Do Cardiac Biomarkers NT-proBNP and hsTnT Predict Microvascular Events in Patients With Type 2 Diabetes? Results From the ADVANCE Trial

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OBJECTIVE

We investigated microvascular event risk in people with type 2 diabetes and assessed whether N-terminal pro–B-type natriuretic peptide (NT-proBNP) and high-sensitivity troponin T (hsTnT) improved prediction.

RESEARCH DESIGN AND METHODS

We performed a case-cohort study, including 439 incident cases of microvascular events (new or worsening nephropathy or retinopathy) and 2,946 noncase subjects identified from participants in the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) trial. NT-proBNP and hsTnT were measured in stored plasma samples using automated commercial assays.

RESULTS

After adjustment for age, sex, and randomized treatment, the hazard ratios for microvascular events per 1-SD increase in the log-transformed hsTnT and NT-proBNP were 1.67 (95% CI 1.51–1.85) and 1.63 (1.44–1.84), respectively. After further adjustment for classical and diabetes-related cardiovascular disease risk factors, the hazard ratios attenuated to 1.40 (1.24–1.58) and 1.41 (1.24–1.60), respectively. While the *C* statistic did not improve on addition of hsTnT or NT-proBNP for the total microvascular end point, a combination of both markers improved the prediction of nephropathy (*P* = 0.033) but not retinopathy (*P* = 0.72). The corresponding net reclassification indices in a three–risk category model (<10%, 10–15%, and >15% 5-year risk) for all microvascular events were 7.31% (95% CI 2.24–12.79) for hsTNT addition, 6.23% (1.74–11.5) for NT-proBNP addition, and 7.1% (1.5–12.9) for both markers together.

CONCLUSIONS

These data suggest that cardiac biomarkers moderately improve microvascular event risk prediction, in particular the risk of nephropathy. Further studies examining the value of this approach for trial design and clinical use are warranted.

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Measurement of circulating concentrations of B-type natriuretic peptide or N-terminal pro–B-type natriuretic peptide (NT-proBNP) and high-sensitivity troponin assays improve the prediction of cardiovascular disease (CVD) in general populations (1–3). Recent data from the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) trial of intensive glucose control and blood pressure lowering also show that NT-proBNP and high-sensitivity troponin T (hsTnT) improve CVD risk prediction in those with type 2 diabetes (4). Microvascular diseases also cause significant morbidity in patients with diabetes; for instance, diabetic nephropathy is a leading cause of end-stage renal disease (5). Clinical risk scores to predict the microvascular complications of diabetes are not in routine use but may be valuable to help clinicians determine which patients are at highest risk and to aid trial design.

Clearly, there is some interrelationship between increased microvascular and macrovascular risk (6).Therefore, putative microvascular risk scores might be expected to contain some macrovascular risk factors, as has been recently demonstrated (7). However, the mechanisms underlying elevation in risk of both microvascular and macrovascular risk are not clear. Microvascular disease in the myocardium might be a major cause of cardiac morbidity among people with type 2 diabetes (8,9), and such subtle changes in cardiac function (e.g., arising from subclinical ischemia) might often precede frank clinically detectable peripheral microvascular disease. Indeed, elevated natriuretic peptides in acute coronary syndromes may be correlated with abnormal perfusion in patients undergoing angioplasty (10). Further, cross-sectional data in people with diabetes suggest a positive association among cardiac biomarkers, cardiac function, and "peripheral" microvascular disease (11). Finally, prospective data from ADVANCE have recently demonstrated that an elevated resting heart rate (potentially an early marker of cardiac overload) is strongly associated with increased risk of nephropathy and retinopathy (12), and data from Zwolle Outpatient Diabetes project Integrating Available Care (ZODIAC)-33 show that copeptin elevation predicts albuminuria (13). Thus, early changes in cardiac biomarkers may precede presentation with frank microvascular disease and yield prognostic information that enables intensive interventions to be targeted to those at greatest risk. Developing a clearer understanding of any relationship between cardiac function/metabolism and microvascular end points is important both from an aetiological perspective and potentially to develop risk scores to guide clinical management. We thus aimed to *1*) investigate the association between biomarkers of cardiac stress and incident microvascular outcomes in patients with type 2 diabetes and *2*) investigate the incremental ability of cardiac biomarkers to predict microvascular events.

RESEARCH DESIGN AND METHODS

We performed a nested case-cohort study (an efficient prospective study design that has power similar to that of a full cohort study) (14) to investigate the association between circulating levels of NT-proBNP and hsTnT and microvascular events in patients with type 2 diabetes who participated in the ADVANCE study (clinical trial reg. no. NCT00145925, clinicaltrials.gov). The case-cohort design has specific advantages over a matched nested case-control study, including the ability to develop risk scores in which the effect of important variables (such as age and sex) are not minimized and the ability to investigate several distinct end points simultaneously (such as macrovascular [4] and microvascular outcomes).

The ADVANCE study recruited 11,140 patients with type 2 diabetes from 215 centers in 20 countries between June 2001 and March 2003 (12,13). Participants were ≥55 years of age and had been diagnosed with type 2 diabetes after the age of 30 years. In addition, they were required to have a history of CVD or one or more additional cardiovascular risk factors. The study made two randomized comparisons: a double-blind assessment of the efficacy of perindopril-indapamide (2 mg/0.625 mg for 3 months increasing, if tolerated, to 4 mg/1.25 mg) versus placebo and an open-label evaluation of an intensive glucose-lowering regimen using modified-release gliclazide, with a target HbA_{1c} of ≤6.5% (48 mmol/mol) vs. standard, guideline-based glycemic control. The study was approved by the ethics committee for each participating center, and all participants provided written informed consent. Detailed study methods, including demographic and clinical measurements, and the main results of the ADVANCE study have previously been reported (15,16) (Supplementary Data). Plasma samples were obtained from all study participants at baseline and stored at −80°C for a median of 7.8 years.

The primary trial outcomes were composites of major macrovascular and microvascular events (15,16). Major macrovascular events were cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke. Major microvascular events were defined, a priori, as a composite of new or worsening nephropathy or retinopathy. Nephropathy was defined as the development of macroalbuminuria (i.e., a urinary albumin [micrograms]–to–creatinine [milligrams] ratio (ACR) of >300, confirmed by two results), doubling of the serum creatinine level to ≥200 μmol/L (2.3 mg/dL) (with nonqualifying exceptions of acute illness and subsequent recovery of renal function or terminal illness) (15), the need for renal replacement therapy due to kidney disease (in the absence of other medical causes requiring transient dialysis), or death due to renal disease. Participants had their creatinine levels measured as part of the study protocol at baseline, 4 months, and 1 year and annually thereafter until completion of the study, with further tests at the discretion of treating clinicians. Urinary ACR was measured as part of the study protocol at baseline, 2 years, 4 years, and completion of the study. Glomerular filtration rate was estimated (eGFR) using the Modification of Diet in Renal Disease formula. New or worsening retinopathy was defined as the development of proliferative retinopathy (identified by the incidence of new blood vessels on the disc or elsewhere, vitreous hemorrhage, preretinal hemorrhage, and fibrous proliferations on the disc or elsewhere in a participant found not to have this condition at entry), macular edema (characterized by a retinal thickening within 1 disc diameter of the macular center in a participant not found to have this condition at entry), diabetes-related blindness (corrected

visual acuity 3/60 or worse, persisting for ≥3 months and known to not be due to non–diabetes-related causes in a participant not found to have this condition at entry), or the use of retinal photocoagulation therapy. Participants underwent formal eye examination and visual acuity testing at baseline, 2 years, 4 years, and completion of the study. Secondary end points were the individual components of the primary outcome (new or worsening nephropathy and new or worsening retinopathy). An End Point Adjudication Committee, unaware of treatment allocation, reviewed source documentation for all individuals who had a suspected primary end point of the ADVANCE study. In a post hoc analysis, we defined baseline microvascular disease as those with previous history of any microvascular event, ACR >300, or eGFR <60 mL/min/1.73 m^2.

Stored plasma samples were available from all countries involved in ADVANCE, except China and India, giving a total base population of 7,376. A case-cohort study was designed for biomarker measurement (Fig. 1).

Biomarkers of interest were measured on stored EDTA plasma samples. NT-proBNP and hsTnT were measured using electrochemiluminescence immunoassays, performed on a Roche Elecsys 2010 automated platform (Roche Diagnostics, Burgess Hill, U.K.). The NT-proBNP and hsTnT assays have lower detection limits of 5 pg/mL and 3 ng/L, respectively (17). For both assays, results below the limit of detection were reported as 50% of the functional limit of detection for use in linear models. Assays were performed using the manufacturer's calibrators and quality controls. NT-proBNP and hsTnT had assay coefficients of variation of 6.5% and 4.5% for the low control and 3.8% and 9.1% for the high control, respectively.

Statistical Analysis

Categorical data are presented as number (percentage) and continuous data as mean (SD), where symmetrically distributed, or median (interquartile range) where skewed. Data for distributions of risk factors were derived in the overall subcohort and split by whether the participant experienced a primary or secondary microvascular event during follow-up.

Hazard ratios for a 1-SD increase in each of hsTnT and NT-proBNP, after log transformation, on incident microvascular events (a statistical model that maximizes power and has been previously used in ADVANCE [4] and elsewhere [1–3]) were obtained from weighted Cox regression models using the STSELPRE procedure for full case-cohort analyses (StataCorp, College Station, Texas). Nonlinearity was tested by comparing the deviances of linear and categorical models and by the inclusion of polynomial components (quadratic and cubic terms). Other analyses were performed using SAS, version 9.2 (SAS Institute, Cary, NC). All *P* values reported are two sided, with the 5% threshold used to determine significance.

Three models, with different potential confounding variables, were fitted for each cardiac biomarker/outcome combination (Supplementary Data). In addition, age, sex, and randomized treatment-adjusted survival curves were modeled by quarters of the distribution of the cardiac biomarkers in the random subcohort only (*n* = 3,500). This restriction limits the potential biases in prediction potentially caused by taking a nonrandom sample.

Prediction metrics for microvascular events were investigated in the random subcohort. *C* statistics for 5-year risk, accounting for censoring (18), were determined for the baseline clinical model and for this model plus each biomarker individually and in combination. The *C* statistic has been criticized for insensitivities to changes in clinical decisions yielded for information gained. Therefore, we also used net reclassification index (NRI), which estimates correct changes in clinical classification across risk thresholds (19). We also measured the integrated discrimination index and

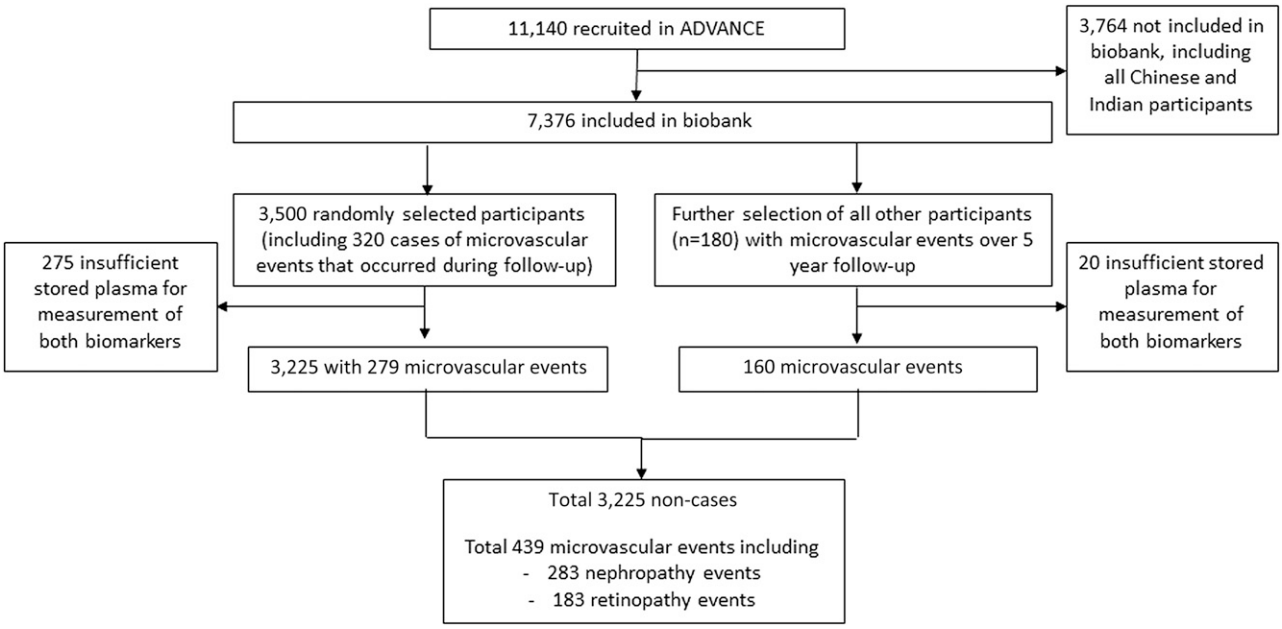


Figure 1—The ADVANCE trial: the case-cohort study design and data availability.

the relative integrated discrimination index, which can be considered as a continuous version of NRI with probability differences used instead of categories (19). We estimated the ability of cardiac biomarkers to appropriately reclassify 5-year risk, using these metrics by methods suitable for survival data, with bootstrapping to estimate 95% CI (19). NRI was derived using a continuous model for changes in risk classification and from a categorical (or threshold) model based on 10%, 10–15%, and >15% 5-year risk.

RESULTS

In the subcohort of 3,500 randomly selected participants, 320 (9.1%) experienced a primary microvascular event during a median of 5 years' follow-up. The entire case-cohort study comprised 3,680 participants (Fig. 1). Of these, 3,385 participants had data for plasma NT-proBNP and hsTnT results (92.0%), of whom 439 experienced a microvascular event, and 2,946 were noncases (Fig. 1). The mean age of the study cohort was 67 years, and 61% were male (Table 1). Median hsTnT was 5 ng/L (64.6% of the cohort had a detectable troponin), and median NT-proBNP was 90 pg/mL. hsTnT concentrations were high (>14 ng/L) in 15.4% and NT-proBNP concentrations high (>400 pg/mL) in 12.8% of the case-cohort study. Crude baseline predictors of incident microvascular risk included male sex, increased duration of diabetes, systolic blood pressure, HDL cholesterol, triglycerides, resting heart rate, HbA_{1c}, fasting glucose, ACR, and eGFR ($P < 0.01$ for all). NT-proBNP and hsTnT were also higher among case subjects ($P < 0.01$). Data are also available for nephropathy and retinopathy end points separately (Supplementary Data).

Both NT-proBNP and hsTnT showed broadly expected associations with risk factors for CVD and diabetes, as previously reported (4) (Supplementary Data). NT-proBNP and hsTnT levels were moderately associated with each other ($r = 0.404$, $P < 0.001$)

There was a log-linear association between both biomarkers and primary and secondary microvascular outcomes (P values <0.001), with no evidence of nonlinearity. After adjustment for age, sex, and randomized treatment, both NT-proBNP and hsTnT associated significantly with the risk for primary microvascular events (Figs. 2 and 3; Table 2), with each parameter being more strongly associated with risk for nephropathy than retinopathy. These associations remained but were attenuated after adjustment for other clinical risk factors for microvascular events. There was no strong evidence of an interaction effect for any of the main adjustment variables (data not shown). The association of quarters of both cardiac biomarkers with the nephropathy end point had no interaction with presence of baseline microvascular disease ($P = 0.155$ for hsTnT and $P = 0.552$ for NT-proBNP).

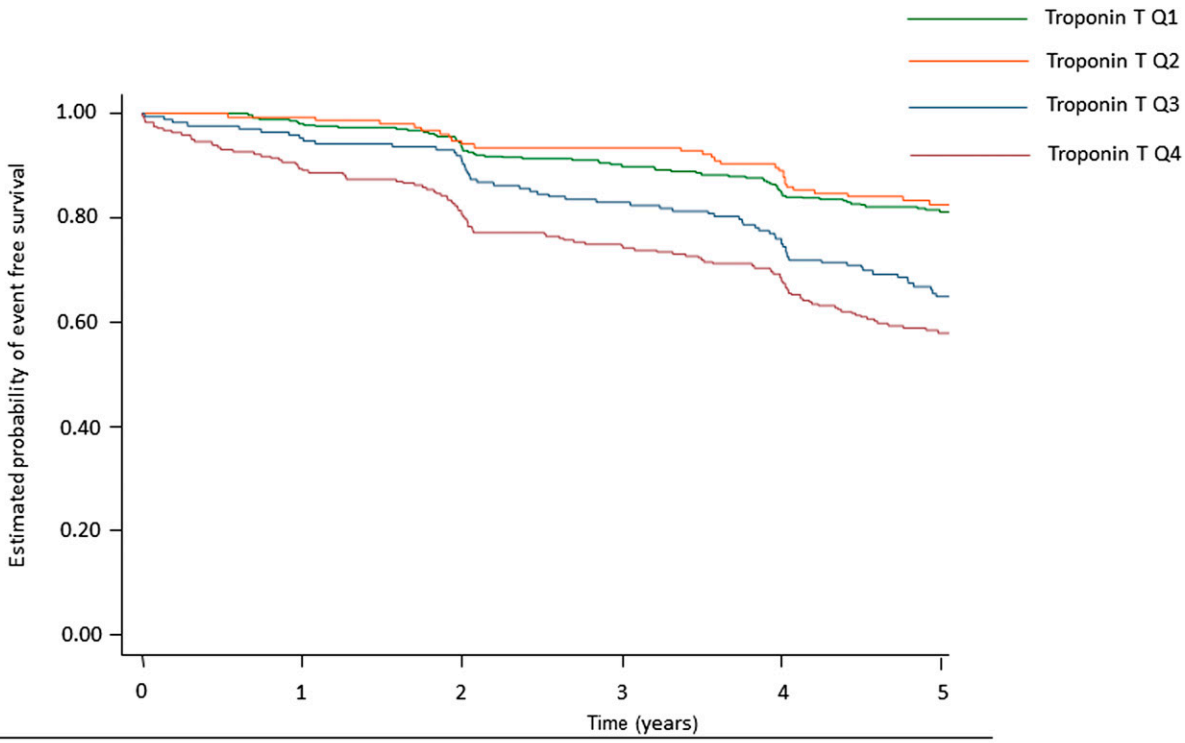
The strongest single predictor of future nephropathy was ACR; *C* statistic 0.812 (95% CI 0.780–0.845). In contrast, retinopathy was most strongly predicted (univariately) by duration of diabetes and HbA_{1c}; *C* statistics 0.636 (0.595–0.678) and 0.604 (0.557–0.650), respectively. A baseline model including all relevant routinely collected baseline clinical variables (age, sex, randomized treatment, country, duration of diabetes, current smoking, systolic blood pressure, BMI, ACR, eGFR, HbA_{lc}, glucose, total and HDL cholesterol, triglycerides, resting heart rate, and history of CVD) discriminated those who experienced any microvascular events moderately well (*C* statistic = 0.731) and the nephropathy end point very well (*C* statistic = 0.829) (Table 3). Addition of hsTnT to the model predicting any microvascular complication did not improve the *C* statistic ($P = 0.201$) but improved the continuous NRI ($P < 0.001$), with the improvement most evident for the prediction of nephropathy. Likewise, NT-proBNP showed some evidence of improving the continuous NRI ($P < 0.001$) but not the *C* statistic ($P = 0.153$). Improvements in the integrated discrimination index were also significant for both biomarkers (Supplementary Data).

Building a categorical NRI for theoretical clinical treatment decisions, we used three cutoffs similar to those

Table 1—Baseline characteristics classified by microvascular event outcome status

	Microvascular events			
	Yes	No	Overall	*P*
N	439	2,946	3,385	
Age (years)	66.5 (6.5)	66.6 (6.6)	66.6 (6.6)	0.86
Male sex	295 (67.2%)	1,729 (58.7%)	2,024 (59.8%)	<0.01
Duration of diabetes (years)	9.8 (6.9)	7.4 (6.2)	7.7 (6.3)	<0.01
Current smokers	61 (13.9%)	444 (15.1%)	505 (14.9%)	0.52
History of CVD	156 (35.5%)	978 (33.2%)	1,134 (33.5%)	0.33
BMI (kg/m^2)	30.2 (5.4)	30.1 (5.3)	30.1 (5.3)	0.69
Systolic BP (mmHg)	150.5 (22.0)	146.9 (21.4)	147.3 (21.5)	<0.01
Diastolic BP (mmHg)	81.1 (11.3)	81.9 (10.7)	81.8 (10.8)	0.18
Total chol. (mmol/L)	5.12 (1.10)	5.15 (1.18)	5.15 (1.17)	0.62
HDL chol. (mmol/L)	1.18 (0.34)	1.24 (0.33)	1.23 (0.33)	<0.01
Triglycerides (mmol/L)	1.79 (1.22, 2.56)	1.70 (1.20, 2.32)	1.70 (1.20, 2.36)	0.03
Resting heart rate (bpm)	74.2 (12.7)	72.4 (12.1)	72.6 (12.2)	<0.01
HbA_{1c} (%)	7.79 (1.57)	7.34 (1.37)	7.39 (1.40)	<0.01
HbA_{1c} (mmol/mol)	61.70 (17.15)	56.68 (14.92)	57.33 (15.32)	<0.01
Glucose (mmol/L)	9.1 (3.4)	8.4 (2.6)	8.5 (2.7)	<0.01
ACR (μg/mg)	48.4 (14.1, 132.6)	12.4 (5.9, 30.0)	14.1 (6.2, 38.9)	<0.01
eGFR (mL/min/1.73 m^2)	67.1 (19.5)	72.6 (16.2)	71.9 (16.8)	<0.01
CRP (mg/L)	1.72 (0.91, 3.52)	1.83 (0.87, 4.06)	1.80 (0.87, 4.03)	0.38
hsTnT (ng/L)	8 (4, 15)	5 (1.5, 10)	5 (1.5, 11)	<0.01
NT-proBNP (pg/mL)	119 (46, 324)	78 (31, 182)	84 (33, 200)	<0.01
log-transformed hsTnT	2.1 (1.4, 2.7)	1.6 (0.4, 2.3)	1.6 (0.4, 2.4)	<0.01
log-transformed NT-proBNP	4.8 (3.8, 5.8)	4.4 (3.4, 5.2)	4.4 (3.5, 5.3)	<0.01

Values are *n* (%), mean (SD), or median (interquartile range). chol., cholesterol; CRP, C-reactive protein.



N at risk by time in years						
Overall	3,225	3,140	3,016	2,920	2,793	1,445
Q1: ≤3 pg/ml	1,226	1,211	1,176	1,155	1,120	575
Q2: >3 to ≤5 pg/ml	527	525	513	505	491	264
Q3: >5 to ≤10 pg/ml	595	583	566	541	518	272
Q4: >10 pg/ml	877	821	761	719	664	334

Figure 2—Kaplan-Meier curve for event-free survival by quarters (Q) of the hsTnT distribution.

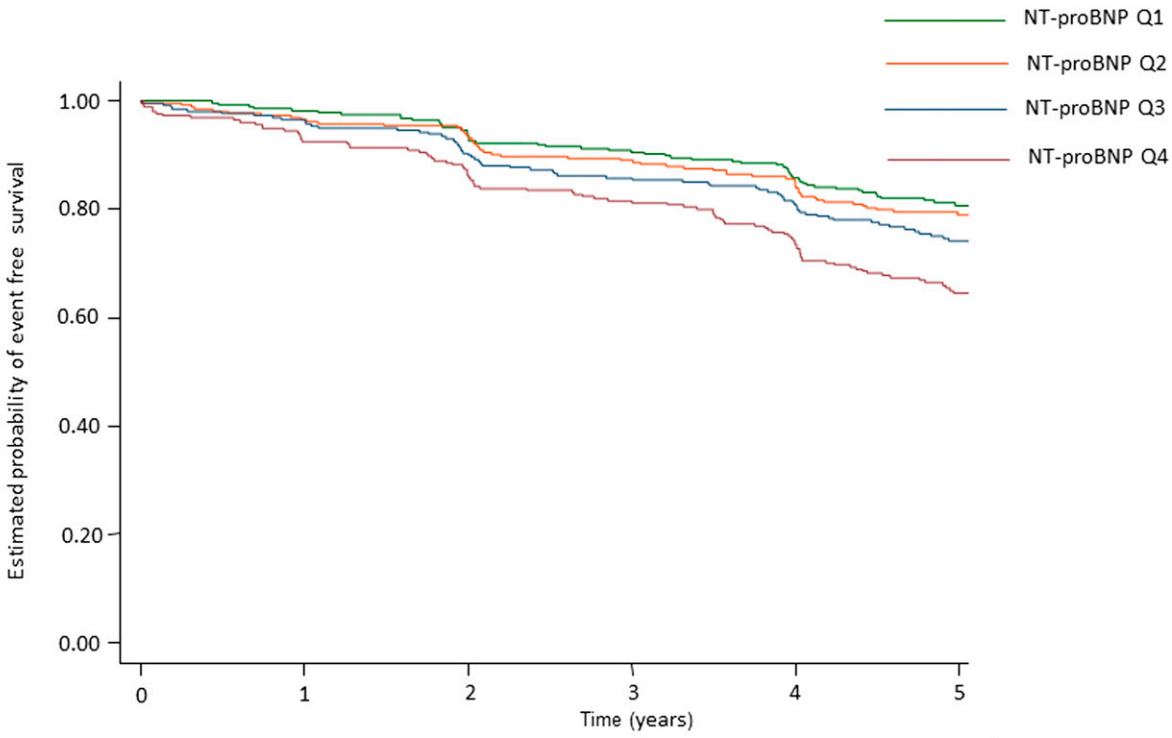
used for CVD risk prediction (cutoffs of 10%, 10–15%, and >15% 5-year risk). Among noncase subjects at baseline, 75.2% were at <10% risk, 12.8% were at 10–15% risk, and 12.0% were at 15% risk of any microvascular end point. The corresponding proportions for case subjects were 40.4%, 21.0%, and 38.6% (Supplementary Data). In this model, addition of hsTNT, and to a lesser extent NT-proBNP, improved categorical classification for predicting microvascular events (Table 3); categorical NRI was 7.3% (95% CI 2.2–12.8) and 6.2% (1.7–11.5), respectively. Neither marker showed any evidence of improving retinopathy prediction in any model. There was no strong evidence of incremental benefit from adding both markers to any model.

CONCLUSIONS

The current study demonstrates that in patients with type 2 diabetes, higher levels of NT-proBNP and hsTnT are associated with an increased risk of developing microvascular complications, particularly nephropathy. This association was attenuated after accounting for conventional risk factors for, and mediators of, macrovascular complications; the relationship between NT-proBNP and hsTnT and established CV risk factors may, at least in part, explain their prognostic value. Models designed to simulate a clinical score for the prediction of microvascular events showed an ability to discriminate between patients who will and will not develop microvascular complications. We suggest that subclinical cardiac disease might be an antecedent of peripheral microvascular disease. Thus, an approach that combines clinical predictors with biomarkers such as NT-proBNP and hsTnT may help to identify patients with type 2 diabetes who are at greatest risk of microvascular complications (particularly nephropathy) and may benefit most from strategies to reduce this risk. However, our data also illustrate that prediction of nephropathy and retinopathy are dependent on different risk factors, reflecting distinct mechanistic pathways. As such, specific prediction of retinopathy (without retinal scanning data) remains challenging using conventional risk factors and cardiac biomarkers.

Potential Mechanisms

Emerging evidence suggests that functional abnormalities in the microvasculature can lead to ischemia (20,21), which would be consistent with possible rises in troponin and NT-proBNP and compensatory changes in cardiac structure and function. Indeed, several studies have recently investigated an association between troponin elevation and microvascular complications after myocardial infarction and percutaneous coronary intervention (22,23). It is important to note that the additional



N at risk by time in years						
Overall	3,225	3,140	3,016	2,920	2,793	1,445
Q1: ≤32 pg/ml	815	809	793	778	757	390
Q2: >32 to ≤81 pg/ml	794	782	763	747	722	380
Q3: >81 to ≤189 pg/ml	805	789	748	720	693	370
Q4: >189 pg/ml	811	760	712	675	621	305

Figure 3—Kaplan-Meier curve for event-free survival by quarters (Q) of the NT-proBNP distribution.

predictive information added by cardiac biomarkers noted herein occurred despite the addition of previous history of CVD, which is a well-known risk factor (6). Renal function is likely to be an important determinant of circulating levels of biomarkers (24), and as such there may be some reverse causality, although this study is prospective and we have adjusted for baseline eGFR and ACR. Therefore, although cardiac biomarkers undoubtedly increase with progressive renal dysfunction, that cardiac biomarkers predict future decline in renal function independently of

Table 2—Hazard ratios (95% CI) for a 1-SD increment in circulating cardiac biomarkers

	Microvascular events (*N* = 3,385, *n* = 439)	New or worsening nephropathy (*N* = 3,363, *n* = 283)	New or worsening retinopathy (*N* = 3,261, *n* = 183)
hsTnT (log scale) (1 SD = 1.05)			
Model 1	1.67 (1.51–1.85); $P < 0.001$	1.81 (1.60–2.05); $P < 0.001$	1.48 (1.26–1.73); $P < 0.001$
Model 2	1.40 (1.24–1.58); $P < 0.001$	1.44 (1.23–1.67); $P < 0.001$	1.32 (1.09–1.60); $P = 0.005$
Model 3	1.30 (1.15–1.47); $P < 0.001$	1.31 (1.11–1.54); $P = 0.001$	1.24 (1.03–1.50); $P = 0.026$
NT-proBNP (log scale) (1 SD = 1.58)			
Model 1	1.63 (1.44–1.84); $P < 0.001$	1.96 (1.67–2.31); $P < 0.001$	1.30 (1.08–1.56); $P = 0.0044$
Model 2	1.41 (1.24–1.60); $P < 0.001$	1.52 (1.29–1.80); $P < 0.001$	1.34 (1.11–1.61); $P = 0.002$
Model 3	1.31 (1.15–1.48); $P < 0.001$	1.40 (1.17–1.66); $P < 0.001$	1.26 (1.05–1.51); $P = 0.012$

N, total number of participants (event and nonevent) in analysis; *n*, total number of events in analysis. Model 1: adjusted for age, sex, and randomized treatment allocations. Model 2: additionally adjusted for country, duration of diabetes, current smoking, systolic blood pressure, BMI, ACR, eGFR, HbA_{1c}, glucose, total and HDL cholesterol, triglycerides, C-reactive protein, resting heart rate, and history of CVD. Model 3: additionally adjusted for the other biomarker (i.e., NT-proBNP/hsTnT).

Table 3—Reclassification and discrimination statistics (95% CI) for circulating cardiac biomarkers after inclusion in a model with clinical markers of risk*

	All microvascular events (N = 3,225, n = 279)	New or worsening nephropathy (N = 3,225, n = 145)	New or worsening retinopathy (N = 3,225, n = 147)
Base *C* statistic*	0.731 (0.701, 0.762)	0.829 (0.795, 0.864)	0.727 (0.685, 0.769)
hsTnT			
C statistic†	0.738 (0.707, 0.769); P = 0.201	0.840 (0.808, 0.872); P = 0.045	0.728 (0.685, 0.771); P = 0.937
NRI: continuous	0.3279 (0.2048, 0.4518); $P < 0.001$	0.3415 (0.1728, 0.5107); $P < 0.001$	0.2221 (0.0532, 0.3943); P = 0.010
NRI: categorical	0.0731 (0.0224, 0.1279); P = 0.006	0.0549 (−0.0156, 0.1259); P = 0.130	0.0407 (−0.0075, 0.0911); P = 0.104
NT-proBNP			
C statistic†	0.735 (0.704, 0.766); P = 0.292	0.835 (0.801, 0.869); P = 0.153	0.731 (0.689, 0.773); P = 0.311
NRI: continuous	0.3363 (0.2207, 0.4667); $P < 0.001$	0.3892 (0.2240, 0.5575); $P < 0.001$	0.1784 (0.0069, 0.3539); P = 0.044
NRI: categorical	0.0623 (0.0174, 0.11485); P = 0.002	0.0561 (−0.0142, 0.1292); P = 0.124	0.0663 (0.0113, 0.1228); P = 0.020
hsTnT and NT-proBNP			
C statistic†	0.738 (0.707, 0.769); P = 0.193	0.842 (0.809, 0.874); P = 0.033	0.729 (0.686, 0.772); P = 0.715
NRI: continuous	0.3097 (0.1915, 0.4337); $P < 0.001$	0.4440 (0.2808, 0.6072); $P < 0.001$	0.1929 (0.0274, 0.3762); P = 0.028
NRI: categorical	0.0712 (0.0154, 0.1293); P = 0.014	0.0795 (0.0058, 0.1588); P = 0.030	0.0183 (−0.0331, 0.0724); P = 0.904

Results for nonmissing data from the random subcohort (N = 3,500). N = total number of participants (event and nonevent) in analysis; n = total number of events in analysis. categories were 10% and 15% 5-year risk. Biomarkers were all analyzed in continuous form after log transformations for NT-proBNP and hsTnT. The P value for the *C* statistic relates to the increase when adding the biomarker to the base model. *Using model 2 (without C-reactive protein) described in Table 2. †P value refers to increase in concordance compared with base model.

baseline renal function suggests potential clinical utility. The findings also hint at a potential for cardiac dysfunction to contribute to renal dysfunction.

Why Predict Microvascular Complications?

Clinical prediction of microvascular events is not as yet advocated by guidelines. This is largely due to a lack of evidence at present regarding evidence-based therapies to prevent onset of microvascular events. ADVANCE reported that intensive glucose and blood pressure control reduce the risk of nephropathy (25), and although data from the ACCORD (Action to Control Cardiovascular Risk in Diabetes) trial and VADT (Veterans Affairs Diabetes Trial) were less positive, results were consistent with reduction or delay in microalbuminuria and macroalbuminuria (26,27). A recent meta-analysis concluded there was inconsistent evidence to promote intensive interventions to prevent microvascular disease (28). Future trials in this area would benefit from better identification of high-risk patients to enable more effective evaluation of interventions. From our reclassification model (using the >15% 5-year risk predicted risk cutoff after addition of cardiac biomarkers), 13.2% of noncase subjects would be in the highest risk category and 44.9% of case subjects. Our results are thus important, since prediction of microvascular events, in particular nephropathy, would be useful for future trials of new agents designed to prevent or slow progression of nephropathy, an area of intense interest and activity (29). Indeed, there is a raft of ongoing work in the field of proteomics to improve the prediction of kidney disease (30,31). While -omics based technology is not yet ready for use in routine biochemistry in terms of standardization, quality control, or technical pragmatism, our data illustrate that simple clinical biomarkers predict nephropathy very well and that other simple and far less costly blood-based tests already in routine use, such as cardiac biomarkers, offer incremental predictive benefit. While our data are hypothesis generating, hinting at the potential for cardiac biomarkers to enhance prediction of diabetic nephropathy, it should be recognized that cardiac biomarkers are predictive of macrovascular risk (4); therefore, such biomarkers have the potential to be routinely assessed for other purposes. Further studies are required to assess the full potential clinical implications of our findings for microvascular risk prediction.

Recent data in a case-control study from the Prevention of REnal and Vascular End-stage Disease (PREVEND) hypertensive cohort also suggested that hsTnT is associated with incident microalbuminuria (32), lending external support to our results. We expand on these data by showing associations of NT-proBNP and troponin with renal and retinopathy end points, as well as building prediction models. The majority of microvascular events in ADVANCE, as in most other cohorts of people with diabetes, were renal. It remains possible that cardiac biomarkers are capturing information about renal function that are not otherwise available and thus mainly predict renal events. From a risk prediction perspective, however, the additional information gained in predicting events is nevertheless potentially clinically useful.

Strengths and Limitations

The current prospective study describes a cohort derived from a large

population, which was well characterized and closely followed up. All end points were independently adjudicated according to predefined criteria, including separate reporting of nephropathy and retinopathy end points. This is one of the first studies to show that subtle subclinical differences in cardiac biomarkers may run concurrently with or even precede peripheral microvascular disease. Prediction of microvascular end points is a novel application of the data. Participants in the ADVANCE study, like those from other randomized controlled trial populations, represent a selected cohort, and our results may not be generalizable to all patients with diabetes. For instance, ADVANCE participants were required to have a history of CVD or CVD risk factors, and although efforts to adjust for these confounding factors were made, residual confounding remains possible. As such, our findings should be considered hypothesis generating. Our results relating to clinical risk predictors are, however, consistent with other data from an ethnically distinct cohort (7), and the baseline characteristics of the ADVANCE cohort are very comparable with those in several observational studies at the community level (33). Although the large study population and case-cohort design ensure considerable statistical power and allow reliable correction for many potential confounding factors, other possible confounders may be present, leading to an overestimate of risk associations. Levels of hsTnT and NT-proBNP were measured only once, and it would be useful to address the effects of serial changes in levels in the future. We speculate on the mechanisms linking cardiac biomarkers to microvascular outcomes but have no data to examine cardiac perfusion specifically. The NRI models that we have constructed are necessarily based on somewhat artificial cutoffs, but they contain most of the pertinent information that might typically be used to estimate the cardiovascular risk of patients with diabetes. NT-proBNP and hsTnT were measured on stored serum samples, and although we cannot rule out the potential for differential sample degradation that biases our results, both of these markers appear fairly stable in long-term storage (34,35).

In conclusion, we report that clinical risk scores to predict microvascular events in patients with type 2 diabetes can be developed using classical CVD and diabetes-related risk factors. We further show that NT-proBNP and hsTnT can add moderate information to these clinical risk scores for nephropathy events. Further work is required to establish whether the prediction of microvascular risk can be improved using a variety of biomarkers.

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