Diabetic Retinopathy and Skin Tissue Advanced Glycation End Products Are Biomarkers of Vascular Events in Type 2 Diabetic Patients

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Original investigation

Abstract

BACKGROUND AND AIMS: Vascular events are the main cause of mortality in patients with type 2 diabetes. However, the risk of vascular events is not homogeneous in subjects with type 2 diabetes and, therefore, an early identification of patients at high risk of developing vascular events remains a challenge to be met. The aim of this study is to evaluate whether the presence of diabetic retinopathy (DR) and accumulation of advanced glycation end products (AGEs) in subcutaneous tissue can help to identify those patients at high risk of vascular events.

MATERIAL AND METHODS: It was a prospective study comprising 200 subjects with type 2 diabetes with no history of clinical cardiovascular disease and 60 non-diabetic controls, matched by age and sex (PRECISED study: ClinicalTrials.gov NCT02248311). The inclusion period began on September 2014 and finished on June 2017. We collected basal features of the subjects, classical cardiovascular risk factors (i.e. age, sex, hypertension, dyslipidemia and coronary artery calcium score [CACs]), presence and degree of DR, and the accumulation of AGEs in subcutaneous tissue using the AGE readerTM device (DiagnOptics Technologies). We followed these subjects until December 2020, collecting any coronary, cerebrovascular or peripheral arterial event.

RESULTS: After a follow up of 4.35 ± 1.43 years, a total of 24 vascular events were registered. There was no significant difference regarding age and gender between individuals with type 2 diabetes and the control group. The number of vascular events was higher in type 2 diabetes group than in the control group (12.3% vs. 1.75%). When analysing the risk factors we found that apart from classic risk factors such as age, gender and CACs, subjects with type 2 diabetes and vascular events presented a higher prevalence of DR (47.8% vs. 24.4%; $p = 0.018$) and AGEs in subcutaneous tissue (63.15% vs 26.71% of values in the higher tertile, $p = 0.001$). DR and AGEs in subcutaneous tissue remain as independent variables related to the development of vascular events in the Cox proportional hazard multiple regression analysis (HR 2.58, 95%CI 1.14−5.85, $p = 0.023$, and HR 4.68, 95%CI 1.83−11.96, $p = 0.001$; respectively).

CONCLUSIONS: As we expected, patients with type 2 diabetes have significantly more VE than non-diabetic subjects. Apart from the classic factors such as age, sex and CACs, we observed that the presence of DR and high levels of AGEs in subcutaneous tissue were predictors of vascular events.

Introduction

Type 2 diabetes confers a substantial burden of macrovascular disease, with two-to four-fold higher increased risk of any cardiovascular event in comparison with non-diabetic patients (1, 2). Although type 2 diabetes is recognized as an independent risk factor for cardiovascular disease (CVD), not all patients with diabetes appear to be at equal risk. In fact, a high percentage of these patients will never experience vascular complications (3, 4). Therefore, the early identification of diabetic patients at risk of developing CVD remains as a challenge to be met (5, 6).

It is well known that chronic hyperglycaemia is related with vascular complications of diabetes. However two large studies revealed that tight glucose control slightly but no significantly reduced the risk of cardiovascular disease (CVD) in either type 1 (7) or type 2 diabetes patients (8). Furthermore, the exaggerated risk for CVD in this population is not fully explained by conventional risk factors such obesity, hyperglycaemia, dyslipidaemia and hypertension and, in fact, a substantial proportion of this risk remains unexplained (5, 6). Therefore, specific diabetes-related risk factors should be involved in the excess risk for CVD, and tissue accumulation of advanced glycation end products (AGEs) could be one of them.

AGEs are formed by the Maillard process, a non-enzymatic glycation of proteins. Early-stage reactions lead to formation of the early glycation adducts (as HbA1c), and later-stage reactions subsequently form AGEs (9). AGEs accumulate in the body during aging, and this process is accelerated by chronic hyperglycemia and oxidative stress (10), two conditions commonly present in type 2 diabetes. Therefore, the formation and accumulation of AGEs is accelerated by the diabetic milieu.

AGEs may play a critical role in the development of diabetic complication by two pathways. First, AGEs can form cross-links with proteins that affect the three-dimensional structure and thereby the functions of these proteins, for example collagen; its modification leads to an increase in vascular and myocardial stiffness. Second, AGEs can cause deleterious effects by the activation of receptors for AGEs (RAGEs), which in turn can lead to activation of second messengers and transcription factors that up-regulate pro-inflammatory cytokines and mediators of oxidative stress. These effects modify pathways which contribute to vascular dysfunction and accelerated development of atherosclerotic processes (11).

In recent years, it has been developed a simple and non-invasive method for AGEs assessment through skin autofluorescence (SAF) based on specific fluorescence of some AGEs. SAF has a strong correlation with the specific AGEs content in skin biopsies, as shown by validation studies (12, 13). There is accumulating evidence of the relationship between SAF and the presence of micro and macroangiopathy in individuals with type 2 diabetes (14). We had previously reported that SAF was a good predictor of a calcium score (CACs) > 400AU, a reliable marker of coronary atherosclerosis (15).

Emerging data indicates link between diabetic microvascular complications such as retinopathy, nephropathy and neuropathy, and cardiovascular disease (16, 17). In addition, a population-based cohort study showed that cumulative burden of microvascular disease increases the risk of future CVD among individuals with type 2 diabete(18). DR has been linked with an increase in risk for all-cause and cardiovascular mortality in patients with diabetes (19, 20). The degree of DR is related to several classical cardiovascular risk factors including hyperglycaemia, blood pressure, renal insufficiency, etc. In addition, we provide evidence that the presence and the degree of DR was a powerful and independent risk factor for identifying subjects with subclinical CVD (21). It should be noted that the diabetic-induced microvascular abnormalities that occurs in the retina may also happen in other vascular beds, such as myocardial microcirculation (22, 23). Nevertheless, DR is often missing as a risk factor in studies addressed to evaluate CVD.

On this basis, the aim of this study is to evaluate whether the presence of diabetic retinopathy (DR) and accumulation of advanced glycation end products (AGEs) in subcutaneous tissue can help to identify those patients with type 2 diabetes at high risk of developing vascular events.

Material And Methods

Study design and subjects

It was a prospective case-control study comprising 200 subjects with type 2 diabetes and 60 non-diabetic controls matched by age and sex, all of them with no history of clinical CVD. The included subjects were enrolled in the PRECISED study (ClinicalTrial. gov NCT02248311).

All subjects enrolled must meet the following criteria: 1) type 2 diabetes diagnosed at least one year before to the day of screening; b) Age from 50–79 years; c) No history of vascular event; d) No contraindication for the performance of CT scan or SAF assessment; and e) No concomitant disease associated with a short life expectancy.

All included subjects were selected from the Outpatient Diabetic Clinic of Vall d'Hebron University Hospital and the Primary Healthcare centers within its catchment area (North Barcelona). The recruitment period began on September 2014 and finished on June 2017. Of the 200 patients with type 2 diabetes, 13 withdrawn the consent, and the same occurred in 3 out 60 of the control group. Consequently, 187 subjects with type 2 diabetes and 57 non-diabetic controls were followed until December 2020.

The study was conducted according to the declaration of Helsinki and was approved by the local ethics committee. All subjects provided written informed consent before study entry.

Data collection and laboratory tests

Basal features of the subjects and classical cardiovascular risk factors (age, sex, ethnicity, current smoking, body mass index, systolic and diastolic blood pressure, clinical characteristic of diabetes disease and comorbidities associated) were collected at the first visit. In addition, a fasting venous blood sample was obtained from each recruited patient.

Anthropometric data were obtained by standardized protocols. A balance with a fixed stadiometer was used to measure height and weight. Waist circumstance was measured between the 10th rib and the iliac crest.

The assessment of the classic risk factors was carried out as follows: A history of smoking habits (non-smoker/current smoker/ex-smoker) was recorded. Hypertension was established as systolic blood pressure $\geq$ 140 mm Hg or diastolic blood pressure $\geq$ 90 mm Hg, or when subjects were under treatment with antihypertensive agents. Dyslipidaemia was defined by the use of lipid-lowering drugs, decreased values of high-density lipoprotein (HDL) cholesterol (men < 0.9mmol/L, women < 1mmol/L), or by at least

one increased value of total cholesterol (> 5.2mmol/L), low-density lipoprotein (LDL) cholesterol (> 4.3mmol/L), or triglycerides (> 1.7mmol/L)

Fasting venous blood sample was collected from the antecubital vein, separated by centrifugation (2000xg at 4ºC for 20 min) and frozen at − 80ºC for batched storage and analysis. HbA1c was by the Cobas B 101 (Roche) system. The remaining biochemical parameters were measured using an Olympus AU5400 automatic biochemistry analyzer (Olympus, Tokyo, Japan).

Fundus Eye examination

DR was evaluated by experienced ophthalmologists in mydriasis using slit-lamp biomicroscopy and retinography with the same camera (Topcon-DRI-OCTTRITON). The examiners classified DR according to the International Clinical Diabetic Retinopathy Disease Severity Scale (24): (1) no apparent retinopathy, (2) mild non-proliferative retinopathy (NPDR), (3) moderate NPDR, (4) severe NPDR, and (5) proliferative diabetic retinopathy (PDR).

Measurement of Skin Autofluorescence

SAF was measured using the AGE Reader™ (DiagnOptics TechnologiesBV, Groningen, the Netherlands), a non-invasive desktop device. AGE ReaderTM detects the characteristic fluorescence of some AGEs and was used to estimate the level of AGEs in the skin. Technical and optical details of this non-invasive method have been described more extensively elsewhere (12). In short, the AGE ReaderTM illuminates a skin surface of 4cm^2 guarded against surrounding light, and uses an excitation light source with a peak excitation of 370. Subsequently, the emitted fluorescence light (within the wavelength range of 420−600 nm) and the reflected excitation light (within the wavelength range of 300−420 nm) from the skin are measured with a spectrometer. SAF is calculated in arbitrary units (AU) as the ratio between the emitted light and the reflected light, multiplied by 100. A series of 3 consecutive measurements was carried out, taking less than a minute. The mean SAF was calculated from these 3 measurements on the ventral side of the forearm and these. We create a variable according SAF value in this population (Higher or Lower SAF): Higher SAF included 3rd tertile SAF values, while lower SAF included 1st and 2nd tertile SAF values.

CT-CAC scanning

First, patient was prepared with beta blockers to decrease the heart rate, and nitroglycerin for vasodilatation if needed, then an ECG synchronized prospective contrast-enhanced coronary CT was performed with SiemensBiograph mCT 64s equipment. Automatic coronary vessel extraction of all coronary vessels with visual analysis of coronary stenosis was performed by researchers' blind to the patient's condition with "Syngo.Via" cardiac CT software as described elsewhere (25). The subjects were divided into two groups according their CACs: CAC < 400 AU and CAC > 400 AU. A value of CACs ≥ 400 AU was considered as "high coronary risk".

Outcome

The primary outcome was the time to first vascular event. We defined vascular event as a composite of myocardial infarction, coronary revascularization, stroke, lower limb amputation or cardiovascular death.

Statistical analysis

Quantitative variables were expressed as the mean ± standard deviation (SD) except for triglycerides, homocysteine and lipoprotein(a) in which median and range were used due to their skewed distribution. Categorical variables were expressed as the absolute number (percentage). Differences among groups were performed using the Student's t test for quantitative variables with a normal distribution and the Pearson's chi-squared test for categorical variables. Non-parametric tests were used for those quantitative variables without normal distribution. In view of the skewed distribution of CACs values they were logarithmically converted to use parametric tests. We used the Kaplan-Meier method to create vascular event free survival and the log-likelihood test to examine differences in survival.

The differences between diabetic subjects who presented a vascular event and those without it were assessed. Cox proportional hazard multiple regression analysis was used to determine independent predictors of vascular events during the follow-up period. The model included all variables that showed association with vascular events in univariate analysis with a p value < 0.05. ROC curves were calculated and the χ^2 test for ROC area comparison was performed. Statistical analyses was performed with Stata statistical package 15. Significance was accepted at the level of p < 0.05 for all the analyses.

Results

Basal characteristics of the sample

The clinical characteristics and the main laboratory findings of both groups (type 2 diabetes and controls) and the specific characteristics of subjects with type 2 diabetes are shown in Table 1. We did not find any significant differences between groups regarding age, gender, ethnicity, smoking habit or family history of cardiovascular disease. The specific characteristics of subjects with type 2 diabetes are shown in Table 2. Individuals with type 2 diabetes included in the study had a relative good metabolic control (HbA1c 56 ± 8.9mmol/mol (7.4 ± 1.18%)) and exhibited a long-term duration of the disease (14 ± 9.4y). More than a half of patients with type 2 diabetes were under treatment with insulin alone or in combination with antidiabetic agents. Regarding microangiopathic complications: 26.73% had DR, 33.9% had urine albumin/creatinine ratio > 3.39mg/mmol, and 18.37% had clinical neuropathy.

Table 1
Characteristics of subjects with type 2 diabetes and non-diabetic control subjects

	Type 2 diabetes (n = 187)	Control group (n = 57)	P
Sex (woman) (n,%)	108 (57.75%)	37 (64.91%)	0.33
Ethnicity (Caucasian n,%)	179 (95.72%)	56 (98.25%)	0.65
Age (years)	65.63 ± 6.52	66.01 ± 6.63	0.85
BMI (kg/m^2)	30.23 ± 4.89	26.83 ± 3.11	< 0.001
Waist circumference (cm)	103.9 ± 13.53	91.2 ± 13.92	< 0.001
Smoking	99(48.13%)	34 (59.65%)	0.59
No (n,%)	25 (13.36%)	7 (12.3%)	
Current Smoker (n, %)	62 (33.15%)	15 (26.32%)	
Ex-smoker (n, %)			
CV family history (n, %)	22 (11.76%)	8 (14.04%)	0.65
Hypertension (n, %)	135 (71.19%)	28 (49.12%)	0.001
Use of ACEi/ARB (n, %)	118 (63.1%)	18 (31.58%)	< 0.001
Dyslipidemia (n, %)	149 (79.67%)	25 (43.86%)	< 0.001
Use of statins (n,%)	133 (71.51%)	19 (31.67%)	< 0.001
Use of ezetimibe (n,%)	10 (5.38%)	0	0.074
Total cholesterol (mmol/L)	4.78 ± 0.92	5.57 ± 0.91	< 0.001
HDL cholesterol (mmol/L)	1.28 ± 0.32	1.28 ± 0.29	< 0.001
LDL cholesterol(mmol/L)	2.72 ± 0.78	3.43 ± 0.81.14	< 0.001
Triglycerides (mmol/L)	1.73 [0.50−5.67]	1.24 [0.46−5.27]	0.012
HbA1c (mmol/mol)	56.33 ± 9.01	42.02 ± 3	< 0.001
HbA1c (%)	7.44 ± 1.19	5.55 ± 0.31	< 0.001
Creatinine (mmol/l)	0.725 ± 0.021	0.067 ± 0. 0.017	0.075
GFR ml/min	81.76 ± 16.00	85.57 ± 10.88	0.09
AST (UI/L)	25.51 ± 15.71	23.48 ± 5.73	0.34
ALT (UI/L)	25.94 ± 16.88	21.12 ± 10.55	0.043
GGT (UI/L)	44.46 ± 71.82	31.04 ± 29.77	0.17

	Type 2 diabetes (n = 187)	Control group (n = 57)	P
Skin AF (AU)	2.68 ± 0.65	2.41 ± 0.60	0.001
Log CACs	2.11 ± 0.81	1.59 ± 0.72	0.002
CCsA ≥ 400 AU (n, %)	41 (21.93%)	0	< 0.001

Table 2
Diabetes features and comorbidities in type 2 diabetes subjects

	N = 187
Diabetes duration (years)	14 ± 9.4
HbA1c (mmol/mol)	56.3 ± 9.01
HbA1c (%)	7.44 ± 1.19
Microvascular complications	
Retinopathy (n, %)	50 (26.73%)
Non-proliferative	44 (23.52%)
Mild	23 (12.29%)
Moderate	16 (8.65%)
Severe	5 (2.67%)
Proliferative	6 (3.21%)
Urine albumin/creatinine ratio (mg/g)	120 (64.52%)
<3.39mg/mmol (n, %)	54 (29.03%)
3.39-33.9mg/mmol (n, %)	9 (4.84%)
>33.9mg/mmol (n, %)	
Neuropathy	36 (18.37%)
Diabetes treatment	
Oral agents	82 (43.85%)
Insulin ± Oral agents	105 (56.14%)

Follow-up

187 subjects with type 2 diabetes and 57 non-diabetic controls were followed until December 2020. After a follow up of 4.35 ± 1.43 years, a total of 24 vascular events were registered, 23 vascular events (12.3%)

in type 2 diabetes group, and 1 (1.75%) in non-diabetic control group. The Kaplan-Meier analysis shows vascular event free-survival regarding groups (p = 0.031), (Fig. 1).

In our type 2 diabetes cohort we found an incidence rate of vascular events of 28.27 per 1000 persons-years. The main basal clinical characteristics of patients with type 2 diabetes according the presence of primary outcome (first vascular event) are shown on Table 3. The multivariate Cox's regression (Table 4) including selected variables that were significant at the univariate analyses and known risk factors of CVD, showed that only age (HR 1.09, 95%CI 1.01−1.18, p = 0.024), gender (HR 0.35, 95%CI 0.15−0.83, p = 0.0174), the presence of retinopathy (HR 2.58, 95%CI 1.14−5.85, p = 0.023), CACS > 400AU (HR 4.16, 95%CI 1.14−10.26, p = 0.002), and a value of SAF on 3rd tertile (HR 4.68, 95%CI 1.83−11.96, p = 0.001) were independently associated with the presence of vascular event.

Table 3
Clinical characteristics of patients with type 2 diabetes according the presence of primary outcome (first vascular event)

	Vascular event + (n = 23)	Vascular event - (n = 164)	p
Follow up (y)	5.09 ± 1.20	5.21 ± 0.95	0.564
Sex (woman) (n, %)	8 (34.7%)	100 (60.9%)	0.017
Age (years)	68.61 ± 6.04	65.22 ± 6.49	0.019
BMI (kg/m^2)	30.18 ± 4.19	30.23 ± 4.99	0.961
Diabetes duration (years)	17.69 ± 9.44	14.08 ± 9.34	0.084
Waist circumference (cm)	105.6 ± 11.89	103.69 ± 13.7	0.552
Smoking	11 (47.8%)	88 (53.65%)	0.943
No (n, %)	03 (13.04%)	22(13.41%)	
Current smoker (n, %)	08(34.37%)	55(33.53%)	
Ex-smoker (n, %)			
Hypertension (n, %)	17 (73.9%)	118 (71.9%)	0.844
Dyslipidemia (n, %)	16 (69.76)	133 (81.1%)	0.198
Insulin treatment (n,%)	17 (73.9%)	91 (54.48%)	0.198
Fast plasma glucose (mmol/L)	7.99 ± 2.43	8.73 ± 2.79	0.232
HbA1c (mmol/mol)	58.45 ± 8.10	56.1 ± 9.08	0.234
HbA1c (%)	7.72 ± 1.07	7.41 ± 1.20	0.234
Total cholesterol (mmol/L)	4.69 ± 0.66	4.78 ± 0.95	0.682
HDL cholesterol (mmol/L)	1.33 ± 0.38	1.27 ± 0.30	0.399
LDL cholesterol (mmol/L)	2.73 ± 0.47	2.71 ± 0.82	0.906
Triglycerides (mmol/L)	1.39[0.51−2.5]	1.53 [0.6−5.7]	0.046
Homocysteine (µmol/L)	12.5 [8.1−17.4]	11.3 [5.8−127]	0.765
Lipoprotein (a) (mg/dl)	7.21 [1-91.2]	8.45 [1-162.9]	0.745
GFR (ml/min)	86.5 ± 11.18	81.12 ± 16.46	0.285
Creatinine (mmol/l)	0.068 ± 0.01	0.0734 ± 0.02	0.278

	Vascular event + (n = 23)	Vascular event - (n = 164)	p
Albumin/creatinine ratio <3.39 mg/mmol (n, %) 3.39−33.9 mg/mmol (n, %) >33.9 mg/mmol (n, %)	9 (40.9%) 10 (47.6%) 2(9.5%)	111 (68.5%) 44 (27.2%) 7 (11.3%)	0.06
Log albumin/creatinine ratio	1.50 ± 0.70	1.25 ± 0.61	0.085
Diabetic Retinopathy (n,%)	11 (47.82%)	40 (24.40%)	0.018
Diabetic Neuropathy (n,%)	3(13.04%)	32(19.451)	0.450
CACS > 400AU (n, %)	10 (52.63%)	31(19.562)	0.001
Log CACs (AU)	2.55 ± 0.84	2.05 ± 0.78.7	0.013
AGEs 3rd Tertil (AU)	12 (63.15%)	39 (26.71%)	0.001
AAS (n,%)	6(27.27%)	54 (32,92%)	0.594
Statines (n,%)	14 (63.63%)	119 (72567%)	0.384

Table 4
Results of the multivariate Cox's regression for predicting a vascular event.

	HR	CI95%	p
Sex (female)	0.35	0.15−0.83	0.017
Age (y)	1.09	1.01−1.18	0.024
BMI (kg/m2)	0.99	0.91−1.08	0.820
Diabetes duration (y)	1.04	0.99−1.08	0.093
Waist (cm)	1.01	0.98−1.04	0.526
Hypertension (yes)	1.13	0.45−2.88	0.792
Dyslipedemia (yes)	0.59	0.24−1.44	0.244
Insulin treatment (yes)	2.11	0.83−5.36	0.116
HbA1c (mmol/mol)	1.20	0.88−1.66	0.255
GFR (ml/min)	1.02	0.99−1.05	0.170
Creatinine (mg/dl)	0.33	0.04−2.44	0.275
Diabetic Retinopathy (yes)	2.58	1.14−5.85	0.023
CACS > 400 AU (yes)	4.16	1.69−10.26	0.002
AGEs 3rd Tertil (yes)	4.68	1.83−11.96	0.001
HR: hazard ratio; CI: confidence interval.			

Discussion

In the present study we confirmed that individuals with type 2 diabetes had significantly more risk of having a vascular event than non-diabetic subjects. Furthermore, we provide evidence that DR and SAF (as a measure of tissue AGE accumulation) are powerful predictors of vascular events in subjects with type 2 diabetes.

We found that patients with type 2 diabetes had significantly more risk of suffering a vascular event than non-diabetic subjects (12.29% VS 1.75%). Consistent with our findings, previous reports have documented that subjects with type 2 diabetes have a higher risk of developing a vascular event and with a worse outcome in comparison with non-diabetic subjects (1, 2).

Previously, we had already provide evidence that DR is an independent predictor of subclinical CVD (21), and SAF was good predictor of a CACs > 400AU (a reliable marker of coronary atherosclerosis) (15). The

current study is important, because we confirm that both, DR and SAF, are not only related to subclinical cardiovascular disease but also are capable of predicting vascular events in type 2 diabetes population.

Several studies had suggested that the burden of microvascular disease is determinant of future cardiovascular risk (18–20). In our study, only DR is a powerful predictor of vascular events in subjects with type 2 diabetes. According with our findings, previous reports have documented an increase in CV risk in patients with DR, mostly in those with advanced DR (26–29). Although the underlying molecular mechanisms linking DR and cardiovascular disease are still a matter of debate, there are notable similarities in their pathophysiology. In this regard, recent evidence indicates that, in individuals with type 2 diabetes, the *vasa vasorum* (a network of small blood vessels that supply the walls of large blood vessels) present evolutionary changes similar to those observed in the retina: an initial stage in which endothelial dysfunction and loss of capillaries predominate (23), and more advanced stages in which ischemia plays a key role, leading to angiogenesis and inflammation in response to the progressive enlargement of the necrotic core within the plaque (30). This change in plaque phenotype results in a more inflamed and unstable plaque, favoring plaque rupture and a poor outcome of cardiovascular events. Thus, microcirculation represents a "common soil" between DR and vascular event, and would explain why DR is a good predictor of vascular events as we reported.

SAF was also a good predictor of vascular events in subjects with type 2 diabetes. There are multiples studies that reported significant associations between SAF and the development of late diabetic complications (both micro and macrovascular), most of them being cross-sectional studies (31–40). To the best of our knowledge, only two prospective studies have examined the usefulness of SAF as a predictor of CVD (35, 41). Both, supports our data and concluded that SAF is a measure of metabolic burden but it is also strongly associated with the presence of CVD and cardiac mortality, as well as a biomarker of vascular damage before it becomes clinically apparent. Therefore, SAF could be a useful clinical tool to identify diabetic individuals with preclinical vascular damage who have a particularly high risk of developing vascular events. It is important to remark, that our study is the only one that includes exclusively subjects with type 2 diabetes and no history of clinical cardiovascular disease, apparently those with less cardiovascular risk, and yet we have obtained similar results.

Mulder et al. (42) showed that SAF is elevated in acute ST-elevation myocardial infarction compared with healthy controls, and higher values of SAF were related with more risk to die or a new myocardial infarction or heart failure in the following one year. This finding suggests that SAF may play an important role in the progression of atherosclerosis. Basic research has shown that in atherosclerotic plaques AGEs interact with RAGE, resulting in increased production of inflammatory mediators, causing the plaques more vulnerable to rupture (43). Data on the important role of oxidative stress markers in endothelial dysfunction and clinically over coronary artery disease are extensive (44, 45). However, most markers for oxidative stress are not readily available for clinical practice. By contrast, skin AGES are stable and could be non-invasively assessed, thus serving as a reliable biomarker of cardiovascular disease.

In our study, we show that higher values of SAF were independently associated with the presence of macrovascular complications. Most of the classical cardiovascular risk factors such as hypertension, dyslipidemia and HbA1c were not significantly associated with the occurrence of a vascular event. However, this does not mean that they are not influencing the development of vascular events, but just that are currently under control. In fact, we would need biomarkers that inform us regarding long-term deleterious effect than those reflecting a short-term impairment. In this regard, skin AGEs are mainly accumulated in collagen, which has a low turnover and represents hyperglycaemia over a longer time period than HbA1c, so SAF may reflect the impact of oxidative stress and history of hyperglycaemic episodes better than classical risk factors. In fact, SAF is considered as a measure of metabolic memory in subjects with type 2 diabetes.

In addition to DR and SAF, we found that other classical factors such age, male sex and CACs > 400AU also were related with the presence of a vascular event. Age is an important determinant of cardiovascular risk, and it is known that the prevalence of inducible ischemia is significantly higher in type 2 diabetes patients over 65 years old (46). Furthermore, it is well documented that the absolute risk of cardiovascular events is higher in men than women (47). CACs is a well-recognized biomarker myocardial ischemia and a good predictor of cardiovascular events (48, 49). In fact, guideliness recommend that assessment of CACs could be considered in asymptomatic patients with diabetes mellitus who are over the age of 40 (5). However, CACs assessment needs of a CT scan examination, which can be inconvenient and rather expensive for routine practice in subjects with type 2 diabetes.

Our study has several limitations. First our sample was relatively small and the results could have been impacted by variables such as ambient factors or diet not considered in this analysis. Second, and probably the major limitation, was the low rate of vascular events in our population. However, it should be noted that there is a clear trend toward a decrease in events in diabetic subjects in the last 20 years, as reported Rawshani et al. (50). This is probably due to the better management of the chronic patient with diabetes, associated with better comprehensive control of the rest of the cardiovascular risk factors, with greater use of statins and antihypertensive drugs.

In conclusion, this study confirms that patients with type 2 diabetes have significantly more vascular events than non-diabetic subjects. In addition, DR and higher values of SAF are powerful predictors of vascular events in subjects with type 2 diabetes and, therefore, could be included as meaningful variables in stratification risk of cardiovascular disease.

Abbreviations

AGEs advanced glycation end products, AU arbitrary units, CACs coronary artery calcium score, CVD cardiovascular disease, DR Diabetic retinopathy, HDL high-density lipoprotein cholesterol, LDL low-density lipoprotein cholesterol, NPDR non proliferative retinopathy, PDR proliferative diabetic retinopathy, RAGE receptors for AGEs, SAF skin autofluorescence, SD standard deviation.

Declarations

Ethics approval and consent to participate.

The study was conducted according to the declaration of Helsinki and was approved by the local ethics committee: Comité Ético de Investigación Clínica del Hospital Universitario de Vall d'Hebron (Ethical Committee for Clinical Research of the Vall d'Hebron University Hospital), with a reference number PR(AG)127/2014. All subjects provided written informed consent before study entry.

Consent for publication: Not applicable.

Data availability. All data relevant to the study are included in the article. The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests. The authors declare that they have no competing interests

Author Contributions. CH, IF-G, and RS conceived the study concept and design, interpreted data, and contributed to critically revising the manuscript. AP, OS-S, AO-Z, JRM and JRH collected and analyzed data. AP drafted the manuscript. All authors approved the final article. CH, IF-G, and RS obtained funding. RS is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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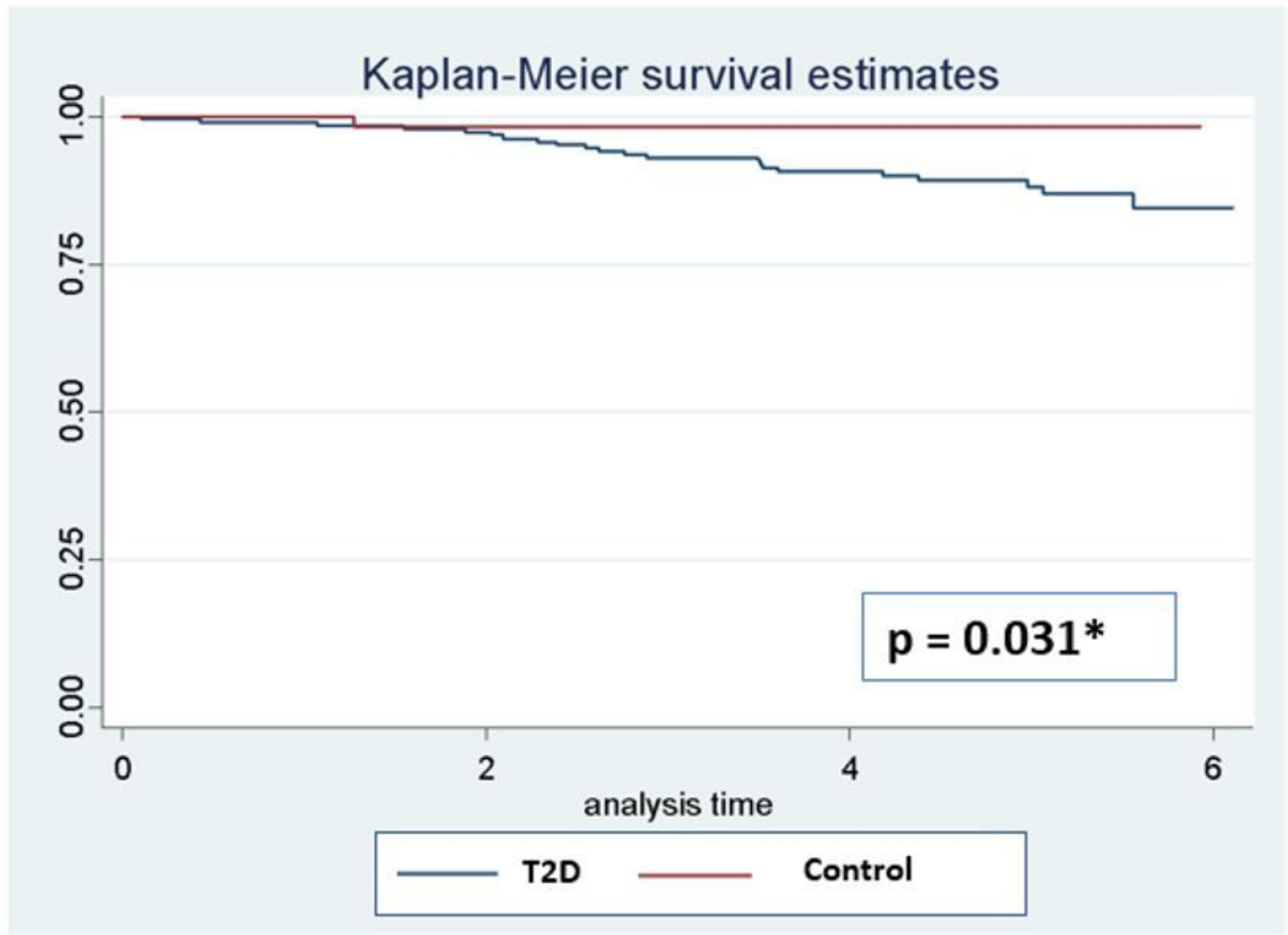
Figures

Figure 1

Kaplan-Meier analysis predicting vascular event free-survival regarding groups.

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