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ARTICLE

ANGPTL2 is associated with an increased risk of cardiovascular events and death in diabetic patients

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Abstract

Aims/hypothesis A high serum angiopoietin-like 2 (ANGPTL2) concentration is an independent risk factor for developing diabetes and is associated with insulin resistance and atherosclerosis. In this work, we have examined the impact of serum ANGPTL2 on improving cardiovascular (CV) risk stratification in patients with type 2 diabetes.

Methods A prospective, monocentric cohort of consecutive type 2 diabetes patients (the SURDIAGENE cohort; total of 1353 type 2 diabetes patients; 58% men, mean ± SD age 64 ± 11 years) was followed for a median of 6.0 years for death as primary endpoint and major adverse CV events (MACE; i.e. CV death, myocardial infarction or stroke) as a secondary endpoint. Patients with end-stage renal disease, defined as a requirement for dialysis or a history of kidney transplantation,

Eric Thorin and Samy Hadjadj contributed equally to this study.

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were excluded. Patients were grouped into quartiles according to ANGPTL2 concentrations at inclusion: <11.2 (Q1), 11.2–14.7 (Q2), 14.8–19.5 (Q3) or >19.5 (Q4) ng/ml.

Results During follow up, 367 patients (representing 4.5% of the total person-years) died and 290 patients (representing 3.7% of the total person-years) presented with MACE. Both the survival and MACE-free survival rates were significantly different between ANGPTL2 quartiles (logrank 82.12, $p < 0.0001$ for death; and logrank 65.14, $p < 0.0001$ for MACE). Patients with ANGPTL2 concentrations higher than 19.5 ng/ml (Q4) had a significantly higher risk of death and MACE than those with ANGPTL2 levels of 19.5 ng/ml or less (Q1–3) (HR for death 2.44 [95% CI 1.98, 3.00], $p < 0.0001$; HR for MACE 2.43 [95% CI 1.92, 3.06], $p < 0.0001$) after adjustment for sex, age and established CV risk factors. Using ANGPTL2 concentrations, prediction of the risk of mortality, as assessed by integrated discrimination improvement (IDI), was significantly improved (IDI 0.006 ± 0.002, $p = 0.0002$).

Conclusions/interpretation In patients with type 2 diabetes, serum ANGPTL2 concentrations were independently associated with death and MACE. Therefore, ANGPTL2 is a promising candidate biomarker for improving risk stratification in type 2 diabetes patients, and may prove to be a valuable therapeutic target.

Keywords Angiopoietin-like 2 · ANGPTL2 · Biomarker · Death · MACE · Type 2 diabetes

Abbreviations

ANGPTL2	Angiopoietin-like 2 protein
CV	Cardiovascular
CVD	Cardiovascular disease
IDI	Integrated discrimination improvement
MACE	Major adverse cardiovascular events
NT-proBNP	N-terminal pro-B-type natriuretic peptide
TNFR1	TNF receptor 1

Introduction

Cardiovascular (CV) disease (CVD) constitutes the major determinant of outcomes in patients with type 2 diabetes mellitus in terms of mortality and morbidity [1]. As compared with individuals without diabetes, type 2 diabetes mellitus is associated with a twofold increased risk of mortality and a threefold increased risk of coronary artery disease [2]. CVD risk stratification in type 2 diabetes patients is based on the one hand on the evaluation of classical CV risk factors such as LDL-cholesterol concentrations and smoking, and on the other hand on diabetes-related variables such as HbA_{1c} [3–5].

In addition to established risk factors, several biomarkers associated with atherosclerosis, inflammation and congestive heart failure [6–11] have recently been proposed to improve risk stratification in type 2 diabetes mellitus patients. Promising data have been obtained using biomarkers associated with the closely interlinked processes of atherosclerosis and low-grade systemic inflammation [12]. However, none of these biomarkers has been introduced into online risk-stratification engines or international guidelines.

Angiopoietin-like 2 (ANGPTL2) is a proinflammatory circulating protein [13] that has been related to chronic inflammatory diseases such as atherosclerosis [14, 15], diabetes [15, 16], cancer [17] and many others [18]. It is produced by endothelial cells [14, 15], adipocytes [15, 19] and macrophages [20]. Abundantly expressed in visceral fat [15], ANGPTL2 is involved in chronic low-grade inflammation, and might contribute to obesity-related systemic insulin resistance [15, 21]. In healthy individuals, increased serum ANGPTL2 concentrations have been found to be associated with a higher risk of developing type 2 diabetes mellitus [16]. Furthermore, patients with heart failure have been found to have increased ANGPTL2 concentrations, as compared with a healthy reference population [22]. Recently, higher ANGPTL2 levels have also been observed in patients with acute coronary syndrome [23]. These findings suggest a link between serum ANGPTL2 concentrations and CV risk factors and CV disease, and it has recently been proposed that ANGPTL2 may be a promising therapeutic target [18].

In the present study, we examined whether measurement of serum ANGPTL2, in addition to established risk markers, is useful in the long-term risk assessment of type 2 diabetes mellitus patients with regard to all-cause mortality and CV morbidity and mortality.

Methods

Patients were prospectively included in this single-centre cohort (the 'SURDIAGENE' cohort) from 2002 to 2011 and followed until 2013 [24]. The study design was approved by the local ethics committee, and written informed consent was obtained from all participants.

Inclusion and exclusion criteria Adult patients with type 2 diabetes mellitus were consecutively recruited and followed regularly at the University Hospital of Poitiers, France. The main exclusion criteria were residence outside of the Poitiers region and evidence of non-diabetic renal disease. For this analysis, additional exclusion criteria were the presence at study admission of a stage 4 or worse chronic kidney disease (GFR <30 ml min^{-1} [1.73 $m]^{-2}$; renal replacement therapy) and a follow-up duration of less than 3 months.

Demographic, clinical and biological variables Clinical data were obtained at inclusion from patient records, and morphometric measurements, BP and ECG recordings were taken at inclusion. A self-reported history of myocardial infarction or symptomatic peripheral artery disease was noted. Biological data were also collected at inclusion. HbA_{1c} and serum creatinine concentrations were determined centrally in the fasting state using a chromatography method (ADAMS A_{1c} HA-8160 analyser; Menarini, Florence, Italy) and a colorimetric method on an automated analyser (KONE Optima; Thermo Clinical Labsystems, Vantaa, Finland), respectively. The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration formula [25]. Urinary creatinine was measured on a Hitachi 911 automatic analyser (Roche Diagnostics, Meylan, France), and urinary albumin by nephelometry on a Modular System P (Roche Diagnostics).

Serum concentrations of TNF receptor 1 (TNFR1), a marker of inflammation, were measured using a human soluble TNFR1 ELISA kit (EKF Diagnostics, Dublin, Ireland). All serum samples were tested in duplicate, and the mean of the two measurements was taken for statistical analysis. Serum ANGPTL2 concentrations were measured using a human ANGPTL2 ELISA kit (Cloud-Clone Corp., Houston, TX, USA) according to the manufacturer's instructions. Serum samples were diluted (1:2, or 1:4 if needed) using the kit standard diluent solution. A serum sample from one healthy volunteer was always included on each ELISA plate in order to validate the assay; inter- and intra-assay variability were <15% and <10%, respectively. Optical densities (450 nm) of two standard curves were averaged and a model of non-linear regression with four variables was used to calculate ANGPTL2 concentrations. This model allowed for the calculation of ANGPTL2 concentrations up to 60 ng/ml in samples diluted 1:2; above this threshold, samples were re-taken and diluted 1:4.

Study outcomes Whether each patient was alive or dead and CV endpoints were determined from patients' hospital records, interviews with their general practitioners and inquiry to the French National Death Registry. The present analysis takes the most recent available data (2013) into account. The primary endpoint was all-cause mortality. The secondary endpoint was a composite of CV death, non-fatal myocardial infarction and non-fatal stroke (major adverse CV events [MACE]) [26]. Each event was reviewed by an adjudication committee according to the international definitions of clinical outcomes. The adjudication committee was blinded with regard to ANGPTL2 concentrations. We compared the observed risk for all-cause death in our cohort with the risk predicted by the latest version of the UK Prospective Diabetes Study outcome equation using the following variables: age, diabetes duration, sex, ethnicity, current smoking status, systolic BP, HbA_{1c}, HDL- and LDL-cholesterol, BMI, eGFR, heart rate, atrial fibrillation, albuminuria and peripheral vascular disease.

Statistical analysis Qualitative variables are reported as absolute values and percentages, while quantitative variables are described by mean ± SD or median (interquartile range), as appropriate. Associations between qualitative variables were evaluated using the χ^2 test. Quantitative variables were analysed using the Student's *t* test or Mann–Whitney *U* test, as appropriate.

We considered time to occurrence of the first event as the primary endpoint. Survival curves were built using the Kaplan–Meier method and compared using the logrank test. Risk-prediction models established by Cox proportional hazard models were used to analyse the effect on study outcomes. HRs and their 95% CIs are presented.

We tested for non-linear associations using cubic splines for ANGPTL2 and non-adjusted risk of all-cause death. Quantitative variables were log-transformed if appropriate in order to meet the application requirement for the Cox model. Variables associated with all-cause death at $p < 0.10$ in the univariate Cox model were selected for the multivariate model. The final model was determined using multiple stepwise regression analysis. To assess improvements in the Cox model achieved by adding ANGPTL2, we used the integrated discrimination improvement (IDI) index and Harrell's C index, in addition to established risk markers.

All hypotheses were tested at the 5% level of significance. Statistical analyses were carried out using the SAS version 9.3 software package (SAS, Cary, NC, USA).

Results

The median follow-up duration was 72 months (interquartile range 67 months), corresponding to 8143 person-years. The demographic, clinical and biological characteristics of the study population are summarised in Table 1.

The mortality rate was 4.5% of total person-years (95% CI 4.0, 5.0; $n = 367$) and the MACE rate was 3.7% of total person-years (95% CI 3.3, 4.1; $n = 290$). The major causes of death were CVD ($n = 211$; 57%), cancer ($n = 56$; 15%) and infections ($n = 33$; 9%).

When compared with the survivor group, patients who died during follow-up had more CV risk factors (including greater impairment of renal function) and also had more prior CV events (Table 1). With regard to type 2 diabetes mellitus, the duration of diabetes was longer and insulin use was more common in those who died, whereas HbA_{1c} levels were comparable between the groups (Table 1).

Serum ANGPTL2 protein concentrations at inclusion were significantly associated with the risk of both death (HR 8.60 [95% CI 5.36, 13.78]; $p < 0.0001$ per log ng/ml) and MACE

Table 1 Baseline patient characteristics according to death

Variable	All ($n = 1353$)	Event ($n = 367$)	No event ($n = 986$)	p value
Men/women, *n* (%)	782 (58)/571 (42)	241 (66)/126 (34)	541 (55)/445 (45)	0.0003***
Age, years	64 ± 11	70 ± 9	63 ± 11	<0.0001***
Non-white ethnicity, *n* (%)	43 (3)	10 (3)	33 (3)	0.5620
BMI, kg/m^2	31 ± 6	31 ± 6	32 ± 6	0.0107*
Active smoking, *n* (%)	148 (11)	43 (12)	105 (11)	0.5738
Hypertension, *n* (%)	1112 (82)	329 (90)	783 (79)	<0.0001***
History of myocardial infarction, *n* (%)	200 (15)	85 (23)	115 (12)	<0.0001***
History of stroke, *n* (%)	73 (5)	32 (9)	41 (4)	0.0010***
Diabetes duration, years	12 (14)	16 (15)	11 (13)	<0.0001***
HbA_{1c}, %	7.8 ± 1.5	7.9 ± 1.5	7.8 ± 1.6	0.1841
HbA_{1c}, mmol/mol	62 ± 12	63 ± 12	62 ± 13	
LDL-cholesterol, mmol/l	2.72 ± 0.96	2.79 ± 1.01	2.69 ± 0.93	0.1043
Serum creatinine, μmol/l	81 (29)	89 (38)	79 (26)	<0.0001***
eGFR, ml min^{-1} (1.73 m)$^{-2}$	76.7 ± 20.9	68.9 ± 21.2	79.6 ± 20.0	<0.0001***
uACR, mg/mmol	2.7 (9.7)	6.9 (31.5)	2.1 (6.7)	<0.0001***
ANGPTL2, ng/ml	14.8 (8.3)	18.1 (10.6)	13.9 (7.6)	<0.0001***
NT-proBNP, pg/ml	102.9 (214.3)	240.5 (514.4)	77.4 (155.8)	<0.0001***
Soluble TNFR1, pg/ml	1817 (696)	2109 (954)	1744 (576)	<0.0001***
Systolic BP, mmHg	132 ± 17	136 ± 19	131 ± 17	<0.0001***
Diastolic BP, mmHg	72 ± 11	72 ± 11	72 ± 11	0.9780
Resting heart rate, bpm	71 ± 14	72 ± 15	71 ± 13	0.1156
Sinus rhythm, *n* (%)	1281 (95)	330 (90)	951 (97)	<0.0001***
Treatment with, *n* (%):				
Beta-blockers	456 (34)	135 (37)	321 (33)	0.1434
ACE inhibitor	505 (37)	170 (46)	335 (34)	<0.0001***
ARB	375 (28)	85 (23)	290 (29)	0.0224*
Diuretics	602 (44)	192 (52)	410 (42)	0.0004***
Insulin	799 (59)	272 (74)	527 (53)	<0.0001***
Aspirin	377 (28)	112 (31)	265 (27)	0.1841
Statin	619 (46)	162 (44)	457 (46)	0.04687*

Quantitative variables are described as mean ± SD or median (interquartile range), unless otherwise specified

*$p < 0.05$, ***$p < 0.001$ for those who died vs those who survived

ARB, angiotensin II receptor blocker; uACR, urinary albumin/creatinine ratio

(HR 7.15 [95% CI 4.19, 12.18]; $p < 0.0001$ per log ng/ml). The non-adjusted risks of death and MACE according to serum ANGPTL2 concentrations at inclusion, expressed as a continuous variable, are presented in electronic supplementary material (ESM) Figs 1 and 2, respectively.

In order to identify a clinically meaningful threshold for serum ANGPTL2, the cohort was divided into quartiles (<11.2 [Q1], 11.2–14.7 [Q2], 14.8–19.5 [Q3] and >19.5 [Q4] ng/ml) and the mortality rate was determined for each quartile. While the mortality rate was not significantly different among the first three quartiles (Q1–3), it was significantly higher for patients in Q4 (i.e. those with the highest ANGPTL2 concentrations) (Fig. 1). Next, survival rates (Fig. 2a) and MACE-free survival rates (Fig. 2b) were calculated for each quartile, and significantly worse outcomes were again shown for patients in Q4 as compared with Q1–3. The HR for death in Q4 as compared with Q1 was 3.18 (95% CI 2.33, 4.34). Of note, patients with ANGPTL2 above 19.5 ng/ml (Q4) compared with those with ANGPTL2 of 19.5 ng/ml or less (Q1–3) were older, had more CV risk factors (hypertension) and longer diabetes duration, were more frequently on insulin, and had higher N-terminal pro-B-type natriuretic peptide (NT-proBNP) and TNFR1 levels (although HbA_{1c} levels were comparable), and more patients had a history of stroke and impaired renal function (Table 2). Taken together, a serum ANGPTL2 level of >19.5 ng/ml at inclusion was associated with a significantly increased risk of adverse events independent of established CV or diabetes-associated risk factors and/or markers.

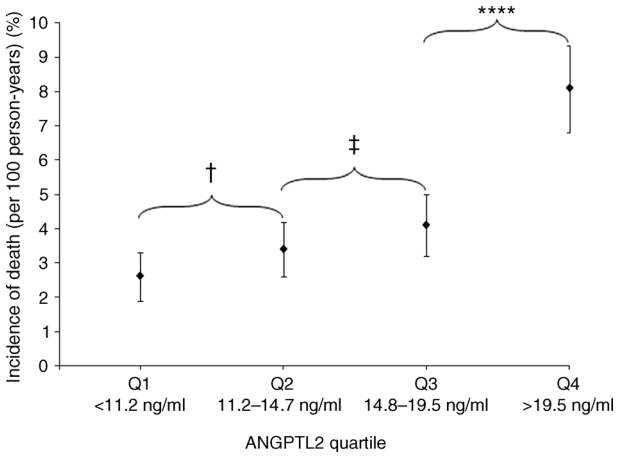

Fig. 1 Incidence of death per 100 person-years according to ANGPTL2 quartiles. Vertical bars show the 95% CIs. ****$p < 0.0001$; $^{\dagger}p = 0.1470$; $^{\ddagger}p = 0.2463$

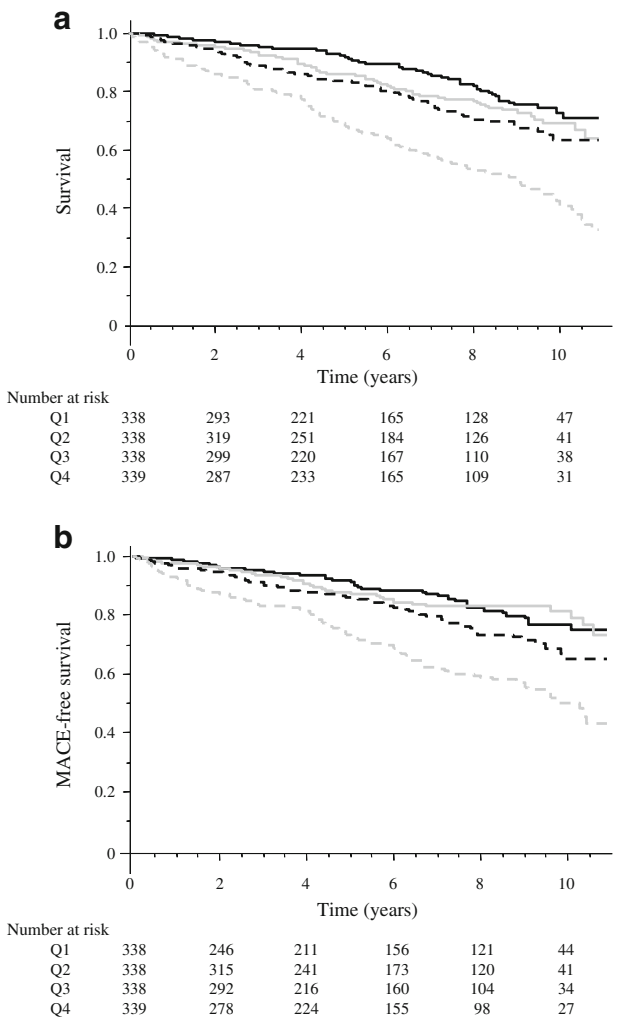

Fig. 2 (**a**) Survival rates and (**b**) MACE-free survival rates according to ANGPTL2 quartiles. Black solid line, Q1; grey solid line, Q2; black dashed line, Q3; grey dashed line, Q4

Beyond male sex and advanced age, increased NT-proBNP, history of MI, and ANGPTL2 concentrations remained the variables the most strongly associated both with increased risk of death (Table 3) and MACE (Table 4) in the multivariate analysis after adjustment for confounders. The association between ANGPTL2 concentrations and adverse outcomes remained statistically significant when considering ANGPTL2 concentrations as a continuous variable.

By using ANGPTL2 concentrations above 19.5 ng/ml in addition to established prognostic markers in type 2 diabetes mellitus, as presented in Tables 3 and 4, the risk assessment could be significantly improved for death (IDI 0.006 ± 0.002, $p = 0.0002$) and for MACE (IDI 0.007 ± 0.002 $p = 0.0014$), respectively. Considering another complementary approach with Harrel's C index, the addition of ANGPTL2 concentration in the model non-significantly improved the C index from 0.766 to 0.767 for death, and from 0.757 to 0.760 for MACE, respectively. The prognostic value of this ANGPTL2 threshold was comparable among different patient subgroups (ESM Fig. 3). We considered the UK Prospective Diabetes Study outcome equation for death in order to evaluate the clinical relevance of ANGPTL2 concentrations in addition to established risk factors, yielding a significant improvement on the prediction of mortality (IDI 0.0012; $p = 0.0003$).

Discussion

In this study we have shown, for the first time, a significant association between serum ANGPTL2 concentrations and all-cause mortality in type 2 diabetes mellitus patients, reinforcing the concept that ANGPTL2, a proinflammatory and pro-oxidative factor, may be causal to the development of CVD associated with type 2 diabetes.

Type 2 diabetes patients represent a high-risk population for mortality and CV morbidity, which are driven by macro- and microvascular complications [27, 28]. Despite optimal medical treatment, type 2 diabetes is still associated with a higher risk of more rapid coronary artery disease progression and the development of diabetic cardiomyopathy [29]. CV risk stratification in type 2 diabetes is still essentially based on the assessment of traditional CV risk factors, and the presence of micro- and macrovascular complications including impaired renal function [30].

Since inflammation plays a key role in the pathophysiology of insulin resistance and diabetes, and because patients with chronic low-grade inflammation are at particularly high risk of adverse events related to atherosclerosis [31], the usefulness of several inflammation and atherosclerosis markers has been evaluated so as to improve CV risk stratification in patients with type 2 diabetes mellitus [10, 12]. In the present study, we investigated the prognostic value of the proinflammatory protein ANGPTL2 for risk stratification. The prognostic value of this biomarker, beyond

Table 2 Comparison of characteristics according to serum ANGPTL2 concentration (Q1–3 vs Q4)

Variable	Q1–3 ($n = 1014$)	Q4 ($n = 339$)	p value
ANGPTL2, ng/ml	12.9 (5.7)	25.2 (9.7)	
Men/women, *n* (%)	578 (57)/436 (43)	204 (60)/135 (40)	0.3055
Age, years	63 ± 11	69 ± 9	<0.0001***
BMI, kg/m^2	31 ± 6	32 ± 6	0.1651
Active smoking, *n* (%)	122 (12)	26 (8)	0.0265*
Hypertension, *n* (%)	805 (79)	307 (91)	<0.0001***
History of myocardial infarction, *n* (%)	141 (14)	59 (17)	0.1161
History of stroke, *n* (%)	46 (5)	27 (8)	0.0156*
Diabetes duration, years	11 (14)	15 (15)	<0.0001***
HbA_{1c}, %	7.8 ± 1.6	7.8 ± 1.4	0.6996
HbA_{1c}, mmol/mol	61.7 ± 13	61.7 ± 11	
LDL-cholesterol, mmol/l	2.77 ± 0.96	2.72 ± 0.96	0.2818
Serum creatinine, μmol/l	77 (24)	98 (43)	<0.0001***
eGFR, ml min^{-1} (1.73 m)$^{-2}$	81.8 ± 18.5	61.3 ± 19.9	<0.0001***
uACR, mg/mmol	2.2 (6.6)	7.1 (29.5)	<0.0001***
NT-proBNP, pg/ml	82.0 (169.3)	207.2 (492.4)	<0.0001***
TNFR1, pg/ml	1705 (546)	2328 (1122)	<0.0001***
Systolic BP, mmHg	131 ± 17	134 ± 18	0.0075**
Diastolic BP, mmHg	73 ± 11	72 ± 12	0.3369
Resting heart rate, bpm	71 ± 13	71 ± 15	0.5793
Sinus rhythm, *n* (%)	972 (96)	309 (91)	0.0003***
Treatment with, *n* (%):			
Beta-blocker	320 (32)	136 (40)	0.0039**
ACE inhibitor	360 (36)	145 (43)	0.0166*
ARB	269 (27)	106 (31)	0.0914
Diuretics	405 (40)	197 (58)	<0.0001***
Insulin	558 (55)	241 (71)	<0.0001***
Aspirin	278 (27)	99 (29)	0.5251
Statin	476 (47)	143 (42)	0.1278

Quantitative variables are described by mean ± SD or median (interquartile range), unless otherwise specified

ANGPTL2 cut-off level between Q1–3 and Q4: 19.5 ng/ml

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

ARB, angiotensin II receptor blocker; uACR, urinary albumin/creatinine ratio

traditional variables, is an important question. The IDI approach suggested that ANGPTL2 serum concentration contributes additional information with regard to mortality risk, although the C statistics approach was not significant. Prospective studies on this question will help to resolve this issue. Serum ANGPTL2 concentrations were strongly associated not only with CV risk, but also with all-cause mortality. We suggest a threshold for ANGPTL2 serum concentrations of nearby 20 ng/ml, above which the risk of all-cause death increases by 60%.

The upper threshold for normal serum ANGPTL2 concentrations has not been defined. In healthy and physically active volunteers, serum concentrations of ANGPTL2 range from 1 to 3 ng/ml, with similar concentrations between males and females [18]. In the Hisayama study, median serum ANGPTL2 concentrations of 2.7–3.4 ng/ml were reported [16]; the risk of developing type 2 diabetes was significantly higher in the highest ANGPTL2 quartile (≥3.41 ng/ml). Further studies have reported ANGPTL2 concentrations to average 3.4 vs 2.6 ng/ml in healthy volunteers [15], while ANGPTL2 concentrations of 4.2 ng/ml have been found in obese dyslipidaemic women with insulin resistance [21]. In contrast to the small changes in serum ANGPTL2 reported in the literature in relatively healthy patients, we found high concentrations of ANGPTL2 in patients with established chronic diseases, in accordance with a Chinese group [32] that reported very high ANGPTL2 levels in diabetic patients with established kidney disease, ranging from 2.1 to 72.3 ng/ml. It has been suggested that reduced kidney clearance associated with chronic kidney disease could cause an increase in ANGPTL2 levels [33], explaining the discrepancies with

Table 3 Cox multivariate analysis for the risk of death

Variable	Maximal model			Final model		
	HR	95% CI	*p* value	HR	95% CI	*p* value
Sex (ref. men)	0.69	0.55, 0.87	0.0018	0.71	0.57, 0.89	0.0027
Age (per year)	1.06	1.04, 1.07	<0.0001	1.06	1.05, 1.08	<0.0001
BMI, kg/m^2	1.01	0.99, 1.02	0.6156			
Diabetes duration (per year)	1.01	1.00, 1.02	0.0598			
History of myocardial infarction	1.42	1.06, 1.90	0.0172	1.52	1.18, 1.96	0.0012
Hypertension	0.93	0.61, 1.40	0.7091			
History of stroke	1.51	1.03, 2.21	0.0338			
eGFR, ml min^{-1} (1.73 $m)^{-2}$	1.01	1.00, 1.02	0.0006	1.01	1.00, 1.02	0.0021
ANGPTL2 (ref. Q1–3)	1.23	0.96, 1.58	0.1023	1.30	1.01, 1.66	0.0397
Soluble TNFR1 (per 0.01 log pg/ml)	1.03	1.02, 1.04	<0.0001	1.03	1.02, 1.04	<0.0001
NT-proBNP (per 100 pg/ml)	1.01	1.00, 1.02	<0.0001	1.03	1.02, 1.04	<0.0001
Systolic BP, mmHg	1.00	0.99, 1.01	0.9276			
Sinus rhythm	0.67	0.46, 0.97	0.0352			
Treatment with:						
Beta-blocker	1.09	0.85, 1.41	0.5012			
ACE inhibitor	1.21	0.97, 1.52	0.0935			
Diuretics	1.13	0.90, 1.42	0.3017			
Insulin	1.34	1.04, 1.71	0.0226			
Aspirin	0.80	0.62, 1.02	0.0686			
uACR (ref. <3 mg/mmol)			<0.0001			<0.0001
3–30 mg/mmol	1.36	1.06, 1.76		1.44	1.13, 1.84	
>30 mg/mmol	2.37	1.73, 3.24		2.38	1.78, 3.17	

Variables associated with all-cause death at $p < 0.10$ in the univariate Cox model were selected for the multivariate 'maximal model'. The 'final model' was determined using multiple backwards stepwise regression analysis applied to the 'maximal model'

uACR, urinary albumin/creatinine ratio

other diseases (e.g. coronary heart disease, acute coronary syndrome). As the mechanism of ANGPTL2 clearance is unknown, this remains to be validated. In summary, the higher values measured in this study are most likely due to differences in ethnic background (white), geographical location (France) and/or clinical characteristics (diabetes and kidney disease) compared with previous studies.

Since ANGPTL2 is secreted by adipose tissue, one could expect that increased BMI might be associated with increased serum ANGPTL2 concentrations, as reported in the seminal work by Tabata et al [15]. However, in the present cohort, the fourth quartile of patients, showing the highest ANGPTL2 concentrations and the highest mortality rate, did not have a significantly higher BMI at inclusion than patients in the first three quartiles. In other words, obese type 2 diabetes mellitus patients did not have higher ANGPTL2 concentrations and were not necessarily at higher risk of mortality than non-obese patients. Consequently, an association between obesity on the one hand and increased concentrations of ANGPTL2 and an increased risk of adverse events on the other hand was not obvious in the present cohort.

Rather than the absolute quantity of adipose tissue, the degree of low-grade inflammation, in which adipose tissue is substantially implicated in type 2 diabetes, seems to play a determining role in insulin resistance [34] and, subsequently, for CV complications in these patients [35]. Indeed, inflammation plays a pivotal role in the promotion of vascular damage in type 2 diabetes [36], and the implication of ANGPTL2 as a proinflammatory cytokine favouring macrophage accumulation and activation in this process therefore seems plausible [15]. However, data derived from preclinical research regarding the beneficial [37] or deleterious [15, 34] role of ANGPTL2 in insulin resistance remain somewhat contradictory.

As we described in a previous publication, low-grade inflammation as assessed by soluble TNFR1 concentrations was significantly associated with adverse events in the present cohort [10], thereby confirming and underscoring the link between inflammatory markers and poor outcomes in type 2 diabetes. Interestingly, the additive diagnostic value of serum ANGPTL2 remained highly significant despite adjustment for TNFR1. This finding suggests that ANGPTL2, beyond its role

Table 4 Cox multivariate analysis for the risk of MACE

Variable	Maximal model			Final model		
	HR	95% CI	*p* value	HR	95% CI	*p* value
Sex (ref. men)	0.72	0.56, 0.94	0.0139	0.70	0.55, 0.90	0.0058
Age (per year)	1.04	1.03, 1.06	<0.0001	1.04	1.03, 1.06	<0.0001
BMI, kg/m^2	1.00	0.98, 1.03	0.7886			
Diabetes duration (per year)	1.01	1.00, 1.02	0.1821			
History of myocardial infarction	1.74	1.27, 2.38	0.0006	1.73	1.32, 2.27	<0.0001
Hypertension	0.92	0.57, 1.47	0.7129			
History of stroke	1.52	1.00, 2.32	0.0508			
HbA_{1c}, %	1.10	1.01, 1.20	0.0248			
eGFR, ml min^{-1} (1.73 $m)^{-2}$	1.01	1.00, 1.02	0.1261			
ANGPTL2 (ref. Q1–3)	1.40	1.05, 1.85	0.0198	1.46	1.14, 1.88	0.0027
TNFR1 (log pg/ml)	2.62	0.81, 8.50	0.1093			
NT-proBNP (per 100 pg/ml)	1.01	1.00, 1.02	<0.0001	1.03	1.02, 1.04	<0.0001
Systolic BP, mmHg	1.00	1.00, 1.01	0.4738			
Sinus rhythm	0.68	0.45, 1.03	0.0679			
Treatment with:						
Beta-blocker	1.03	0.77, 1.38	0.8267			
ACE inhibitor	1.20	0.93, 1.55	0.1702			
Diuretics	1.28	0.98, 1.66	0.0660	1.32	1.04, 1.68	0.0239
Insulin	1.49	1.12, 1.98	0.0059	1.63	1.24, 2.14	0.0005
Aspirin	0.89	0.67, 1.17	0.3841			
uACR (ref. <3 mg/mmol)			0.0004			<0.0001
3–30 mg/mmol	1.17	0.96, 1.69		1.35	1.03, 1.78	
>30 mg/mmol	2.04	1.43, 2.90		2.29	1.68, 3.14	

Variables associated with MACE at $p < 0.10$ in the univariate Cox model were selected for the multivariate 'maximal model'. The 'final model' was determined using multiple backwards stepwise regression analysis applied to the 'maximal model'

uACR, urinary albumin/creatinine ratio

as a proinflammatory marker, might be related to adverse outcomes by its implication in pathophysiological processes independent of that of TNF. This further supports the concept that ANGPTL2 may be a valuable target in CVD.

Beyond ANGPTL2, three other members of the ANGPTL family have been shown to be associated with CV risk factors and/or CVD. A significant association between ANGPTL3 and atherosclerosis has been reported [38]. The potential of ANGPTL3 inhibition to reduce atherosclerosis is still under evaluation [39]. ANGPTL3 might also be implicated in insulin sensitivity [40] and thereby modulate both lipid and glucose metabolism. ANGPTL4 has also been associated with dyslipidaemia and atherosclerosis in preclinical studies, with interesting data derived from genetic studies [41, 42]. Data concerning the role of ANGPTL8 in diabetes and obesity are conflicting. In mice, ANGPTL8 has been proposed to improve glucose tolerance [43], but increased ANGPTL8 levels in diabetic patients have not been correlated with glucose or insulin levels [44, 45]. Taken together, only ANGPTL4 has been demonstrated to be associated with CV events [46], and none of the ANGPTL family members has been demonstrated to be associated with all-cause death.

Limitations and strengths The major limitation of the present study is that the proposed ANGPTL2 threshold for improving risk stratification in diabetic patients has not yet been confirmed by a validation cohort. Another limitation is that, in the absence of a clinical indication, the majority of patients did not have cardiac imaging at inclusion, which means that the pre-existence of cardiac pathologies such as myocardial hypertrophy or valvular diseases cannot be excluded with certainty. Finally, recruitment in the SURDIAGENE cohort was hospital-based, with a possible recruitment bias for high-risk patients, and might therefore not be representative for an all-comers type 2 diabetes population.

Major strengths of this study are the low dropout rate, the relatively long follow-up, the predefined and clinically highly relevant endpoints, and the robustness of the outcome data in various subgroups.

Conclusions and perspectives We have shown a strong and independent association of serum ANGPTL2 concentrations with death and MACE in patients with type 2 diabetes mellitus, with or without a history of CV events. Furthermore, we have proposed a clinically meaningful threshold of ANGPTL2, above which the risk of all-cause death is significantly higher. If our results are confirmed, measurement of ANGPTL2 could become a promising biomarker for improving risk stratification in type 2 diabetes mellitus patients.

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