

SUPPLEMENTARY DATA

Welsh et al: Prediction of microvascular events in patients with type 2 diabetes and the role of cardiac biomarkers: results from the ADVANCE trial.

Demographic and clinical measurements

Baseline data included demographic and clinical information. Blood pressure was recorded as the mean of two measurements made after the patient was rested for at least 5 minutes in the seated position, using a standardised automated sphygmomanometer (Omron HEM-705CP, Tokyo, Japan). Weight, height, urinary albumin/creatinine ratio (AC ratio), serum creatinine, fasting lipid levels, fasting glucose, glycated haemoglobin (HbA1c) were measured at baseline.

Multivariable adjustment models

Three models, with different potential confounding variables, were fitted for each cardiac biomarker/outcome combination: model 1 with age, sex and randomised treatment; model 2 with, additionally, a prior macrovascular complication of diabetes (myocardial infarction, stroke, hospital admission for a transient ischemic attack or for unstable angina, coronary or peripheral revascularization or amputation secondary to peripheral vascular disease), duration of diabetes, current smoking, systolic blood pressure, body mass index (BMI), albumin/creatinine (AC) ratio, estimated glomerular filtration rate (eGFR), HbA1c, plasma glucose, total and HDL-cholesterol, triglycerides, high-sensitivity C reactive protein, and resting heart rate; and model 3, additionally to model 2 the other biomarker (i.e. NT-proBNP or hsTnT). When adjusting for treatment randomisation blood pressure groups and glucose treatment were adjusted for separately. There was no evidence of interaction and no interaction term was included.

Risk prediction model

We utilised a pragmatic model, using only cases obtained in the randomly selected subcohort for risk prediction. As well as the C-statistic and NRI, we measured the integrated discrimination index (IDI) and relative IDI¹. We also explored the use of recently developed methods that allow for more efficient use of the full cohort, rather than basing the analysis only on the data in the subcohort². While these methods did indeed lead to increased precision for estimating coefficients of the adjustment variables in our models, they had no impact on the coefficients of the biomarkers of interest. This was because our data did not include any additional variables that could be used to reliably predict the biomarkers. Indeed, the best identified model for predicting the biomarkers resulted in an R-squared of only 8%. This same phenomenon has been well described elsewhere in the literature.

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Supplementary Table 1. Baseline characteristics classified by nephropathy outcome status (based on non-missing plasma samples for cardiac biomarkers).

	New or worsening nephropathy		Overall	
	Yes	No		
	n=283	n=3080	n=3363	p-value
Age (years)	66.4 (6.7)	66.6 (6.5)	66.5 (6.6)	0.74
Male sex	202 (71.4%)	1807 (58.7%)	2009 (59.7%)	<0.01
Duration of diabetes (yrs)	9.2 (6.7)	7.6 (6.2)	7.7 (6.3)	<0.01
Current Smokers	51 (18.0%)	451 (14.6%)	502 (14.9%)	0.13
History of CVD	108 (38.2%)	1013 (32.9%)	1121 (33.3%)	0.07
BMI (kg/m ²)	30.6 (5.6)	30.0 (5.2)	30.1 (5.3)	0.08
Systolic BP (mmHg)	150.2 (22.6)	147.0 (21.3)	147.3 (21.5)	0.02
Diastolic BP (mmHg)	81.2 (11.7)	81.8 (10.7)	81.8 (10.8)	0.34
Total chol. (mmol/L)	5.07 (1.12)	5.16 (1.18)	5.15 (1.17)	0.25
HDL chol. (mmol/L)	1.16 (0.34)	1.24 (0.33)	1.23 (0.33)	<0.01
Triglycerides (mmol/L)	1.87 (1.30, 2.70)	1.69 (1.20, 2.31)	1.70 (1.20, 2.36)	<0.01
Resting heart rate (bpm)	73.7 (13.6)	72.5 (12.1)	72.6 (12.3)	0.14
HbA1c (%)	7.70 (1.54)	7.36 (1.38)	7.39 (1.40)	<0.01
HbA1c (mmol/mol)	60.69(16.83)	56.99(15.13)	57.30(15.31)	<0.01
Glucose (mmol/L)	8.8 (3.4)	8.4 (2.6)	8.5 (2.7)	0.10
AC ratio (μ /mg)	75.1 (30.9, 160.9)	12.4 (6.0, 30.1)	14.1 (6.2, 38.9)	<0.01
eGFR	65.3 (19.9)	72.5 (16.4)	71.9 (16.8)	<0.01
CRP (mg/L)	1.83 (1.07, 3.89)	1.80 (0.85, 4.04)	1.81 (0.87, 4.03)	0.26
hs Troponin T (pg/ml)	9 (5, 16)	5 (1.5, 10)	5 (1.5, 10)	<0.01
NT-proBNP (pg/ml)	149 (53, 469)	78 (31, 182)	84 (33, 197)	<0.01

Values are n (%), mean (standard deviation), or median (interquartile range)

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Supplementary Table 2. Baseline characteristics classified by retinopathy outcome status (based on non-missing plasma samples for cardiac biomarkers).

	New or worsening retinopathy		Overall	
	Yes	No		
	n=183	n=3078	n=3261	p-value
Age (years)	66.2 (6.1)	66.6 (6.6)	66.5 (6.5)	0.48
Male sex	111 (60.7%)	1819 (59.1%)	1930 (59.2%)	0.68
Duration of diabetes (yrs)	10.8 (7.0)	7.5 (6.2)	7.7 (6.3)	<0.01
Current Smokers	11 (6.0%)	479 (15.6%)	490 (15.0%)	<0.01
History of CVD	58 (31.7%)	1037 (33.7%)	1095 (33.6%)	0.58
BMI (kg/m ²)	29.6 (5.0)	30.1 (5.3)	30.0 (5.2)	0.28
Systolic BP (mmHg)	151.4 (22.0)	146.9 (21.5)	147.1 (21.5)	<0.01
Diastolic BP (mmHg)	81.4 (11.4)	81.8 (10.8)	81.7 (10.8)	0.65
Total chol. (mmol/L)	5.20 (1.03)	5.16 (1.19)	5.16 (1.18)	0.57
HDL chol. (mmol/L)	1.22 (0.32)	1.23 (0.33)	1.23 (0.33)	0.65
Triglycerides (mmol/L)	1.7 (1.20, 2.30)	1.70 (1.20, 2.35)	1.70 (1.20, 2.34)	0.67
Resting heart rate (bpm)	75.2 (11.2)	72.4 (12.2)	72.6 (12.2)	<0.01
HbA1c (%)	7.95 (1.64)	7.36 (1.38)	7.39 (1.40)	<0.01
HbA1c (mmol/mol)	63.43(17.92)	56.92(15.04)	57.29(15.28)	<0.01
Glucose (mmol/L)	9.5 (3.4)	8.4 (2.6)	8.5 (2.7)	<0.01
AC ratio (μ /mg)	20.3 (8.8, 65.4)	13.2 (6.2, 34.5)	13.3 (6.2, 8.1)	<0.01
eGFR	69.5 (18.5)	72.3 (16.5)	72.1 (16.6)	0.05
CRP (mg/L)	1.44 (0.74, 2.59)	1.83 (0.87, 4.06)	1.80 (0.86, 4.03)	<0.01
hs Troponin T (pg/ml)	7 (1.5, 14)	5 (1.5, 10)	5 (1.5, 10)	<0.01
NT-proBNP (pg/ml)	87 (33, 230)	80 (32, 189)	81 (32, 190)	0.15

Values are n (%), mean (standard deviation), or median (interquartile range)

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Supplementary Table 3. Baseline characteristics classified by quarters of the high sensitivity Troponin T distribution.

	Troponin T hs (pg/ml)				
	1st: <3*	2nd: ≥3-<7	3rd: ≥7-<14	4th: ≥14	p-trend
	n=1252	n=754	n=799	n=580	
Age (years)	64.8 (6.1)	66.2 (6.1)	67.7 (6.5)	69.2 (6.9)	<0.0001
Male sex	526 (42.0%)	473 (62.7%)	581 (72.7%)	444 (76.5%)	<0.0001
Duration of diabetes (years)	7.1 (5.8)	7.3 (6.0)	8.1 (6.5)	9.2 (7.2)	<0.0001
Current Smokers	219 (17.5%)	106 (14.1%)	107(13.4%)	73 (12.6%)	0.0021
History of CVD	338 (27.0%)	245 (32.5%)	290 (36.3%)	261 (45.0%)	<0.0001
BMI (kg/m ²)	30.2 (5.4)	30.2 (5.4)	29.9 (4.9)	30.1 (5.2)	0.458
Systolic BP (mmHg)	143.6 (20.1)	147.9 (21.1)	149.3 (22.1)	151.7 (22.8)	<0.0001
Diastolic BP (mmHg)	81.0 (10.5)	82.4 (10.5)	82.5 (11.2)	81.6 (11.1)	0.061
Total chol. (mmol/L)	5.32 (1.26)	5.13 (1.15)	5.02 (1.10)	4.98 (1.08)	<0.0001
HDL chol. (mmol/L)	1.26 (0.34)	1.24 (0.33)	1.20 (0.32)	1.19 (0.33)	<0.0001
Trigs (mmol/L)	1.7 (1.2-2.4)	1.70 (1.20-2.30)	1.68 (1.20-2.35)	1.70 (1.20-2.37)	0.351
Heart rate (bpm)	73.2(11.6)	72.7(12.6)	72.0(12.9)	71.9(12.2)	0.015
HbA1c (%)	7.33 (1.33)	7.39 (1.41)	7.48 (1.43)	7.44(1.50)	0.024
HbA1c (mmol/mol)	56.58(14.52)	57.24(15.45)	58.25(15.59)	57.83(16.39)	0.024
Glucose (mmol/L)	8.4 (2.6)	8.5 (2.6)	8.5 (2.6)	8.5 (3.1)	0.442
AC ratio (μ /mg)	11.5 (6.1-27.5)	15.0 (5.9-34.9)	14.9 (7.0-43.3)	23.0 (8.0-76.9)	<0.0001
eGFR	75.6 (15.3)	73.7 (15.4)	70.1 (16.5)	63.9 (18.9)	<0.0001
CRP (mg/L)	1.82 (0.87-4.05)	1.73 (0.84-4.06)	1.84 (0.87-3.76)	1.83 (0.93-4.24)	0.015
NT-proBNP (pg/ml)	50 (20-114)	76 (31-162)	106 (47-250)	223 (88-624)	<0.0001
Log-transformed NT-proBNP	3.9 (3.0-4.7)	4.3 (3.4-5.1)	4.7 (3.8-5.5)	5.4 (4.5-6.4)	<0.0001

Values are n (%), mean (standard deviation), or median (interquartile range)

* Values are below the limit of sensitivity (3pg/ml)

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Supplementary Table 4. Baseline characteristics classified by quarters of the NT-proBNP distribution.

	NT-proBNP (pg/ml)				p-trend
	1st: ≤35	2nd: >35-≤90	3rd: ≥90-<223	4th: >223	
	n=899	n=878	n=840	n=768	
Age (years)	63.9 (6.1)	66.2 (6.1)	67.3 (6.3)	69.2 (6.6)	<0.0001
Male sex	584 (64.9%)	508 (57.9%)	472 (56.2%)	460 (59.9%)	0.0176
Duration of diabetes (years)	7.1 (5.9)	7.5 (6.1)	7.8 (6.4)	8.7 (6.9)	<0.0001
Current Smokers	173 (19.2%)	136 (15.5%)	115 (13.7%)	81 (10.6%)	<0.0001
History of CVD	193 (21.5%)	224 (25.5%)	323 (38.5%)	394 (51.3%)	<0.0001
BMI (kg/m ²)	29.8 (4.9)	30.2 (5.4)	30.5 (5.3)	29.9 (5.4)	0.479
Systolic BP (mmHg)	142.6 (18.8)	146.8 (20.5)	149.1 (22.6)	151.5 (23.3)	<0.0001
Diastolic BP (mmHg)	82.2 (9.8)	81.7 (10.7)	81.9 (11.1)	81.2 (11.6)	0.118
Total chol. (mmol/L)	5.24 (1.27)	5.17 (1.10)	5.14 (1.16)	5.04 (1.14)	0.001
HDL chol. (mmol/L)	1.20 (0.31)	1.25 (0.33)	1.25 (0.34)	1.22 (0.34)	0.205
Trigls (mmol/L)	1.72 (1.25-2.50)	1.70 (1.24-2.30)	1.69 (1.20-2.40)	1.62 (1.17-2.20)	0.0006
Heart rate (bpm)	75.8(11.8)	72.8(11.2)	71.3(12.2)	70.1(13.0)	0.015
HbA1c (%)	7.44 (1.36)	7.39 (1.34)	7.35 (1.43)	7.40 (1.49)	0.393
HbA1c (mmol/mol)	57.81(14.83)	57.33(14.66)	56.81(15.59)	57.34(16.31)	0.393
Glucose (mmol/L)	8.7 (2.6)	8.5 (2.6)	8.4 (2.6)	8.3 (3.00)	0.002
AC ratio (μ/mg)	12.4 (6.0-30.0)	11.6 (6.0-30.9)	13.3 (6.2-34.5)	22.1 (8.1-70.2)	<0.0001
eGFR	77.5 (14.9)	74.0 (15.6)	70.8 (16.2)	64.0 (17.7)	<0.0001
CRP (mg/L)	1.52 (0.79-3.47)	1.65 (0.83-3.56)	2.01 (0.92-4.38)	2.17 (1.06-4.63)	<0.0001
Troponin T (pg/ml)	1.5 (1.5-6)	4 (1.5-9)	5 (1.5-10.5)	11 (5-18.5)	<0.0001
Log-transformed Troponin T	0.4 (0.4-1.8)	1.4 (0.4-2.2)	1.6 (0.4-2.4)	2.4 (1.6-2.9)	<0.0001

Values are n (%), mean (standard deviation), or median (interquartile range)

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Supplementary Table 5. Integrated discrimination indices (IDI) and relative IDI (95% confidence intervals) for circulating cardiac biomarkers after inclusion in a model with clinical markers of risk*. Results for non-missing data from the random sub-cohort ($N=3500$).

	All microvascular events (N=3225 n=279)	New or worsening nephropathy (N=3225 n=145)	New or worsening retinopathy (N=3225 n=147)
Troponin T			
IDI	0.0225 (0.0164, 0.0291) p-value<0.001	0.0357 (0.0197, 0.0541) p-value<0.001	0.0116 (0.0084, 0.0159) p-value<0.001
Relative IDI (%)	31.4 (23.6, 40.0)	25.5 (14.1, 38.1)	26.2 (20.6, 32.0)
NT-proBNP			
IDI	0.0169 (0.0126, 0.0216) p-value<0.001	0.0305 (0.0180, 0.0443) p-value<0.001	0.0103 (0.0075, 0.0138) p-value<0.001
Relative IDI (%)	23.5 (17.8, 29.2)	21.7 (12.6, 31.2)	23.2 (18.7, 28.4)
Troponin T & NT-proBNP			
IDI	0.0264 (0.0193, 0.0344) p-value<0.001	0.0441 (0.0258, 0.0643) p-value<0.001	0.0123 (0.0089, 0.0165) p-value<0.001
Relative IDI (%)	36.8 (27.1, 47.6)	31.4 (18.4, 45.9)	27.7 (21.7, 33.8)

N: total number of participants (event and non-event) in analysis; n: total number of events in analysis

Base model: age, sex, treatment randomisation, duration of diabetes, current smoking, systolic blood pressure, BMI, AC ratio, eGFR, HbA1c, glucose, total and HDL cholesterol, triglycerides, heart rate, and history of CVD

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Supplementary Table 6. Categorical reclassification model for microvascular risk on addition of high sensitivity Troponin T among those included in the random subcohort only.

Without events				
Base Model (n and percentage)	Base Model + Troponin			
	≤10%	>10% to ≤15%	>15%	Total
≤10%	1939	110	3	2052
	94.5%	5.4%	0.2%	
>10% to ≤15%	96	190	62	348
	27.6%	54.6%	17.8%	
>15%	1	36	292	329
	0.3%	10.9%	88.8%	
Total	2036	336	357	2729
With events				
Base Model (n and percentage)	Base Model + Troponin			
	≤10%	>10% to ≤15%	>15%	Total
≤10%	96	12	0	108
	88.9%	11.1%	0.0%	
>10% to ≤15%	4	30	22	56
	7.1%	53.6%	39.3%	
>15%	1	5	97	103
	1.0%	4.8%	94.2%	
Total	101	47	119	267

Base model: As table 5

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Supplementary Table 7. Categorical reclassification model for microvascular risk on addition of NT-proBNP among those included in the random subcohort only.

Without events				
Base Model (n and percentage)	Base Model + NT-proBNP			
	≤10%	>10% to ≤15%	>15%	Total
≤10%	1968	84	0	2052
	95.9%	4.1%	0.0%	
>10% to ≤15%	73	228	47	348
	21.0%	65.5%	13.5%	
>15%	1	30	298	329
	0.3%	9.1%	90.6%	
Total	2042	342	345	2729
With events				
Base Model (n and percentage)	Base Model + NT-proBNP			
	≤10%	>10% to ≤15%	>15%	Total
≤10%	96	12	0	108
	88.9%	11.1%	0.0%	
>10% to ≤15%	3	37	16	56
	5.4%	66.1%	28.6%	
>15%	0	6	97	103
	0.0%	5.8%	94.2%	
Total	99	55	113	267

Base model: As table 5

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Supplementary Table 8. Categorical reclassification model for microvascular risk on addition of Troponin and NT-proBNP among those included in the random subcohort only.

Without events				
Base Model (n and percentage)	Base Model + Troponin and NT-proBNP			
	≤10%	>10% to ≤15%	>15%	Total
≤10%	1931	116	5	2052
	94.1%	5.7%	0.2%	
>10% to ≤15%	102	182	64	348
	29.3%	52.3%	18.4%	
>15%	1	37	291	329
	0.3%	11.3%	88.5%	
Total	2034	335	360	2729
With events				
Base Model (n and percentage)	Base Model + Troponin and NT-proBNP			
	≤10%	>10% to ≤15%	>15%	Total
≤10%	96	11	1	108
	88.9%	10.2%	0.9%	
>10% to ≤15%	5	28	23	56
	8.9%	50.0%	41.1%	
>15%	1	6	96	103
	1.0%	5.8%	93.2%	
Total	102	45	120	267

Base model: As table 5

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Supplementary References.

1. Pencina MJ, D'Agostino RB, Sr., Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. *Stat Med* 2011;30:11-21.
2. Breslow N, Lumley T, Ballantyne C, Chambless, L, Kulich, M. Using the Whole Cohort in the Analysis of Case-Cohort Data. *Am J Epidemiol* 2009;169:1398–1405.