathy in their medical history or in the clinical and electrophysiological examinations listed below.

Clinical and Electrophysiological Examination

A detailed medical history was documented for every patient, and the examination of neuropathic symptoms was performed according to the guidelines of the German Diabetes Association (Deutsche Diabetes Gesellschaft [DDG]), which included the evaluation of the neuropathy symptom score (NSS) and the neuropathy disability score (NDS) (16–18). DN was determined with one or both of the following two criteria (16–18): *1*) a score of ≥4 in NSS or NDS (if a discrepancy between NSS and NDS was found, we chose the higher score), and/or *2*) abnormal results of nerve conduction parameters as mentioned below in at least two different nerves.

The electrophysiological examination (VikingQuest; Viasys Healthcare GmbH, Höchberg, Germany) of the right leg was conducted by two specially trained medical technical assistants with >5 years of experience in electrophysiological examinations on patients with diabetes. Examinations included distal motor latencies of the right tibial and peroneal nerve, motor and sensory amplitudes (compound muscle action potentials [CMAPs] and sensory nerve action potentials [SNAPs], respectively) of the tibial, peroneal, and sural nerves, and nerve conduction velocities (NCVs) of the tibial, peroneal, and sural nerves. It was ensured that skin temperature was at least 32°C throughout the examination. Intima-media thickness (IMT) of both carotid arteries and the diameter of the abdominal aorta were measured with duplex ultrasound examination (device: Samsung Sono ACE ×8). A resting-state electrocardiogram (ECG) and 24-h blood pressure were recorded (TM-2430 with CA11 blood pressure cuff, adapted in size to the circumference of each participant's upper arm; Boso d.o.o.). The ankle-brachial index (ABI) and pulse-wave velocity (PWV) were calculated using noninvasive blood pressure measurements of arms and ankles (ABI

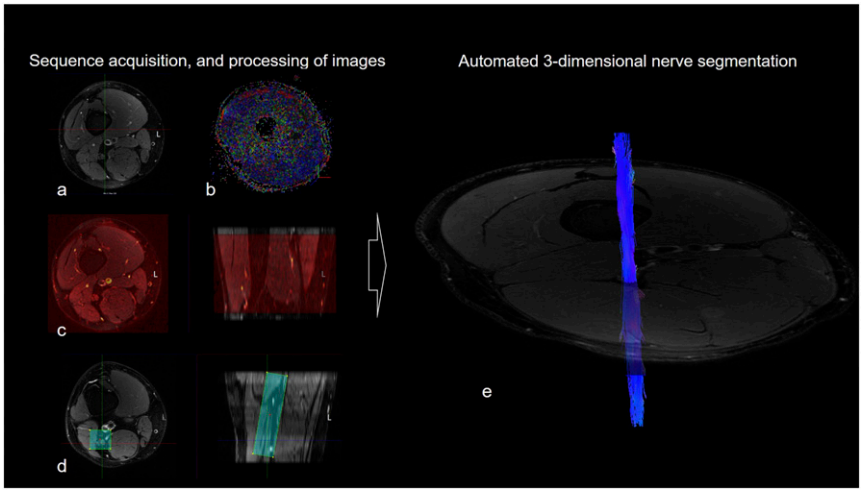

Figure 1—Acquisition and coregistration of MRI sequences with subsequent automated segmentation of the sciatic nerve. *A*: Acquisition of anatomical T2-weighted (T2w), fat-suppressed (FS) sequences of the right thigh. *B*: Automated coregistration of T2wFS and DTI sequences. *C*: Manual selection of nerve region on anatomical T2wFS images. *D* and *E*: Automated three-dimensional segmentation of the sciatic nerve (*D*) and resulting fiber tracts of the sciatic nerve (*E*).

Table 1—MRN, clinical, epidemiological, and serological data of control subjects, patients with T2D with DN, and patients T2D without DN

	Control subjects	T2D no DN	T2D DN	*P* (ANOVA)
FA	0.549 ± 0.052	0.531 ± 0.038	0.473 ± 0.056	<0.001***
Age (years)	55.9 ± 12.6	63.1 ± 10.5	68.7 ± 8.1	0.001**
Sex				
Female	7	9	9	n.a.
Male	3	16	19	n.a.
Disease duration (years)	n.a.	7.5 ± 5.8	13.5 ± 9.8	0.079^{ns}
NSS	0 ± 0	1.00 ± 1.94	6.24 ± 3.09	<0.001***
NDS	0.80 ± 1.32	0.87 ± 0.97	5.46 ± 1.95	<0.001***
Tibial CMAPs (mV)	17.85 ± 6.90	13.51 ± 5.45	9.26 ± 7.00	0.004**
Tibial NCVs (m/s)	47.33 ± 4.66	42.64 ± 4.82	40.44 ± 4.89	0.002**
Tibial DMLs (ms)	3.53 ± 0.73	3.93 ± 0.79	5.32 ± 2.86	0.006**
Peroneal CMAPs (mV)	7.66 ± 1.25	6.56 ± 3.94	3.64 ± 2.72	<0.001***
Peroneal NCVs (m/s)	45.86 ± 4.02	40.09 ± 6.031	37.67 ± 5.96	0.004**
Peroneal DMLs (ms)	3.79 ± 0.31	3.93 ± 0.90	5.40 ± 2.34	0.021*
Sural SNAPs (μV)	7.20 ± 3.00	5.71 ± 3.61	4.20 ± 2.01	0.043*
Sural NCVs (m/s)	48.00 ± 4.50	47.78 ± 5.38	27.86 ± 22.55	0.031*
hsTNT (pg/mL)	6.00 ± 1.70	8.21 ± 5.11	11.64 ± 5.38	<0.001***
proBNP (pg/mL)	60.30 ± 43.69	115.8 ± 146.4	109.4 ± 87.78	0.283^{ns}
HbA_{1c} (%)	5.48 ± 0.44	6.94 ± 1.49	6.98 ± 1.14	<0.001***
HbA_{1c} (mmol/mol)	36.28 ± 2.22	52.34 ± 2.75	47.39 ± 2.5	<0.001***
Creatinine (mg/dL)	0.78 ± 0.14	0.85 ± 0.24	0.84 ± 0.16	0.577^{ns}
GFR–CKD-EPI (mL/min)	89.95 ± 11.89	89.02 ± 17.11	85.84 ± 14.72	0.750^{ns}
Cystatin C (mg/L)	0.837 ± 0.092	0.949 ± 0.205	0.904 ± 0.318	0.174^{ns}
Serum cholesterol (mg/dL)				
Total	211.00 ± 40.73	191.20 ± 38.80	198.00 ± 45.90	0.472^{ns}
HDL cholesterol	67.20 ± 17.31	49.13 ± 14.21	54.11 ± 16.53	0.014*
LDL cholesterol	122.20 ± 38.66	108.60 ± 36.05	111 ± 42.34	0.653^{ns}
Triglycerides (mg/dL)	108.70 ± 44.61	190.40 ± 120.30	170.30 ± 96.78	0.095^{ns}

All values are displayed as mean ± SD. DML, distal motor latencies; GFR, glomerular filtration rate; n.a., not applicable. Level of significance: $^{ns}P > 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

System 1000; Boso d.o.o.). Additionally, the heart rate variability (HRV) test was performed for the assessment of cardiac autonomic neuropathy (CAN) as recommended by the German Society for Diabetology (18,19).

Blood was drawn in the fasting state and processed immediately under standardized conditions in the central laboratory of Heidelberg University Hospital. Albumin excretion in urine was measured in morning spot urine for all participants. The estimated glomerular filtration rate was obtained with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (20). Plasma levels of all serological parameters acquired were analyzed with clinical chemistry automation according to the appropriate standard operating protocol in the central laboratory of Heidelberg University Hospital.

MRN Imaging Protocol

We performed high-resolution MRN of the right thigh in a 3T MR scanner (Magnetom TIM-TRIO; Siemens Healthcare, Erlangen, Germany) for all study participants, using a 15-channel transmit-receive extremity coil and the following sequences:

1) Axial high-resolution T2-weighted turbo spin echo two-dimensional sequence with spectral fat saturation, and the following parameters: relaxation time = 5,970 ms, echo time = 55 ms, field of view = 160 × 160 mm^2, matrix size = 512 × 512, slice thickness = 4 mm, interslice gap = 0.35 mm, voxel size = 0.5 × 0.3 × 4.0 mm^3, 24 slices, 24 acquired images.
2) Axial fat-suppressed, diffusion-weighted two-dimensional echo-planar sequence with the following parameters: relaxation time = 5,100 ms; echo time = 92.8 ms; b = 0 m, and 1,000 s/mm^2; directions = 20; field of view = 160 × 160 mm^2; matrix size = 128 × 128; slice thickness = 4 mm; voxel size = 1.3 × 1.3 × 4 mm^3; no interslice gap, 3 averages, 24 slices, 1,512 acquired images.

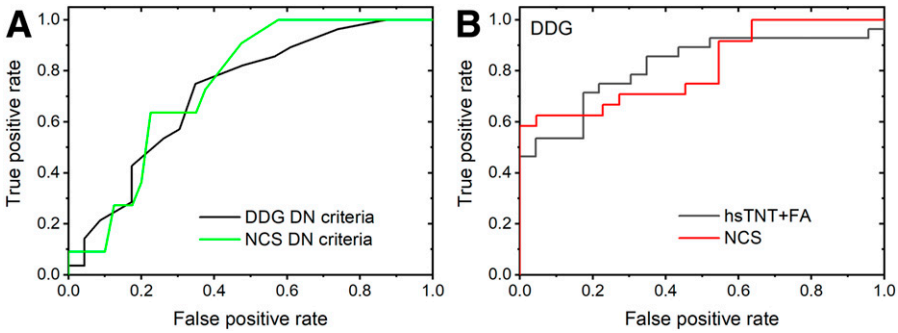

Figure 2—ROC curves to evaluate diagnostic validity. *A*: ROC curves for hsTNT against gold standard DDG DN criteria (including results from NDS, NSS, and abnormal NCS; black curve) and against abnormal NCS results only (green curve). According to the Youden *J* statistic, the sensitivity and specificity for hsTNT against DDG DN criteria to optimally differentiate between DN and no DN are 75.0% and 65.2%, respectively, and for hsTNT against abnormal NCS results only, we find sensitivity and specificity at 72.7% and 62.5%, respectively. AUC values are 0.72 for hsTNT against the gold standard and 0.75 for hsTNT against abnormal NCS results. *B*: ROC curves for combined hsTNT levels and FA against DDG DN criteria (black curve; AUC = 81.83%) and NCS results for the same participant collective (red curve; AUC = 81.44%).

The sequences were centered on the sciatic nerve's bifurcation to ascertain that the anatomical region mapped by MRN was comparable in all participants.

Image Postprocessing and Statistical Analysis

All images were pseudonymized. Images were then analyzed in an automated approach using Nordic BRAINEX, a U.S. Food and Drug Administration–approved processing software designed for automated calculation and reconstruction of fiber tracts in diffusion-weighted imaging (21,22). A total number of 61 × 1,536 = 93,696 images were analyzed accordingly. T2-weighted and diffusion-weighted sequences were coregistered, and the region of the sciatic nerve was marked by two trained neuroradiologists with 3 and 4 years of experience in MRN imaging, respectively. According to the results of former studies on DTI in the sciatic nerve, the nerve was automatically segmented with a threshold of >0.3 for the nerve's fractional anisotropy (FA), a dimensionless quantity that measures directed diffusion, with values between 0 (isotropic diffusion) and 1 (diffusion in only one direction). FA is therefore considered as a general measure for structural nerve integrity and was shown to be a highly sensitive parameter for nerve damage in previous clinical studies (23,24). The average FA of the segmented nerve fibers was calculated automatically (22). A graphic overview of the process of image coregistration and nerve segmentation is given in Fig. 1.

Statistical Analysis

Statistical data analysis was performed with GraphPad Prism 7 and MATLAB 7.14.0.0739 (R2012a) software. We tested for Gaussian normal distribution with the D'Agostino-Pearson omnibus normality test. When a Gaussian normal distribution was given, we used *t* tests for comparisons of two groups, one-way ANOVAs for comparisons of more than two groups, and Pearson correlation coefficients for correlation analysis. When data were not Gaussian distributed, we used the Mann-Whitney test for comparisons of two groups, the Kruskal-Wallis test for multiple comparisons of more than three groups with post hoc Dunn correction, and nonparametric Bonferroni-corrected Spearman correlation for correlation analysis. If multiple significant correlations were found for one parameter, partial correlation analysis controlled for all significant correlations was performed to rule out confounding variables (25). Wilcox-modified Johnson-Neyman intervals, which determine 95% CIs for covariate-controlled group comparisons, were calculated in R 3.6.1 open-source software (www.r-project.org) (26). Receiver operating characteristic (ROC) curves to obtain diagnostic validity (of hsTNT to predict DN, see below), as well as Youden *J* statistics to determine sensitivity and specificity at optimal diagnostic threshold in ROC curves (27), were calculated in MATLAB.

The level of significance for all tests was defined at $P < 0.05$. All results are presented as mean values ± SD.

Data and Resource Availability

The data set and subdata sets generated and/or analyzed in the current study are not publicly available because they contain patient data from Heidelberg University Hospital. Data can be made available after anonymization from the corresponding author upon reasonable request for research purpose after approval by the local ethics committee.

RESULTS

Clinical and Epidemiological Data

There were 51 patients (17 women, 34 men; mean age 66.7 ± 9.7 years; age range 41–86 years) with T2D (23 without DN, 28 with DN) and 10 control subjects (7 women, 3 men; mean age 55.9 ± 12.6 years; age range 31–69 years) who took part in this study. ECGs showed no signs of an acute or previous myocardial infarction in any of the participants. ANOVA revealed no significant difference for age between control subjects and patients with T2D without DN

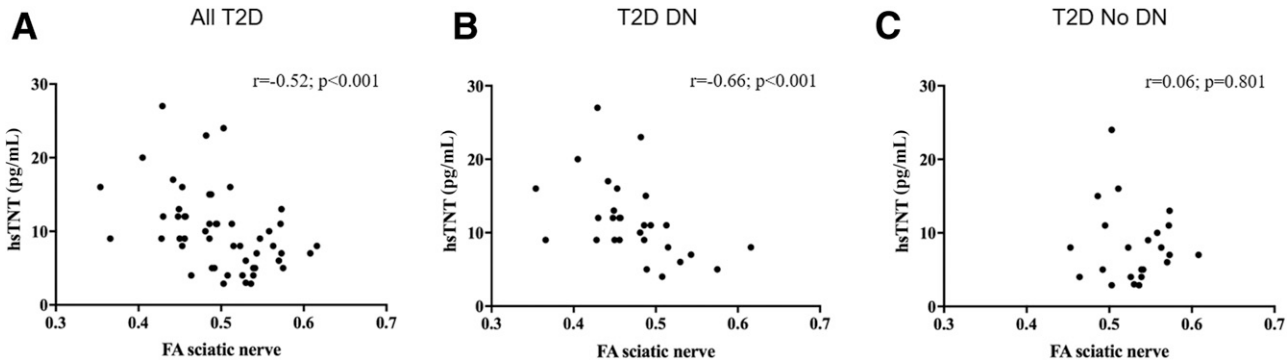

Figure 3—Correlation of hsTNT (in pg/mL) with the sciatic nerve's FA. *A*: There is a negative correlation of hsTNT and FA in all participants with T2D (Pearson $r = -0.52$; $P < 0.001$). *B* and *C*: The negative correlation of hsTNT and FA is increased (Pearson $r = -0.66$; $P < 0.001$) in patients with T2D with DN (*B*), whereas there is no significant correlation of hsTNT and FA in all participants with T2D without DN (Pearson $r = 0.06$; $P = 0.801$) (*C*).

($P = 0.092$) but did for control subjects and patients with T2D with DN ($P < 0.001$). As expected, patients with T2D with DN showed higher values for NSS and NDS scores compared with patients with T2D without DN or control subjects ($P < 0.001$). HbA_{1c} levels differed significantly between patients with T2D and control subjects ($P < 0.001$), but not between patients with T2D with and without DN ($P > 0.999$). No significant differences were found for parameters of renal function or dyslipidemia. An overview of all clinical and serological data are given in Table 1.

hsTNT, proBNP, and Clinical Parameters

A Kruskal-Wallis test with post hoc Dunn correction revealed that patients with DN showed higher values of hsTNT compared with control subjects and patients with T2D without DN ($P < 0.001$). Additional Johnson-Neyman intervals for age as a covariate (i.e., potential confounder) revealed significant differences between the DN group and no DN group and healthy control subjects, respectively (DN vs. no DN: 259.13, 18.27 years, DN vs. healthy controls: 322.09, 21.51 years, where lower bounds larger than upper bounds denote group difference within the covariate's full considered range: 31–86 years). No significant difference was found for no DN versus healthy control subjects for participants with age up to 41.83 years. Thus, increased hsTNT in patients with DN compared with patients without DN or control subjects remained stable when controlled for age. For proBNP, no significant differences between the three groups could be detected ($P = 0.283$). In T2D, hsTNT correlated positively with age ($r = 0.51$, $P < 0.001$). This correlation could only be reproduced in patients with T2D without DN ($r = 0.61$, $P = 0.002$), but not in DN patients ($r = 0.36$, $P = 0.063$) or control subjects ($r = -0.10$, $P = 0.79$). No correlation was found for hsTNT or proBNP with diabetes duration ($r = 0.10$, $P = 0.54$, and $r = 0.25$, $P = 0.164$, respectively). In T2D, hsTNT correlated positively with NDS ($r = 0.39$, $P = 0.005$) but not with NSS ($r = 0.26$, $P = 0.063$). Negative correlations were found with sural ($r = -0.65$, $P < 0.001$), tibial ($r = -0.44$, $P = 0.002$) and peroneal ($r = -0.42$, $P = 0.003$) NCVs and tibial ($r = -0.53$, $P < 0.001$) and peroneal ($r = -0.29$, $P = 0.044$) nerve amplitudes. To determine the diagnostic validity of hsTNT as a marker for DN, ROC curves were obtained for hsTNT against both of the diagnostic criteria for DN issued by the DDG (i.e., condition 1 and/or 2) (Fig. 2*A*, black curve; area under the curve [AUC] = 71.89%) and against DN criteria derived from abnormal nerve conduction study (NCS) results (i.e., condition 2 only) (see Fig. 2*A*, green curve; AUC = 74.66%). According to the Youden *J* statistic, the sensitivity and specificity for hsTNT against DDG DN criteria to optimally differentiate between DN and no DN are 75.0% and 65.2%, respectively, and for hsTNT against abnormal NCS results only, we find sensitivity and specificity at 72.7% and 62.5%, respectively. The combined measure of hsTNT levels and FA outperforms NCS results in diagnostic validity against DDG DN criteria (see Fig. 2*B*) (hsTNT + FA vs. DDG DN criteria: AUC = 81.83%; NCS results vs. DDG DN criteria: AUC = 81.44%).

No correlations were found for proBNP with any of the acquired electrophysiological parameters or neuropathy scores. In the 24-h blood pressure recordings, hsTNT and proBNP were both negatively correlated with the pulse frequency ($r = -0.40$, $P = 0.022$ and $r = -0.58$, $P < 0.001$, respectively), but showed no significant correlations with systolic or diastolic values or the mean arterial blood pressure. Positive correlations were found for hsTNT and proBNP with the PWV ($r = 0.46$, $P = 0.001$ and $r = 0.38$, $P = 0.007$, respectively). In the test for HRV, proBNP was correlated positively with the heart rate in standing ($r = 0.45$, $P = 0.001$) and lying positions ($r = 0.31$, $P = 0.029$). No such correlations were found for hsTNT. No significant correlations were found for hsTNT or proBNP with the ABI, the IMT of the right and left carotid artery, or the diameter of the abdominal aorta. Also, no significant correlations were found for hsTNT with any of the acquired ECG parameters. proBNP was correlated with cystatin C ($r = 0.30$, $P = 0.033$), hemoglobin ($r = -0.42$, $P = 0.002$),

Table 2—Partial correlation analysis for FA with hsTNT, age, and cystatin C

	No controlling		Double controlling for age and cystatin C		Double controlling for age and hsTNT		Double controlling for hsTNT and cystatin C	
	All T2D	T2D DN	All T2D	T2D DN	All T2D	T2D DN	All T2D	T2D DN
FA vs. hsTNT	$r = -0.52$ $P < 0.00^{***}$	$r = -0.66$ $P < 0.00^{***}$	$r = -0.31$ $P = 0.02^{*}$	$r = -0.61$ $P = 0.00^{**}$	n.a.		n.a.	
FA vs. age	$r = -0.42$ $P = 0.00^{**}$	$r = -0.22$ $P = 0.26^{ns}$	n.a.		$r = -0.20$ $P = 0.17^{ns}$	$r = 0.14$ $P = 0.49^{ns}$	n.a.	
FA vs. cystatin C	$r = 0.29$ $P = 0.04^{*}$	$r = -0.40$ $P = 0.03^{*}$	n.a.		n.a.		$r = -0.05$ $P = 0.71^{ns}$	$r = -0.25$ $P = 0.22^{ns}$

n.a., not applicable. Level of significance: $^{ns}P > 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

and hematocrit ($r = -0.43$, $P = 0.002$). No significant correlations were found between hsTNT and any of the other acquired serological parameters.

MRN Imaging

FA of the Sciatic Nerve and Clinical Parameters

The automatically calculated FA of the distal tibial nerve was lower in DN patients compared with control subjects and patients with T2D without DN ($P < 0.001$). FA correlated with NDS ($r = -0.52$, $P < 0.001$) and NSS ($r = -0.36$, $P = 0.009$) scores. FA also correlated with sural ($r = 0.40$, $P = 0.033$), tibial ($r = 0.37$, $P = 0.011$), and peroneal ($r = 0.48$, $P < 0.001$) NCVs, tibial (($r = 0.57$, $P < 0.001$) and peroneal ($r = 0.65$, $P < 0.001$) amplitudes, and tibial ($r = -0.32$, $P = 0.029$) and peroneal distal motor latencies. Further correlations of the FA were found with the PWV ($r = -0.31$, $P = 0.033$). FA showed no significant correlations with any parameters of the 24-h blood pressure measurements, the ABI, the IMT of the right and left carotid artery, the diameter of the abdominal aorta, any parameters of the HRV test, or any ECG parameters.

FA of the Sciatic Nerve and Serological Parameters

For patients with T2D, FA was correlated negatively with hsTNT ($r = -0.52$, $P < 0.001$) (Fig. 3*A*), with a more pronounced correlation in DN patients ($r = -0.66$, $P < 0.001$) (Fig. 3*B*) and no significant correlation in patients without DN ($r = 0.06$, $P = 0.801$) (Fig. 3*C*) or in control subjects ($r = 0.21$, $P = 0.555$). Further correlations for patients with T2D were found between FA and patients' age ($r = -0.42$, $P = 0.002$) and cystatin C levels ($r = -0.29$, $P = 0.040$). The correlation between age and FA could not be reproduced for DN patients ($r = 0.37$, $P = 0.055$). In a double-controlled partial correlation analysis for hsTNT and FA, controlled for cystatin C and patients' age as potential confounders, the correlation between FA and hsTNT remained significant for the patients with T2D ($r = -0.31$, $P = 0.030$) and DN patients ($r = -0.61$, $P = 0.001$), whereas no significant correlation with FA could be found for cystatin C or patients' age when controlled for hsTNT (see also Table 2). No correlations were found between FA and proBNP ($r = -0.12$, $P = 0.411$) or any other of the acquired serological parameters. An overview of correlations of FA, hsTNT, and proBNP with clinical, electrophysiological, and serological parameters is given in Tables 3 and 4.

DISCUSSION

This study is the first to find a strong association of hsTNT assays with measures of diffusion-weighted neuroimaging that codifies structural nerve integrity in T2D DN as well as clinical neuropathy scores and electrophysiological data. Serum levels of hsTNT may therefore serve as a potential marker for structural nerve integrity in T2D DN. In addition, we showed that the results of a fully automated calculation of the sciatic nerve's FA provides close

Table 3—Correlations of FA, hsTNT, and proBNP with clinical, epidemiological, and serological data of control subjects and patients with T2D

	hsTNT		proBNP		FA	
	Control subjects	All T2D	Control subjects	All T2D	Control subjects	All T2D
FA	$r = -0.34$, $P = 0.331^{ns}$	$r = -0.52$, $P < 0.001^{***}$	$r = 0.01$, $P = 0.981^{ns}$	$r = -0.118$, $P = 0.411^{ns}$	n.a.	n.a.
Age (years)	$r = -0.10$, $P = 0.787^{ns}$	$r = -0.51$, $P < 0.001^{***}$	$r = 0.040$, $P = 0.917^{ns}$	$r = 0.170$, $P = 0.233^{ns}$	$r = -0.53$, $P = 0.117^{ns}$	$r = -0.42$, $P = 0.002^{**}$
BMI (kg/m^2)	$r = 0.21$, $P = 0.561^{ns}$	$r = -0.18$, $P = 0.266^{ns}$	$r = 0.45$, $P = 0.202^{ns}$	$r = -0.01$, $P = 0.950^{ns}$	$r = -0.36$, $P = 0.283^{ns}$	$r = 0.05$, $P = 0.721^{ns}$
Disease duration (years)	n.a.	$r = 0.10$, $P = 0.54^{ns}$	n.a.	$r = 0.25$, $P = 0.164^{ns}$	n.a.	$r = -0.15$, $P = 0.245^{ns}$
Sex (female = 1, male = 0)	$r = 0.31$, $P = 0.383^{ns}$	$r = -0.19$, $P = 0.241^{ns}$	$r = 0.06$, $P = 0.861^{ns}$	$r = 0.28$, $P = 0.076^{ns}$	$r = -0.30$, $P = 0.298^{ns}$	$r = 0.06$, $P = 0.756^{ns}$
NDS	$r = 0.01$, $P = 0.99^{ns}$	$r = 0.39$, $P = 0.005^{**}$	$r = 0.06$, $P = 0.867^{ns}$	$r = 0.15$, $P = 0.278^{ns}$	$r = -0.01$, $P = 0.973^{ns}$	$r = -0.52$, $P < 0.001^{***}$
NSS	$r = 0.39$, $P = 0.567^{ns}$	$r = 0.26$, $P = 0.063^{ns}$	$r = -0.36$, $P = 0.263^{ns}$	$r = 0.22$, $P = 0.113^{ns}$	$r = 0.05$, $P = 0.886^{ns}$	$r = -0.36$, $P = 0.009^{**}$
hsTNT (pg/mL)	n.a.	n.a.	$r = -0.12$, $P = 0.748^{ns}$	$r = 0.13$, $P = 0.350^{ns}$	$r = 0.21$, $P = 0.555$	$r = -0.52$, $P < 0.001^{***}$
proBNP (pg/mL)	$r = -0.12$, $P = 0.748^{ns}$	$r = 0.13$, $P = 0.350^{ns}$	n.a.	n.a.	$r = 0.01$, $P = 0.977^{ns}$	$r = -0.12$, $P = 0.411^{ns}$
HbA_{1c} (%/mmol/mol)	$r = 0.39$, $P = 0.303^{ns}$	$r = 0.21$, $P = 0.138^{ns}$	$r = -0.11$, $P = 0.780^{ns}$	$r = 0.20$, $P = 0.161^{ns}$	$r = -0.57$, $P = 0.108^{ns}$	$r = -0.19$, $P = 0.191^{ns}$
Cystatin C (mg/L)	$r = -0.38$, $P = 0.282^{ns}$	$r = -0.41$, $P = 0.003^{**}$	$r = -0.21$, $P = 0.560^{ns}$	$r = 0.30$, $P = 0.033^{*}$	$r = -0.05$, $P = 0.891^{ns}$	$r = -0.29$, $P = 0.040^{*}$
GFR–CKD-EPI (mL/min)	$r = -0.04$, $P = 0.923^{ns}$	$r = -0.23$, $P = 0.013^{*}$	$r = -0.11$, $P = 0.761^{ns}$	$r = -0.13$, $P = 0.101^{ns}$	$r = 0.43$, $P = 0.215^{ns}$	$r = 0.18$, $P = 0.156^{ns}$
Creatinine (mg/dL)	$r = -0.11$, $P = 0.760^{ns}$	$r = 0.15$, $P = 0.284^{ns}$	$r = 0.02$, $P = 0.964^{ns}$	$r < 0.01$, $P = 0.982^{ns}$	$r = 0.16$, $P = 0.663^{ns}$	$r < 0.01$, $P = 0.956^{ns}$
Cholesterol (mg/dL)	$r = 0.14$, $P = 0.700^{ns}$	$r = 0.06$, $P = 0.666^{ns}$	$r = -0.07$, $P = 0.855^{ns}$	$r = -0.08$, $P = 0.592^{ns}$	$r = -0.55$, $P = 0.102^{ns}$	$r = 0.07$, $P = 0.615^{ns}$
HDL cholesterol (mg/dL)	$r = 0.02$, $P = 0.954^{ns}$	$r = 0.11$, $P = 0.453^{ns}$	$r = 0.39$, $P = 0.265^{ns}$	$r = 0.37$, $P = 0.008^{**}$	$r = -0.56$, $P = 0.093^{ns}$	$r = -0.10$, $P = 0.708^{ns}$
LDL cholesterol (mg/dL)	$r = 0.13$, $P = 0.728^{ns}$	$r = 0.02$, $P = 0.911^{ns}$	$r = -0.21$, $P = 0.563^{ns}$	$r = -0.16$, $P = 0.284^{ns}$	$r = -0.49$, $P = 0.152^{ns}$	$r = 0.08$, $P = 0.567^{ns}$
Triglycerides (mg/dL)	$r = 0.03$, $P = 0.927^{ns}$	$r = -0.15$, $P = 0.306^{ns}$	$r = -0.19$, $P = 0.609^{ns}$	$r = -0.07$, $P = 0.618^{ns}$	$r = 0.68$, $P = 0.029^{*}$	$r = 0.22$, $P = 0.127^{ns}$

All values are displayed as mean ± SD. GFR, glomerular filtration rate; n.a., not applicable. Level of significance: $^{ns}P > 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 4—Correlations of FA, hsTNT, and proBNP with neurophysiological data, ultrasound, ECG, and blood pressure measurements

	hsTNT		proBNP		FA	
	Control subjects	All T2D	Control subjects	All T2D	Control subjects	All T2D
Sural NCVs (m/s)	$r = 0.36$, $P = 0.347^{ns}$	$r = -0.65$, $P < 0.001^{***}$	$r = -0.31$, $P = 0.416^{ns}$	$r = -0.30$, $P = 0.452^{ns}$	$r = -0.67$, $P = 0.049^{*}$	$r = 0.40$, $P = 0.033^{*}$
Sural SNAPs (μV)	$r = 0.49$, $P = 0.176^{ns}$	$r = -0.07$, $P = 0.365^{ns}$	$r = 0.46$, $P = 0.216^{ns}$	$r = -0.14$, $P = 0.174^{ns}$	$r = -0.22$, $P = 0.577^{ns}$	$r = 0.22$, $P = 0.199^{ns}$
Tibial NCVs (m/s)	$r = 0.49$, $P = 0.180^{ns}$	$r = -0.44$, $P = 0.002^{**}$	$r = -0.15$, $P = 0.708^{ns}$	$r = 0.09$, $P = 0.536^{ns}$	$r = 0.40$, $P = 0.280^{ns}$	$r = 0.37$, $P = 0.011^{*}$
Tibial CMAPs (mV)	$r = 0.12$, $P = 0.764^{ns}$	$r = -0.53$, $P < 0.001^{***}$	$r = -0.07$, $P = 0.860^{ns}$	$r = -0.01$, $P = 0.954^{ns}$	$r = -0.21$, $P = 0.580^{ns}$	$r = 0.57$, $P < 0.001^{***}$
Tibial DMLs (ms)	$r = 0.27$, $P = 0.480^{ns}$	$r = 0.50$, $P < 0.001^{***}$	$r = -0.32$, $P = 0.405^{ns}$	$r = 0.02$, $P = 0.919^{ns}$	$r = 0.25$, $P = 0.516^{ns}$	$r = -0.32$, $P = 0.029^{*}$
Peroneal NCVs (m/s)	$r = 0.38$, $P = 0.311^{ns}$	$r = -0.42$, $P = 0.003^{**}$	$r = 0.10$, $P = 0.791^{ns}$	$r = -0.03$, $P = 0.832^{ns}$	$r = 0.18$, $P = 0.651^{ns}$	$r = 0.48$, $P < 0.001^{***}$
Peroneal CMAPs (mV)	$r = -0.47$, $P = 0.201^{ns}$	$r = -0.29$, $P = 0.044^{*}$	$r = 0.25$, $P = 0.513^{ns}$	$r = -0.13$, $P = 0.382^{ns}$	$r = 0.24$, $P = 0.530^{ns}$	$r = 0.65$, $P < 0.001^{***}$
Peroneal DMLs (ms)	$r = 0.39$, $P = 0.306^{ns}$	$r = 0.21$, $P = 0.142^{ns}$	$r = -0.32$, $P = 0.406^{ns}$	$r = -0.10$, $P = 0.492^{ns}$	$r = -0.33$, $P = 0.379^{ns}$	$r = -0.37$, $P = 0.009^{**}$
24-h systolic MAP (mmHg)	$r = 0.45$, $P = 0.319^{ns}$	$r = 0.19$, $P = 0.304^{ns}$	$r = 0.21$, $P = 0.662^{ns}$	$r = 0.22$, $P = 0.217^{ns}$	$r = <0.01$, $P > 0.999^{ns}$	$r = 0.04$, $P = 0.843^{ns}$
24-h diastolic MAP (mmHg)	$r = 0.17$, $P = 0.748^{ns}$	$r = -0.10$, $P = 0.570^{ns}$	$r = 0.64$, $P = 0.139^{ns}$	$r = -0.14$, $P = 0.454^{ns}$	$r = 0.107$, $P = 0.840^{ns}$	$r = -0.10$, $P = 0.578^{ns}$
24-h MAP (mmHg)	$r = 0.09$, $P = 0.867^{ns}$	$r = 0.05$, $P = 0.789^{ns}$	$r = 0.20$, $P = 0.714^{ns}$	$r < 0.01$, $P = 0.995^{ns}$	$r = 0.14$, $P = 0.803^{ns}$	$r = -0.04$, $P = 0.950^{ns}$
24-h mean pulse frequency (bpm)	$r = -0.04$, $P = 0.943^{ns}$	$r = -0.40$, $P = 0.022^{*}$	$r = 0.21$, $P = 0.662^{ns}$	$r = -0.58$, $P < 0.001^{***}$	$r = 0.61$, $P = 0.09^{ns}$	$r = -0.10$, $P = 0.585^{ns}$
PWV (m/s)	$r = -0.14$, $P = 0.726^{ns}$	$r = 0.46$, $P = 0.001^{**}$	$r = 0.13$, $P = 0.735^{ns}$	$r = 0.38$, $P = 0.007^{**}$	$r = 0.46$, $P = 0.214^{ns}$	$r = -0.31$, $P = 0.033^{**}$
ABI left	$r = -0.24$, $P = 0.510^{ns}$	$r = -0.07$, $P = 0.649^{ns}$	$r = 0.59$, $P = 0.08^{ns}$	$r = -0.20$, $P = 0.160^{ns}$	$r = 0.11$, $P = 0.766^{ns}$	$r = 0.07$, $P = 0.654^{ns}$
ABI right	$r = -0.29$, $P = 0.417^{ns}$	$r = -0.18$, $P = 0.219^{ns}$	$r = 0.45$, $P = 0.195^{ns}$	$r = -0.28$, $P = 0.046^{*}$	$r = 0.32$, $P = 0.369^{ns}$	$r = 0.08$, $P = 0.572^{ns}$
HRV test pulse frequency						
In lying position (bpm)	$r = 0.16$, $P = 0.697^{ns}$	$r = -0.08$, $P = 0.599^{ns}$	$r = 0.03$, $P = 0.957^{ns}$	$r = 0.31$, $P = 0.029^{*}$	$r = 0.63$, $P = 0.075^{ns}$	$r = -0.01$, $P = 0.933^{ns}$
In standing position (bpm)	$r = 0.07$, $P = 0.879^{ns}$	$r = -0.15$, $P = 0.288^{ns}$	$r = -0.36$, $P = 0.339^{ns}$	$r = 0.45$, $P = 0.001^{**}$	$r = 0.33$, $P = 0.385^{ns}$	$r = 0.06$, $P = 0.666^{ns}$
HRV test E/I quotient	$r = 0.44$, $P = 0.242^{ns}$	$r = -0.21$, $P = 0.148^{ns}$	$r = 0.63$, $P = 0.076^{ns}$	$r = -0.23$, $P = 0.101^{ns}$	$r = -0.288$, $P = 0.459^{ns}$	$r = 0.15$, $P = 0.304^{ns}$
HRV test 30/15 quotient	$r = -0.62$, $P = 0.087^{ns}$	$r = 0.08$, $P = 0.602^{ns}$	$r = 0.32$, $P = 0.388^{ns}$	$r = -0.24$, $P = 0.101^{ns}$	$r = -0.16$, $P = 0.684^{ns}$	$r = -0.17$, $P = 0.244^{ns}$
IMT left carotid artery	$r = 0.26$, $P = 0.477^{ns}$	$r = 0.11$, $P = 0.454^{ns}$	$r = 0.22$, $P = 0.541^{ns}$	$r = 0.11$, $P = 0.454^{ns}$	$r = -0.20$, $P = 0.580^{ns}$	$r = 0.01$, $P = 0.676^{ns}$
IMT right carotid artery	$r = 0.33$, $P = 0.359^{ns}$	$r = 0.19$, $P = 0.194^{ns}$	$r = -0.30$, $P = 0.408^{ns}$	$r = 0.24$, $P = 0.091^{ns}$	$r = -0.09$, $P = 0.803^{ns}$	$r = -0.16$, $P = 0.403^{ns}$
Abdominal aorta diameter (mm)	$r = -0.54$, $P = 0.11^{ns}$	$r = -0.14$, $P = 0.350^{ns}$	$r = -0.11$, $P = 0.768^{ns}$	$r = 0.05$, $P = 0.726^{ns}$	$r = -0.05$, $P = 0.883^{ns}$	$r = -0.10$, $P = 0.607^{ns}$

All values are displayed as mean ± SD. DML, distal motor latencies; E/I quotient, quotient of the longest RR interval under expiration and the shortest RR interval under inspiration in the HRV test; HRV, heart rate variability; MAP, mean arterial pressure; n.a., not applicable; 30/15 quotient, quotient of the longest RR interval between beat 20 and 40 and the shortest RR interval between beats 5 and 25 after standing up in the HRV test. Level of significance: $^{ns}P > 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

correlations with clinical and electrophysiological parameters and that the combination of FA and hsTNT outranks electrophysiological tests as a predictor for neuropathic damage in DN.

Our results are in line with recent studies that focused on hsTNT as a potential marker for microvascular events in T2D (12). Because hsTNT has been shown to be a marker for cardiac microangiopathy, our results support the hypothesis that a decrease in neural blood supply, as a consequence of peripheral microangiopathy after cardiac microvascular disease, is a major contributor to the deterioration of axons and Schwann cells in DN (10,28,29). The finding that proBNP was neither correlated with any of the acquired clinical scores for DN nor with any of the electrophysiological parameters further supports this hypothesis: because proBNP is a peptide released in the left cardiac atrium as a consequence of myocardial distension, it is a marker for cardiac congestion but not for cardiac microvascular disease (30). Our proBNP results are not in line with findings from previous studies on the predictive value of proBNP for microvascular complications in DN (12). This may be explained by the fact that hsTNT and proBNP often show close correlations since cardiac microangiopathy or cardiac ischemia often results in cardiac congestion and heart failure, which makes it hard to distinguish which of the two parameters is the more important factor with regard to the pathogenesis of microangiopathic complications (30). In our cohort, however, there was no significant correlation between hsTNT and proBNP levels. Thus, the fact that only hsTNT as a marker for myocardial microangiopathy was correlated with the acquired parameters of DN suggests that peripheral microangiopathy due to cardiac microvascular disease is a more important contributor to DN than cardiac insufficiency alone. This hypothesis is further supported by the finding that the PWV, as an indicator for the resistance of the smaller peripheral blood vessels, is positively correlated with hsTNT and negatively correlated with the sciatic nerve's FA, indicating that cardiac microangiopathy is paralleled by an increase in the resistance of peripheral microvasculature (12,31).

One may argue, however, that hsTNT and FA also showed correlations with age in participants with T2D and, therefore, may only represent the physiological process of aging in this group. It is indeed well known that symptom severity in DN increases with patients' age and that nerves in healthy subjects without diabetes show an age-related loss of structural integrity (24,32,33). It is important to keep in mind, however, that the correlation between hsTNT and FA remained significant for all patients with T2D as well as for DN patients in a double-controlled partial correlation analysis with age and cystatin C as potential confounders, whereas the same analysis showed no significant effect for age or cystatin C. Also, hsTNT and FA both showed a much stronger correlation with clinical and electrophysiological parameters than patients' age alone. It should further be considered that a correlation of hsTNT and age was only found in patients with T2D without DN but not in patients with T2D with DN. This suggests that in T2D without DN, hsTNT indeed represents an age-related structural decline of nerves; however, because hsTNT was not significantly correlated with age in DN and, more importantly, was higher in DN compared with T2D without DN or control subjects, the age-related process of nerve deterioration appears to be accelerated in patients with DN in T2D as a consequence of microangiopathy, which is represented by hsTNT. Further supporting this hypothesis is that none of the other serological values acquired in our cohort, such as parameters of renal function, dyslipidemia, and glycemic control, showed an equally strong correlation with FA or any clinical and electrophysiological parameters.

Based on the finding that hsTNT and FA were both negatively correlated with the participants' heart rate in 24-h blood pressure recordings, one may argue that an elevation in hsTNT simply reflects the presence of CAN and, therefore, may not be the consequence of cardiac microangiopathy. One has to consider, however, that in our cohort, neither hsTNT nor FA showed any correlation with parameters of heart rate variability or ECG parameters such as the QTc, both of which have been shown to be associated with CAN (19,34). It therefore seems unlikely that the correlations of hsTNT with clinical and electrophysiological parameters are linked to CAN in our cohort.

With regard to hsTNT as a marker for cardiac microangiopathy, one may further argue that an increase in hsTNT is not exclusively caused by cardiac microangiopathy but may also be the consequence of macroangiopathic changes of coronary blood vessels. Although we cannot fully exclude the presence of cardiac macroangiopathy or subclinical myocardial ischemia in our cohort, one should note that the ECG showed no signs of acute or former myocardial infarction in any of the participants and that no correlations were found between hsTNT and IMT. Since IMT is a strong parameter for the presence of macroangiopathy, this renders macroangiopathy as an unlikely cause for hsTNT elevation in the examined group of participants. Furthermore, although our results strongly indicate that peripheral microangiopathy, paralleled by cardiac microangiopathy, is an important contributor to DN in T2D, this assumption remains hypothetical because we could not assess cardiac or peripheral microangiopathy directly.

Our study is limited by the fact that the sample size precludes multivariate analysis and that only cross-sectional data were used, which do not allow for a prediction of a patient's prognosis. It should be taken into account, however, that double-controlled partial correlation analysis ruled out all potential confounders that were significantly correlated with patients' FA and hsTNT and that all acquired serological parameters except hsTNT showed no significant correlation with any of the acquired clinical neuropathy scores and electrophysiological parameters, which suggests that hsTNT is a potential predictor of nerve damage in T2D. A further

limitation of our study is that the sample size is not equally balanced for men and women, which precludes sex-specific analysis.

In summary, this study is the first to show that hsTNT and the sciatic nerve's FA are both potential indicators for structural nerve damage in T2D. Larger longitudinal studies are required to determine the predictive value of hsTNT and DTI imaging in DN.

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