



Valeurs pronostiques des marqueurs de l'inflammation et du stress oxydatif dans la prédition de l'artériopathie oblitérante des membres inférieurs chez des sujets diabétiques de type 2: une étude prospective monocentrique

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**VALEURS PRONOSTIQUES DES MARQUEURS DE
L'INFLAMMATION ET DU STRESS OXYDATIF DANS LA
PREDICTION DE L'ARTERIOPATHIE OBLITERANTE DES
MEMBRES INFÉRIEURS CHEZ DES SUJETS DIABÉTIQUES DE
TYPE 2 : UNE ETUDE PROSPECTIVE MONOCENTRIQUE**

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TABLE DES MATIERES

ABREVIATIONS

PARTIE I : INTRODUCTION

PARTIE II : VALEURS PRONOSTIQUES DES MARQUEURS DE L'INFLAMMATION ET DU STRESS OXYDATIF DANS LA PREDICTION DE L'ARTERIOPATHIE OBLITERANTE DES MEMBRES INFERIEURS CHEZ DES SUJETS DIABETIQUES DE TYPE 2 : UNE ETUDE PROSPECTIVE MONOCENTRIQUE

PARTIE III : DISCUSSION GENERALE ET PERSPECTIVES

PARTIE IV : CONCLUSION

REFERENCE BIBLIOGRAPHIQUE

RESUME EN FRANÇAIS

SERMENT D'HIPPOCRATE

ABREVIATIONS :

ADVANCE : Action in Diabetes and Vascular Disease: PreterAx and DiamicroN Modified-Release Controlled Evaluation

AOMI : artériopathie oblitérante des membres inférieurs

AVC : accident vasculaire cérébral

CANTOS : *Canakinumab antiinflammatory thrombosis outcome study*

CRP : protéine C réactive

IL-1 : interleukine 1

IL-6 : interleukine 6

IL-8 : interleukine 8

IMA : ischemia-modified albumin

IPS : index de pression systolique

NO : monoxyde d'azote

SURDIAGENE : SURvie, DIAbete de type 2 et GENEtique

TNF : tumor necrosis factor

UKPDS : UK Prospective Diabetes Study

PARTIE I : INTRODUCTION

L’artériopathie oblitérante des membres inférieurs (AOMI) constitue avec la cardiopathie ischémique et l’accident vasculaire cérébral une localisation majeure d’athérosclérose. Il s’agit d’un problème notable de santé publique avec une prévalence en augmentation, due à l’explosion de la démographie mondiale, du vieillissement de la population, et de l’augmentation de la prévalence du tabagisme, de l’hypertension artérielle, et du diabète de type 2 (1).

La prévalence de l’AOMI est plus fréquente chez les patients diabétiques avec une fréquence de 2 à 4 fois plus élevée en comparaison aux sujets non diabétiques (2).

Le pronostic de l’artériopathie périphérique est sévère, dominé par un risque d’amputation au membre inférieur 5 fois plus élevé chez les patients diabétiques en comparaison aux sujets non diabétiques (3). L’artériopathie périphérique diabétique est également associée à un risque élevé de maladie cardiovasculaire et de mortalité (4, 5). La prise en charge complexe génère un coût économique considérable nécessitant des hospitalisations longues et itératives, complétées par des soins ambulatoires (6, 7), mais aussi un lourd retentissement sur la vie des patients au décours avec des séquelles invalidantes, un état dépressif, et une altération de la qualité de vie, engendrant ainsi des difficultés sociales et professionnelles (8, 9).

L’appréciation du risque de développer une maladie artérielle périphérique est essentielle, néanmoins les outils actuels de dépistage et diagnostiques sont à ce jour imparfaits. La prévalence de l’AOMI était estimée à 4,6% à l’inclusion de l’étude ADVANCE (*Action in Diabetes and Vascular Disease: PreterAx and DiamicroN Modified-Release Controlled Evaluation*) avec une incidence de 1,24 par 100 patients-année (10).

Diagnostiquée habituellement lors de la cinquième décennie, la prévalence de l'AOMI augmente de façon exponentielle après 65 ans. Une progression avec l'ancienneté du diabète a été observée dans l'étude UKPDS (*UK Prospective Diabetes Study*) : 1,2 % des patients au moment du diagnostic du diabète et 12,5 % après 18 ans d'évolution (11).

Les principaux facteurs de risque d'artériopathie périphérique diabétique sont comparables aux autres facteurs de risque cardiovasculaire traditionnels notamment l'âge, le sexe, la pression artérielle systolique, la concentration plasmatique des lipoprotéines, et le tabagisme actif (4, 11, 12). Plusieurs études ont montré que l'atteinte microvasculaire diabétique (néphropathie et rétinopathie) était également un facteur de risque indépendant d'AOMI (4, 12, 13).

Le développement de l'AOMI est principalement lié à la rigidité artérielle. L'homéostasie vasculaire dépend de la sécrétion paracrine des cellules endothéliales au niveau pariétal et intravasculaire. En présence de facteurs de risque cardiovasculaire, les troubles hémodynamiques et la dysfonction endothéliale induits, la réactivité et la relaxation des vaisseaux sont altérées, favorisant ainsi l'athérosclérose (14). Les anomalies thrombotiques, les produits avancés de la glycation, le stress oxydant, et l'inflammation de bas grade sont particulièrement impliqués dans la pathogénie de l'AOMI. Plusieurs études ont suggéré le développement d'une réaction inflammatoire aiguë secondaire à l'ischémie lors de l'exercice chez des patients présentant une claudication intermittente avec l'augmentation des concentrations plasmatiques de plusieurs médiateurs (thromboxane, interleukine 8, molécule soluble d'adhésion 1, ou le facteur de von Willebrand) et la libération d'agents vasoconstricteurs telle que l'endothéline 1 (15). Nous avons émis l'hypothèse que des biomarqueurs de l'inflammation et du stress oxydant peuvent être utiles pour le diagnostic d'AOMI et la prédiction des événements vasculaires périphériques. Nous avons dosé plusieurs biomarqueurs (récepteur 1 au TNF-alpha (TNFR-1), angiopoietin like-2, ischemia-modified albumin (IMA), produits avancés de glycation des protéines, carbonyles, et la capacité totale

plasmatique d'oxydo-réduction) chez 1412 patients diabétiques de type 2 à leur inclusion dans la cohorte SURDIAGENE (*SURvie, DIAbete de type 2 et GENEtique*). Les participants de cette cohorte ont été inclus entre 2002 – 2012, et suivis tous les deux ans de 2007 au 31 Décembre 2015. Les participants étaient âgés en moyenne de 64,7 (10,6) années, 58,2% étaient des hommes, avec une durée médiane (25^{ème} et 75^{ème} percentiles) de diabète de 13 [6, 21] années et d'HbA1c moyenne de 7,8 (1,5) %.

Le critère de jugement principal de notre présente étude était de développer un évènement majeur d'AOMI au cours du suivi : revascularisation périphérique et/ou amputation au membre inférieur. Les critères secondaires de jugement étaient revascularisation périphérique ou amputation au membre inférieur considérées séparément. Pendant un suivi médian de 5,6 [3,0 ; 8,6] années, 7,9 (%) patients ont développé un évènement majeur d'AOMI. Les participants ayant développé un événement vasculaire périphérique, comparés à ceux qui ne l'ont pas développé, étaient significativement plus âgés, plus souvent des hommes, avec un diabète plus ancien, une pression artérielle systolique plus élevée, et des valeurs abaissées d'IMC, de DFG et de HDL-cholestérol. Ils étaient moins souvent traités par fibrate, et plus souvent traités par statine, antihypertenseur et antiagrégant plaquettaire. Ils avaient plus souvent une rétinopathie diabétique, un antécédent d'amputation au membre inférieur et de revascularisation périphérique à l'inclusion. Nous avons observé que les concentrations plasmatiques du TNFR-1, angiopoietin like-2, IMA et carbonyles étaient plus élevées à l'inclusion chez les patients avec AOMI pendant le suivi en comparaison aux autres. Après ajustement sur les facteurs confondants, seules les associations avec TNFR1 et IMA persistaient. L'addition des concentrations plasmatiques de TNFR1 améliorait la prédiction du risque d'AOMI. Des résultats assez comparables ont été observés avec les critères secondaires de jugement, ou dans une série d'analyse de sensibilité notamment l'ajustement sur le décès d'origine cardiovasculaire (comme un facteur compétitif), ou les sous-groupes de patients indemnes à

l'inclusion d'AOMI, de maladie cardiovasculaire, ou de maladie rénale chronique. La méthodologie et les résultats complets de notre travail sont présentés plus bas sous forme d'un article original publié dans le dernier numéro du journal de la société Américaine du diabète : Diabetes Care (16).

PARTIE II : VALEURS PRONOSTIQUES DES MARQUEURS DE
L'INFLAMMATION ET DU STRESS OXYDATIF DANS LA
PREDICTION DE L'ARTERIOPATHIE OBLITERANTE DES
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TYPE 2 : UNE ÉTUDE PROSPECTIVE MONOCENTRIQUE



Prognostic Values of Inflammatory and Redox Status Biomarkers on the Risk of Major Lower-Extremity Artery Disease in Individuals With Type 2 Diabetes

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OBJECTIVE

Inflammation and oxidative stress play an important role in the pathogenesis of lower-extremity artery disease (LEAD). We assessed the prognostic values of inflammatory and redox status biomarkers on the risk of LEAD in individuals with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Plasma concentrations of tumor necrosis factor- α receptor 1 (TNFR1), angiopoietin-like 2, ischemia-modified albumin (IMA), fluorescent advanced glycation end products, protein carbonyls, and total reductive capacity of plasma were measured at baseline in the SURDIAGENE (Survie, Diabète de type 2 et Génétique) cohort. Major LEAD was defined as the occurrence during follow-up of peripheral revascularization or lower-limb amputation.

RESULTS

Among 1,412 participants at baseline (men 58.2%, mean [SD] age 64.7 [10.6] years), 112 (7.9%) developed major LEAD during 5.6 years of follow-up. High plasma concentrations of TNFR1 (hazard ratio [95% CI] for second vs. first tertile 1.12 [0.62–2.03; $P = 0.71$] and third vs. first tertile 2.16 [1.19–3.92; $P = 0.01$]) and of IMA (2.42 [1.38–4.23; $P = 0.002$] and 2.04 [1.17–3.57; $P = 0.01$], respectively) were independently associated with an increased risk of major LEAD. Plasma concentrations of TNFR1 but not IMA yielded incremental information, over traditional risk factors, for the risk of major LEAD as follows: C-statistic change (0.036 [95% CI 0.013–0.059]; $P = 0.002$), integrated discrimination improvement (0.012 [0.005–0.022]; $P < 0.001$), continuous net reclassification improvement (NRI) (0.583 [0.294–0.847]; $P < 0.001$), and categorical NRI (0.171 [0.027–0.317]; $P = 0.02$).

CONCLUSIONS

Independent associations exist between high plasma TNFR1 or IMA concentrations and increased 5.6-year risk of major LEAD in people with type 2 diabetes. TNFR1 allows incremental prognostic information, suggesting its use as a biomarker for LEAD.

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Lower-extremity artery disease (LEAD) is one of the major clinical manifestations of atherosclerosis around the world (1). Its prevalence is two- to threefold higher in individuals with type 2 diabetes than in those without diabetes (2,3). LEAD is a leading cause of limb loss and is associated with worse cardiovascular outcomes in patients with type 2 diabetes (4–7). It is also responsible for a worsening of quality of life and a high economic burden (8,9).

Low-grade inflammation and oxidative stress play an important role in the development of atherosclerosis and its presentations in various arterial beds, including the lower-limb arteries (10–13). Several studies have evaluated the association between circulating inflammatory or redox biomarkers with chronic kidney disease (CKD) and major adverse cardiovascular events (MACEs), but few have reliably tested these candidates on the risk of LEAD in individuals with type 2 diabetes. Our team has assessed a broad spectrum set of inflammatory and redox biomarkers to test their ability to predict kidney and vascular complications in the Survie, Diabète de Type 2 et Génétique (SURDIAGENE) type 2 diabetes cohort (14–17). Hence, plasma concentrations of tumor necrosis factor- α receptor 1 (TNFR1) and angiopoietin-like 2 (ANGPTL2), two proinflammatory factors, have been associated with an increased risk of MACEs, CKD, and death (14–16). However, the prediction of MACEs has not been enhanced by measuring circulating levels of redox status surrogates, including ischemia-modified albumin (IMA), fluorescent advanced glycation end products (F-AGEs), protein carbonyls, and total reductive capacity of plasma (TRCP) (17). The aims of the current investigation were to 1) evaluate the relationship between circulating levels of TNFR1, ANGPTL2, IMA, F-AGEs, protein carbonyls, and TRCP and the incidence of major LEAD and 2) test whether these biomarkers improve the prediction of major LEAD over conventional vascular risk factors in the SURDIAGENE cohort.

RESEARCH DESIGN AND METHODS

Participants

SURDIAGENE is a French single-center prospective cohort designed to identify genetic and biochemical determinants of vascular complications in individuals with type 2 diabetes (18). Adults with an established diagnosis of type 2 diabetes for at least 2 years were recruited in

2002–2012 and followed every 2 years from 2007 to 31 December 2015. Non-diabetic kidney disease and short follow-up duration (<1 month) were the main exclusion criteria. The SURDIAGENE study protocol was approved by the Poitiers University Hospital Ethics Committee (CPP Ouest 3), Poitiers, France, and all participants gave written informed consent before enrollment.

Definition of Clinical Parameters at Baseline

History of macrovascular disease was defined as the presence at baseline of at least one of the following: myocardial infarction, stable angina, stroke, transient ischemic attack, coronary, or carotid artery revascularization. Estimated glomerular filtration rate (eGFR) was determined using the Chronic Kidney Disease Epidemiology Collaboration equation. CKD was defined at baseline as eGFR <60 mL/min/1.73 m². Diabetic retinopathy was staged as absent, nonproliferative, preproliferative, or proliferative.

Definition of End Points

The primary end point of major LEAD was defined as the first occurrence during follow-up of lower-limb amputation (minor: toes or mediotarse; major: transtibial or transfemoral) or requirement of a peripheral revascularization procedure (angioplasty, surgery), whichever came first. Requirements of peripheral revascularization procedure and lower-limb amputation were considered separately as secondary end points. An independent committee adjudicated each end point.

Laboratory Procedures

Blood and second morning urine samples were obtained after an overnight fast and stored at –80°C until use in the CHU Poitiers biobanking facility (CRB0033-00068). HbA_{1c} was assessed using a high-performance liquid chromatography method (ADAMS A1c HA-8160 analyzer; Menarini, Florence, Italy). Serum and urine creatinine and urinary albumin were measured by nephelometry on a Modular P system (Roche Diagnostics, Mannheim, Germany). Plasma concentrations of triglycerides and total and HDL cholesterol were measured using enzymatic methods.

Plasma concentrations of TNFR1 (EKF Diagnostics, Dublin, Ireland) and human ANGPTL2 (Cloud-Clone Corp., Houston, TX) were measured using ELISA kits.

Samples were tested in duplicate, and the mean of the two measurements was considered. The intra- and interassay coefficients of variation were, respectively, 1.8–5.3% and 3.6–6.8% for TNFR1 and <10% and <15% for ANGPTL2. The results of both biomarkers are expressed in nanograms per milliliter. Plasma C-reactive protein (CRP) was measured at baseline using an immunoturbidimetric assay (Roche/Hitachi cobas c systems; Roche Diagnostics). Coefficients of variance were 2.07 and 2.85% for CRP concentrations at 8.01 and 36.9 mg/L, respectively.

The comprehensive biological process used to measure the redox biomarkers were recently reported (17). Briefly, plasma IMA index, an early marker of ischemia, was assessed by spectrophotometry. The measurement was based on the decreased capacity of IMA to bind cobalt, and the results are expressed as arbitrary units (AU). Plasma F-AGE concentrations were assessed using a spectrofluorometer (FLUOstar Omega; BMG Labtech), and the results are expressed as 10^{–3} AU. Plasma concentrations of protein carbonyls, reflecting the degree of carbonylation in plasma, were determined by ELISA (OxiSelect Protein Carbonyl ELISA Kit; Cell Biolabs), and the results are expressed as millimoles per milligram. TRCP, a marker of the antioxidant capacity of plasma, was measured using the Folin-Ciocalteu method. Gallic acid (Sigma) was used as a standard, and the results are expressed as gallic acid equivalents.

Analyses and Statistical Methods

Continuous variables are expressed as mean (SD) or as median (25th, 75th percentiles) for those with skewed distribution. Categorical variables are expressed as the number of participants with a corresponding percentage. Participants were categorized into three equally sized groups corresponding to increasing tertiles (T1, T2, and T3) of each biomarker (Supplementary Table 1). Participant characteristics at baseline by the incidence of major LEAD during follow-up were compared using χ^2 test, ANOVA, or Wilcoxon rank sum test.

A complete case method was used to handle missing data. Thus, 56 participants with at least one missing value were omitted from the current study, leaving a complete case study sample size of 1,412 (Supplementary Fig. 1).

Restrictive cubic splines regression analyses were performed (using quantiles as knots, and medians as reference values) to assess nonlinearity in the relationship between each biomarker and the primary end point. Kaplan-Meier curves were plotted to evaluate the primary end point–free survival rates by biomarker tertiles at baseline and compared using the log-rank test. Cox proportional hazards regression models were fitted to estimate hazard ratios (HRs) with associated 95% CIs for end points during follow-up for T2 and T3 of each biomarker compared with T1. Analyses were adjusted for sex and age (model 1) and for all potential confounding covariates at baseline as follows: model 1 plus BMI, duration of diabetes, HbA_{1c}, systolic and diastolic blood pressure, urinary albumin-to-creatinine ratio (ACR), eGFR, diabetic retinopathy stages, plasma concentrations of total and HDL cholesterol and triglycerides, history of macrovascular disease, current smoking, and use of insulin therapy and antihypertensive, statin, fibrate, and antiplatelet drugs (model 2). The Schoenfeld residuals method was used to check the proportional hazards assumption for the association between primary end point and each biomarker. Harrell C-statistic (19), integrated discrimination improvement (IDI), and net reclassification improvement (NRI) were performed for participants with no major LEAD at baseline to compare discrimination and classification of the primary end point, assessed using survival methodology, between two prognostic models: model 2 versus model 2 plus plasma concentrations of relevant (independently associated with major LEAD) biomarkers.

We conducted a series of sensitivity analyses to 1) use the competing risk model of Fine and Gray to estimate the subdistribution HRs for major LEAD while accounting for the competing risk of cardiovascular death (20); 2) evaluate associations between plasma biomarker levels and the primary end point in participants without a history of major LEAD, macrovascular disease, or CKD at baseline; and 3) assess associations between biomarkers and an alternative primary end point defined as the first occurrence during follow-up of one of the following: minor lower-limb amputation with peripheral revascularization, major lower-limb amputation, or requirement

of a peripheral revascularization procedure. Finally, we evaluated the prognostic value of plasma CRP concentrations on the risk of major LEAD in the subset of participants for whom CRP data were available at baseline.

Statistics were performed using SAS 9.4 (SAS Institute, www.sas.com) and Stata 13 (StataCorp, www.stata.com) software. Two-sided $P < 0.05$ was considered significant.

RESULTS

Characteristics of Participants at Baseline According to Incidence of Major LEAD During Follow-up

We investigated 1,412 participants (58.2% men, mean [SD] age 64.7 [10.6] years, median [25th, 75th percentiles] duration of diabetes 13 [6, 21] years at baseline). New cases of major LEAD occurred in 112 (7.9%) participants during a median follow-up duration of 5.6 [3.0, 8.6] years. The incidence rate of major LEAD was 1.4 per 100 person-years. Participants who developed a major LEAD during follow-up, compared with those who did not, were significantly older; were more frequently men; had a longer duration of diabetes and higher systolic blood pressure and ACR; had a lower BMI, eGFR, and HDL cholesterol level; were less likely to use a fibrate drug but more likely to use statin, antihypertensive, and antiplatelet drugs; and had more prevalent diabetic retinopathy, lower-limb amputation, and peripheral revascularization at baseline (Table 1).

Risk of Primary End Point by Plasma Concentrations of Inflammatory and Oxidative Stress Biomarkers at Baseline

Participants who developed a major LEAD during follow-up, compared with those who did not, had higher plasma concentrations of TNFR1, ANGPTL2, IMA, and protein carbonyls (Table 1). The relationship between plasma concentrations of each biomarker at baseline and the primary end point were not linear ($P < 0.0001$ for all) (Supplementary Fig. 2). The Kaplan-Meier estimate of the 6-year cumulative incidence of major LEAD during follow-up by tertiles of each biomarker at baseline are plotted in Fig. 1. The biomarkers were higher in T1 than in the T2 and T3 of TNFR1 (4.2%, 4.7%, and 17.7%, respectively; $P < 0.0001$), ANGPTL2 (4.4%, 6.8%, and 13.9%; $P < 0.0001$), IMA (4.3%, 10.5%, and 9.7%;

$P = 0.002$), and protein carbonyls (7.4%, 6.0%, and 10.4%; $P = 0.03$). No significant association was observed between major LEAD and F-AGE or TRCP tertiles (Fig. 1 and Table 2). Cox proportional hazards regression model 1 confirmed the associations between TNFR1, ANGPTL2, and IMA tertiles and major LEAD (Table 2). However, only TNFR1 and IMA tertiles remained significantly associated with the risk of major LEAD in the fully adjusted model 2. Similar results were observed after including both TNFR1 and IMA tertiles together in model 2 (TNFR1: T2 vs. T1 1.15 [0.63–2.10; $P = 0.64$], T3 vs. T1 2.28 [1.25–4.15; $P = 0.007$]; IMA: T2 vs. T1 2.52 [1.44–4.42; $P = 0.001$], T3 vs. T1 2.09 [1.20–3.66; $P = 0.009$]). No evidence for interaction was observed between plasma concentrations of TNFR1 and IMA on the risk of major LEAD (P for interaction = 0.30).

The findings were reliable after adjusting for cardiovascular death as a competing risk (Supplementary Table 2) and after considering participants with no baseline history of major LEAD ($n = 1,290$), CKD ($n = 1,016$), or macrovascular disease ($n = 905$, except for the absence of significant IMA-LEAD association in this subset of participants) (Supplementary Table 3). The use of the alternative primary outcome did not materially alter the results (Supplementary Table 4).

Plasma concentrations of CRP were measured at baseline in 291 (20.6%) participants. Participants with available CRP measurements at baseline, compared with others, had slightly higher BMI and HbA_{1c} and lower systolic blood pressure, HDL cholesterol, and total-cholesterol and were less likely to use a fibrate drug but more likely to use antihypertensive and antiplatelet drugs (Supplementary Table 5). Among participants for whom CRP measurements were available at baseline, major LEAD occurred during follow-up in 31 (10.6%). Plasma CRP concentrations were higher in participants who experienced major LEAD than in those who did not (8.5 [5.4–22.5] vs. 4.7 [2.0–12.6] mg/L; $P = 0.001$), with a nonlinear relationship (P for nonlinearity < 0.0001) (Supplementary Fig. 3). The Kaplan-Meier estimate of the 6-year cumulative incidence of major LEAD during follow-up was higher in CRP T2 (15.0%) and T3 (18.1%) than in T1 (3.2%; $P = 0.02$) (Supplementary Fig. 4). HRs (95% CIs) for major LEAD increased with growing CRP tertiles

Table 1—Characteristics of participants at baseline according to the incidence of major LEAD during follow-up

	Overall	Major LEAD		<i>P</i> value
		No	Yes	
Number of participants	1,412	1,300	112	
Clinical parameters				
Male sex	822 (58.2)	733 (56.4)	89 (79.5)	<0.0001
Age (years)	64.7 (10.6)	64.5 (10.8)	66.9 (8.9)	0.02
Duration of diabetes (years)	13 (6, 21)	12 (6, 20)	16 (10, 24)	0.0009
BMI (kg/m ²)	31.3 (6.3)	31.4 (6.4)	29.7 (5.0)	0.005
Systolic blood pressure (mmHg)	132 (18)	132 (17)	138 (20)	0.0004
Diastolic blood pressure (mmHg)	72 (11)	72 (11)	73 (12)	0.83
Biological parameters				
HbA _{1c} (%)	7.8 (1.5)	7.8 (1.5)	7.6 (1.5)	0.25
HbA _{1c} (mmol/mol)	62 (17)	62 (17)	60 (16)	0.25
Urinary ACR (mg/mmol)	3 (1, 14)	3 (1, 12)	13 (2, 131)	<0.0001
eGFR (mL/min/1.73 m ²)	73 (24)	74 (24)	62 (28)	<0.0001
Serum total cholesterol (mmol/L)	4.79 (1.15)	4.79 (1.14)	4.81 (1.24)	0.89
Serum HDL cholesterol (mmol/L)	1.21 (0.41)	1.21 (0.42)	1.13 (0.35)	0.03
Serum triglycerides (mmol/L)	1.57 (1.12, 2.30)	1.57 (1.12, 2.30)	1.69 (1.14, 2.27)	0.71
Medical history				
Current smoking	152 (10.8)	135 (10.4)	17 (15.2)	0.15
Diabetic retinopathy	624 (44)	544 (42)	80 (71)	<0.0001
Macrovascular disease	507 (36)	459 (35)	48 (43)	0.12
Major LEAD	122 (9)	83 (6)	39 (35)	<0.0001
Lower-limb amputation	69 (5)	43 (3)	26 (23)	<0.0001
Peripheral revascularization	69 (5)	50 (4)	19 (17)	<0.0001
History of treatment				
Antihypertensives	1,172 (83)	1,067 (82)	105 (94)	0.0009
Statins	638 (45)	576 (44)	62 (55)	0.03
Fibrates	160 (11)	154 (12)	6 (5)	0.04
Antiplatelet drugs	593 (42)	532 (41)	61 (54)	0.007
Insulin	846 (60)	771 (59)	75 (67)	0.13
Plasma concentrations of biomarkers				
TNFR1 (ng/mL)	1.8 (1.5, 2.3)	1.8 (1.5, 2.3)	2.3 (1.8, 3.1)	<0.0001
ANGPTL2 (ng/mL)	15 (11, 21)	15 (11, 20)	19 (13, 28)	<0.0001
IMA (AU)	0.51 (0.33, 0.63)	0.51 (0.32, 0.63)	0.58 (0.48, 0.68)	<0.0001
F-AGE (10 ⁻³ AU)	111 (93, 132)	111 (93, 132)	117 (94, 141)	0.24
Protein carbonyls (mmol/mg)	28 (26, 31)	28 (26, 31)	29 (26, 33)	0.02
TRCP (gallic acid equivalents)	120 (103, 145)	120 (103, 145)	122 (103, 159)	0.45

Categorical variables are *n* (%). Continuous variables are expressed as mean (SD), except for variables with skewed distribution, which are presented as median (25th, 75th percentiles): duration of diabetes, urinary ACR, triglycerides, TNFR1, ANGPTL2, IMA, F-AGE, and TRCP. Comparisons of qualitative and quantitative parameters were performed using χ^2 test and ANOVA, respectively. Wilcoxon rank sum test was used for comparisons of variables with skewed distribution. *P* < 0.05 was significant.

(T2 vs. T1 5.52 [1.38–22.09; *P* = 0.02], T3 vs. T1 7.14 [1.82–27.96; *P* = 0.005]) in the fully adjusted model. A significant interaction was observed between TNFR1 and CRP on the risk of major LEAD (*P* for interaction = 0.03).

Additive Value of Plasma Concentrations of TNFR1 or IMA at Baseline in Discrimination and Classification of Major LEAD During Follow-up

The addition of plasma concentrations of TNFR1 to traditional risk factors (as in model 2) improved the C-statistic (0.036 [95% CI 0.013–0.059]; *P* = 0.002), IDI (0.012 [0.005–0.022]; *P* < 0.001), continuous NRI (0.583 [0.294–0.847]; *P* < 0.001), and categorical NRI (0.171

[0.027–0.317]; *P* = 0.02) for the 5.6-year risk of major LEAD during follow-up. Plasma concentrations of IMA at baseline did not enhance discrimination or classification of the investigated risk (Table 3). No further improvement was observed by the addition of both plasma concentrations of TNFR1 and IMA together into model 2 (data not shown). Plasma CRP concentrations enhanced the C-statistic (0.071 [0.008–0.135]; *P* = 0.03), IDI (1.031 [0.789–1.90]; *P* < 0.001), and continuous NRI (0.291 [0.205–0.385]; *P* < 0.001) for the risk of major LEAD during follow-up. A greater improvement in the C-statistic was observed when both TNFR1 and CRP were introduced together in the final model (0.086 [0.020–0.151]; *P* = 0.01).

Risk of Secondary End Points by Plasma Concentrations of Inflammatory and Oxidative Stress Biomarkers at Baseline

Peripheral revascularization and lower-limb amputation occurred during follow-up in 79 (5.6%) and 58 (4.1%) participants, respectively. Their incidence rates were 1.0 and 0.7 per 100 person-years, respectively. The risk of peripheral revascularization was significantly higher in TNFR1 and IMA T1, whereas the risk of lower-limb amputation was greater in TNFR1 and ANGPTL2 T1 than in T2 and T3 (Supplementary Table 6).

CONCLUSIONS

In the current study, we evaluated the relationship between plasma concentrations of inflammatory and redox status

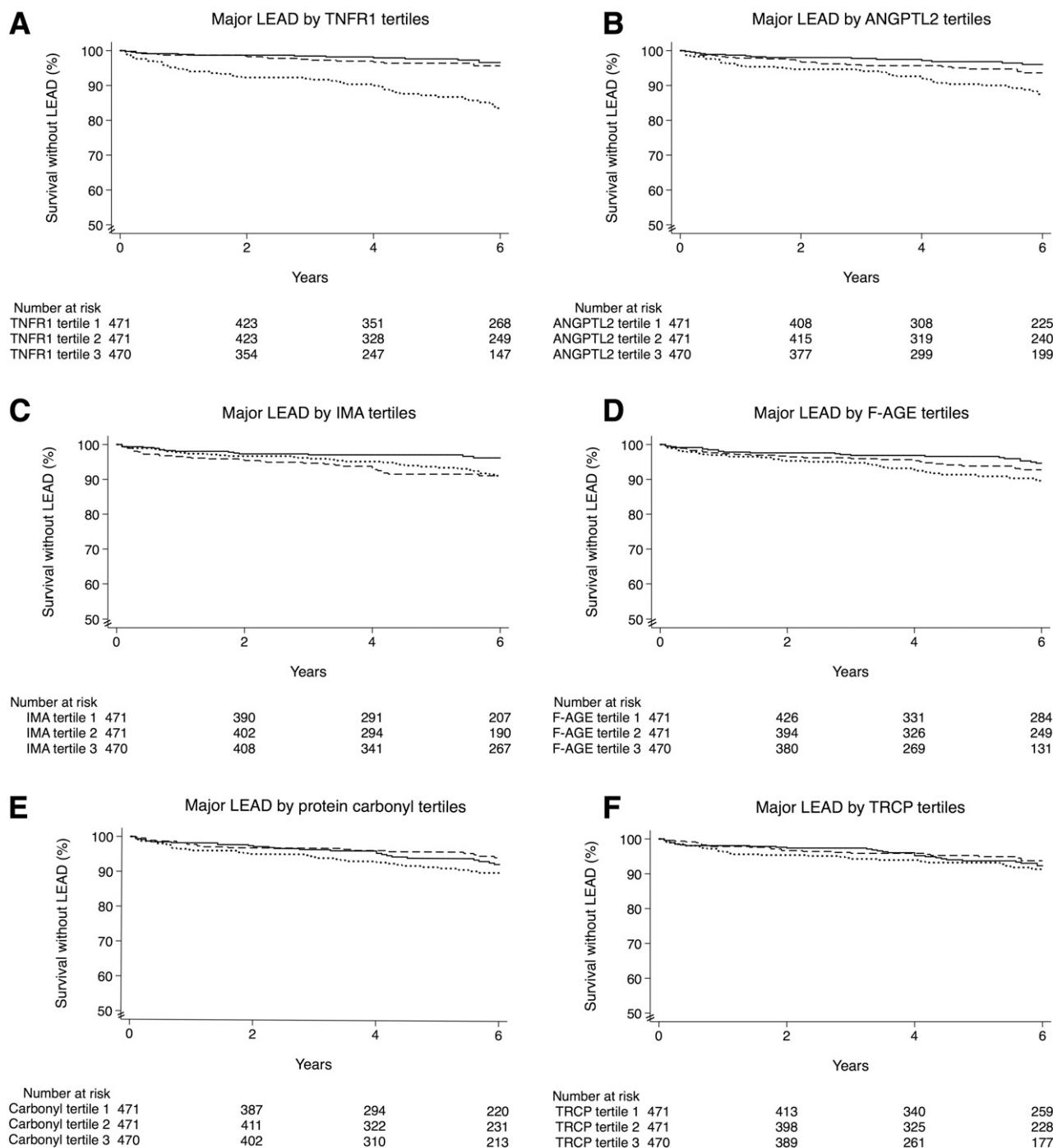


Figure 1—Major LEAD during follow-up by plasma concentrations of inflammatory and oxidative stress biomarkers at baseline. Survival without major LEAD in T3 (dotted line) and T2 (dashed line) compared with T1 (solid line) of TNFR1 ($P < 0.0001$) (A), ANGPTL2 ($P < 0.0001$) (B), IMA ($P = 0.002$) (C), F-AGE ($P = 0.07$) (D), protein carbonyls ($P = 0.03$) (E), and TRCP ($P = 0.48$) (F).

biomarkers and the risk of major LEAD in a prospective cohort of individuals with type 2 diabetes. We observed associations between plasma concentrations of TNFR1 and IMA at baseline and excess risk of major LEAD during follow-up but no independent associations between major LEAD and circulating levels of ANGPTL2, F-AGE, protein carbonyls, or TRCP.

Participants in TNFR1 T1 had a twofold increased risk of major LEAD compared with those in T3. This finding was derived from an analysis of the whole cohort and remained significant in the subset of participants with no history of major LEAD at baseline. This association was independent on potential confounders, including key cardiovascular risk factors.

Similar results were observed with either peripheral revascularization or lower-limb amputation considered individually as secondary end points. Furthermore, plasma concentrations of TNFR1 provided additive prognostic information, beyond conventional risk factors, on the risk of major LEAD. They improved C-statistics, IDI, and NRI.

Table 2—Risk for major LEAD during follow-up according to plasma concentrations of inflammatory and oxidative stress biomarkers at baseline

	Major LEAD		Model 1		Model 2	
	No, n	Yes, n (%)	HR (95% CI)	P value	HR (95% CI)	P value
All	1,300	112 (7.9)				
TNFR1						
T1	448	23 (4.9)	Ref.		Ref.	
T2	447	24 (5.1)	1.25 (0.70–2.24)	0.45	1.12 (0.62–2.03)	0.71
T3	405	65 (13.8)	3.86 (2.34–6.38)	<0.0001	2.16 (1.19–3.92)	0.01
ANGPTL2						
T1	449	22 (4.7)	Ref.		Ref.	
T2	439	32 (6.8)	1.52 (0.87–2.65)	0.14	1.31 (0.74–2.32)	0.36
T3	412	58 (12.3)	2.75 (1.64–4.63)	<0.0001	1.59 (0.88–2.85)	0.12
IMA						
T1	453	18 (3.8)	Ref.		Ref.	
T2	428	43 (9.1)	2.49 (1.43–4.32)	0.001	2.42 (1.38–4.23)	0.002
LEAD	419	51 (10.9)	2.33 (1.36–4.00)	0.002	2.04 (1.17–3.57)	0.01
F-AGE						
T1	437	34 (7.2)	Ref.		Ref.	
T2	433	38 (8.1)	1.26 (0.79–2.02)	0.34	1.10 (0.68–1.80)	0.70
T3	430	40 (8.5)	1.58 (0.99–2.54)	0.05	1.15 (0.69–1.92)	0.59
Protein carbonyls						
T1	436	35 (7.4)	Ref.		Ref.	
T2	443	28 (5.9)	0.75 (0.45–1.25)	0.27	0.66 (0.40–1.12)	0.12
T3	421	49 (10.4)	1.37 (0.88–2.14)	0.16	1.16 (0.73–1.83)	0.53
TRCP						
T1	433	38 (8.1)	Ref.		Ref.	
T2	437	34 (7.2)	0.92 (0.58–1.47)	0.74	0.92 (0.57–1.48)	0.73
T3	430	40 (8.5)	1.23 (0.78–1.93)	0.38	1.08 (0.67–1.75)	0.74

HRs and 95% CIs for the T2 and T3 compared with T1. Analyses adjusted for baseline age and sex (model 1) and for model 1 plus BMI; duration of diabetes; HbA_{1c}; systolic and diastolic blood pressure; urinary ACR; eGFR; diabetic retinopathy stages; plasma concentrations of total and HDL cholesterol and triglycerides; use of insulin therapy and antihypertensive, statin, fibrate, and antiplatelet drugs; and history of current smoking and macrovascular disease (model 2). P < 0.05 was significant. Ref., reference.

As far as we know, this study is the first to report reliable evidence for the prognostic value of plasma TNFR1 concentrations on the risk of major LEAD in

individuals with type 2 diabetes. Few cross-sectional studies have investigated the association between LEAD and TNF- α or its two soluble receptors TNFR1 and

TNFR2 in the general population. Two small studies, including one in an overall sample of 100 participants, showed higher circulating TNF- α , TNFR1, and TNFR2 concentrations in individuals with LEAD than in control subjects (21,22). The Framingham Offspring Study, a larger community-based cohort, showed an association between TNFR2 and LEAD defined as an ankle-brachial index <0.9, intermittent claudication, and/or lower-extremity revascularization (23). In the type 2 diabetes setting, higher circulating TNFR1 concentrations have been reported to be mainly associated with an increased risk of kidney disease, cardiovascular events, or mortality (14,16,24), but no investigation to our knowledge has studied the risk of LEAD. The current findings unlikely were driven by kidney or cardiovascular disease, yet the TNFR1-LEAD association remained significant after adjustment for renal parameters and cardiovascular risk factors as well as in participants with no history of kidney or macrovascular disease at baseline. Furthermore, we did not observe evidence of a competing risk of cardiovascular death in this association.

No etiological conclusions can be drawn from the current findings, but the findings are consistent with previous studies supporting the implication of systemic inflammation in peripheral artery disease (25–27). TNF proinflammatory activities promote atherosclerosis by increasing endothelial cell permeability, inducing the expression of surface leukocyte adhesion molecules, and enhancing the production of cytokines (28,29). Furthermore, TNF decreases the activity of adipocyte-derived lipoprotein lipase and increases the production of hepatic VLDLs in response to acute endotoxin exposure (30,31). Increased TNF- α activity also may reflect oxidative stress (32) and was correlated with pulse wave velocity, an established surrogate for arterial stiffness (33), which plays an important role in the pathophysiology of LEAD (34).

High plasma CRP concentrations were associated with an increased risk of major LEAD and provided additive prognostic information over traditional risk factors. Plasma CRP concentrations significantly interacted with TNFR1 levels on their associations with major LEAD, suggesting that these relationships are related to the inflammatory background. However,

Table 3—Discrimination and classification assessments for risk of major LEAD during follow-up according to traditional risk factors without and with plasma concentrations of TNFR1 or IMA at baseline

	Risk of LEAD	P value
C-statistic (95% CI) for model 2	0.753 (0.688–0.817)	
Change in C-statistic (95% CI) for model 2 + TNFR1	0.036 (0.013–0.059)	0.002
Change in C-statistic (95% CI) for model 2 + IMA	0.007 (−0.009 to 0.022)	0.38
IDI (95% CI) for TNFR1	0.012 (0.005–0.022)	<0.001
Continuous NRI (95% CI) for TNFR1	0.583 (0.294–0.847)	<0.001
Categorical NRI (95% CI) for TNFR1	0.171 (0.027–0.317)	0.02
IDI (95% CI) for IMA	0.001 (−0.006 to 0.009)	0.63
Continuous NRI (95% CI) for IMA	0.239 (−0.043 to 0.508)	0.11
Categorical NRI (95% CI) for IMA	0.055 (−0.021 to 0.134)	0.18

IDI and continuous and categorical (5% and 10% risk thresholds) NRI tests were performed for model 2 plus baseline plasma concentrations of TNFR1 or IMA compared with model 2 alone. Model 2: age; sex; BMI; duration of diabetes; HbA_{1c}; systolic and diastolic blood pressure; urinary ACR; eGFR; diabetic retinopathy stages; plasma concentrations of total and HDL cholesterol and triglycerides; use of insulin therapy and antihypertensive, statin, fibrate, and antiplatelet drugs; and history of current smoking and macrovascular disease. Plasma concentrations of TNFR1 and IMA were introduced into the model as categorical variables (tertiles). All analyses were performed in individuals without a baseline history of major LEAD (n = 1,290).

these findings are limited by the issue that they were derived from a subset of 291 participants with CRP data available at baseline.

This study also shows an independent association between plasma IMA concentrations and an increased risk of major LEAD and peripheral revascularization but not lower-limb amputation. The association between circulating IMA levels and major LEAD remained significant in participants without LEAD or CKD at baseline but not in those with no history of macrovascular disease. IMA reflects ischemia regardless of vascular bed, and it has been suggested as a biomarker of acute myocardial ischemia, skeletal muscle ischemia, and stroke (35–37). In ischemia, structural changes take place in the N-terminus of the human albumin, which reduce its binding capacity (38) possibly as a result of exposure to reactive oxygen species. However, the diagnostic and prognostic values of IMA have not been clearly established. In the current study, circulating IMA levels did not improve discrimination or classification of major LEAD risk. In the same line, IMA did not provide incremental diagnostic information for cardiovascular events in the SURDIAGENE type 2 diabetes cohort or in patients with suspected acute coronary syndrome in the IMAGINE (Ischemia Modified Albumin in Diagnosing Ischemic New Events) multicenter prospective study (17,39).

We also have observed an association between greater circulating ANGPTL2 levels and an increased risk of lower-limb amputation. However, plasma concentrations of ANGPTL2 did not enhance the discrimination or classification of limb loss (data not shown) and were not independently associated with the risk of the primary end point. Although with the absence of strong evidence to support the usefulness of plasma ANGPTL2 as a reliable predictor for major LEAD, our observation is consistent with the role of vascular inflammation in the natural history of lower-limb amputation. Excess ANGPTL2 may accelerate vascular inflammation by activating proinflammatory pathways in endothelial cells and increasing macrophage infiltration, leading to endothelial dysfunction and atherosclerosis progression (40).

The main strength of this study is the use of a contemporary prospective

cohort designed to investigate clinical, biochemical, and genetic determinants of vascular complications in individuals with type 2 diabetes. SURDIAGENE contains comprehensive data on clinical and biochemical parameters at baseline as well as adjudicated vascular end points during follow-up. We assessed wide-ranging biomarkers of such major pathways involved in the pathophysiology of lower-extremity atherosclerosis, including inflammation, oxidative stress, and advanced glycation end products. The major limitation of the study is that the SURDIAGENE cohort was conducted in a single French diabetes department and may not be representative of all populations with type 2 diabetes. The findings can be generalized only for Caucasian people with type 2 diabetes, not for other ethnic groups. The study also lacks data on intermittent claudication and ankle-brachial index, which can lead to an underestimated association between candidate biomarkers and early stages of LEAD. Furthermore, SURDIAGENE lacks data on peripheral neuropathy and foot infection, which can have confounding effects, especially in the risk of lower-limb amputation. Nevertheless, similar associations were observed when we considered the alternative end point including lower-limb amputation (transmetatarsal with need of revascularization, transtibial, or transfemoral) believed to be a result of artery disease. The main association and prognostic value of plasma TNFR1 concentrations were observed not only for the combined LEAD end point but also for peripheral revascularization considered individually as a secondary end point.

Overall, high plasma concentrations of TNFR1 and IMA are independently associated with an increased 6-year risk of major LEAD in individuals with type 2 diabetes. TNFR1 yielded incremental prognostic information on the risk of major LEAD, suggesting that it is a useful biomarker for peripheral arterial disease in this population.

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SUPPLEMENTARY DATA

Prognostic values of inflammatory and redox status biomarkers on the risk of major lower-extremity artery disease in subjects with type 2 diabetes

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Supplementary Material

Supplementary Figure 1. Flow chart of the SURDIAGENE prospective cohort

Supplementary Figure 2. Hazard ratios for major LEAD during follow-up by splines of plasma concentrations of inflammatory and oxidative stress biomarkers at baseline

Supplementary Figure 3. Hazard ratios for major LEAD during follow-up by splines of CRP plasma concentrations

Supplementary Figure 4. Major LEAD during follow-up by tertiles of CRP plasma concentrations

Supplementary Table 1. Characteristics of participants according to tertiles of plasma concentrations of each biomarker at baseline

Supplementary Table 2. Major lower extremity artery disease during follow-up according to plasma concentrations of inflammatory and oxidative stress biomarkers at baseline with correction for competing risk of cardiovascular death

Supplementary Table 3. Risk for major lower extremity artery disease during follow-up according to plasma concentrations of inflammatory and oxidative stress biomarkers in different subsets of participants

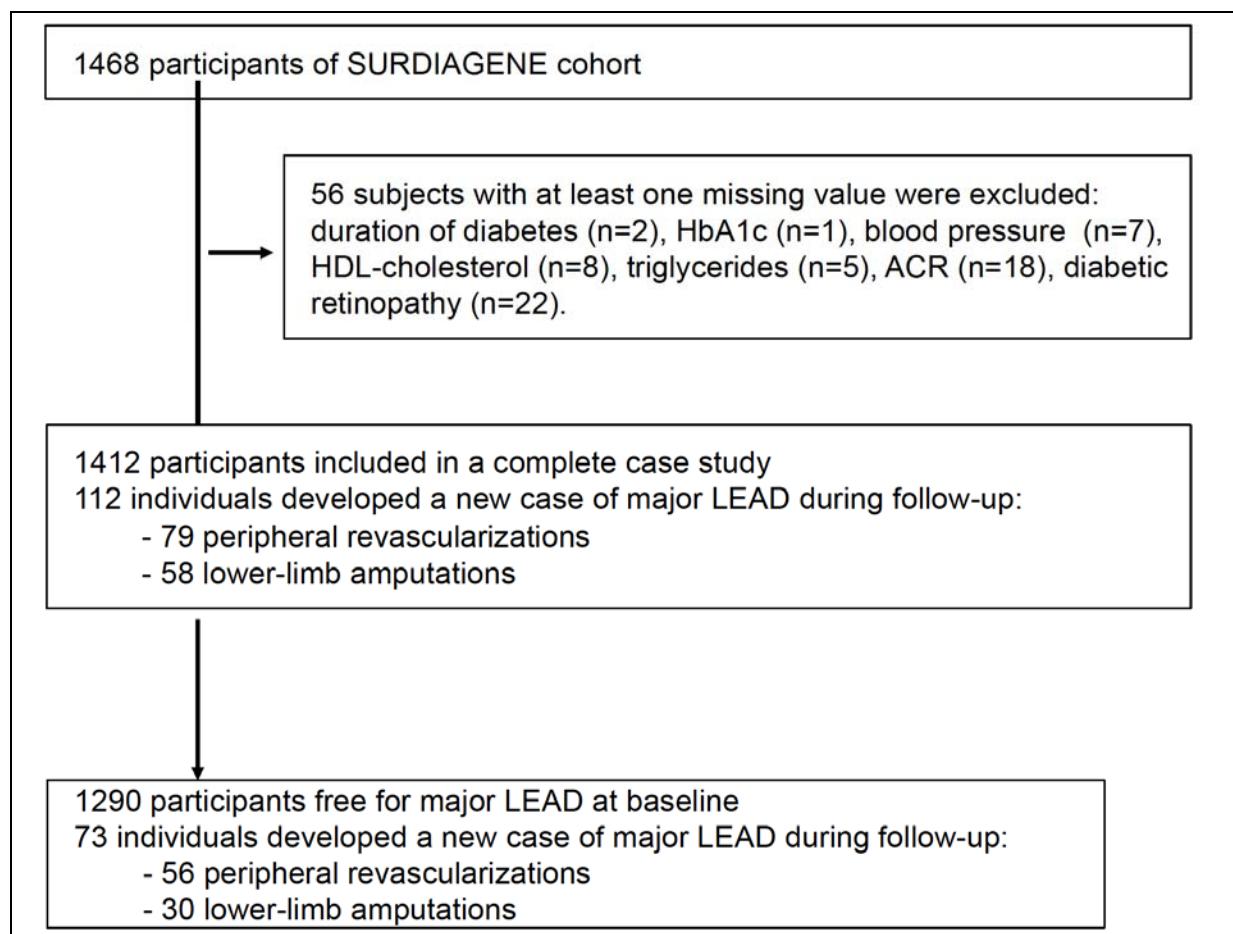
Supplementary Table 4. Alternative primary endpoint during follow-up according to plasma concentrations of inflammatory and oxidative stress biomarkers at baseline

Supplementary Table 5. Characteristics of participants according to the availability of CRP measurements at baseline

Supplementary Table 6. Risks for peripheral revascularisation and lower-limb amputation during follow-up according to plasma concentrations of inflammatory and oxidative stress biomarkers at baseline

SUPPLEMENTARY DATA

Supplementary Figure 1. Flow chart of the SURDIAGENE prospective cohort



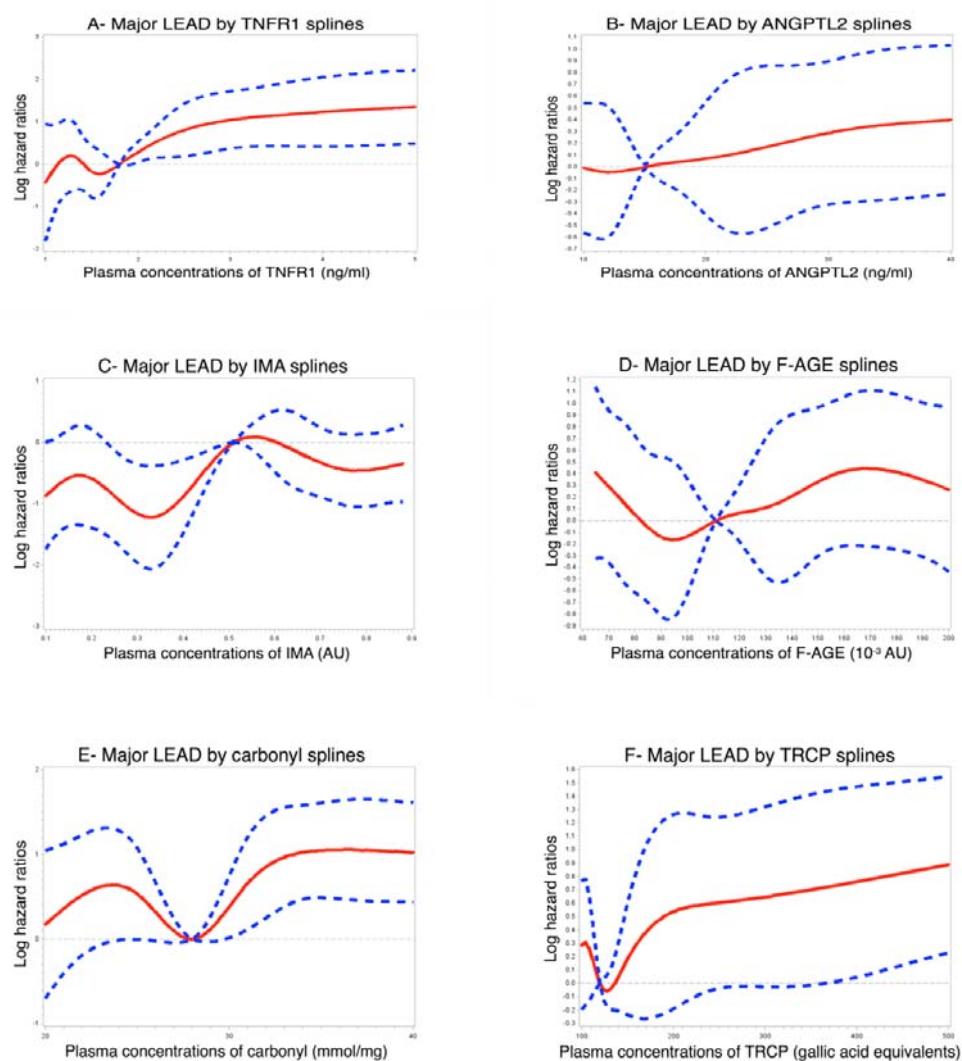
SUPPLEMENTARY DATA

LEGENDS OF SUPPLEMENTAL FIGURE 2

Supplementary Figure 2. Hazard ratios for major LEAD during follow-up by splines of plasma concentrations of inflammatory and oxidative stress biomarkers at baseline.

Multi-adjusted hazard ratios (solid red line) and 95% confidence intervals (dashed blue lines) for major LEAD during follow-up by baseline TNFR1 (knots: 1, 1.5, 2.3, 3.3, 5 and reference at 1.8 ng/ml), ANGPTL2 (knots: 10, 12, 20, 30, 40 and reference at 15 ng/ml), IMA (knots: 0.1, 0.33, 0.63, 0.75, 0.88 and reference at 0.51 AU), F-AGE (knots: 65, 93, 132, 157, 200 and reference at 111×10^{-3} AU), protein carbonyls (knots: 20, 25, 31, 35, 40 and reference at 28 mmol/mg), and TRCP (knots: 100, 102, 145, 241, 500 and reference at 120 gallic acid equivalents).

Analyses were adjusted as in model 2: age, sex, BMI, duration of diabetes, HbA1c, systolic and diastolic blood pressure, urinary ACR, eGFR, diabetic retinopathy stages, plasma concentrations of HDL-cholesterol, total cholesterol and triglycerides, use of insulin therapy, antihypertensive, statin, fibrate, and antiplatelet drugs, and history of current smoking and macrovascular disease (p for non-linearity <0.0001 for all).



SUPPLEMENTARY DATA

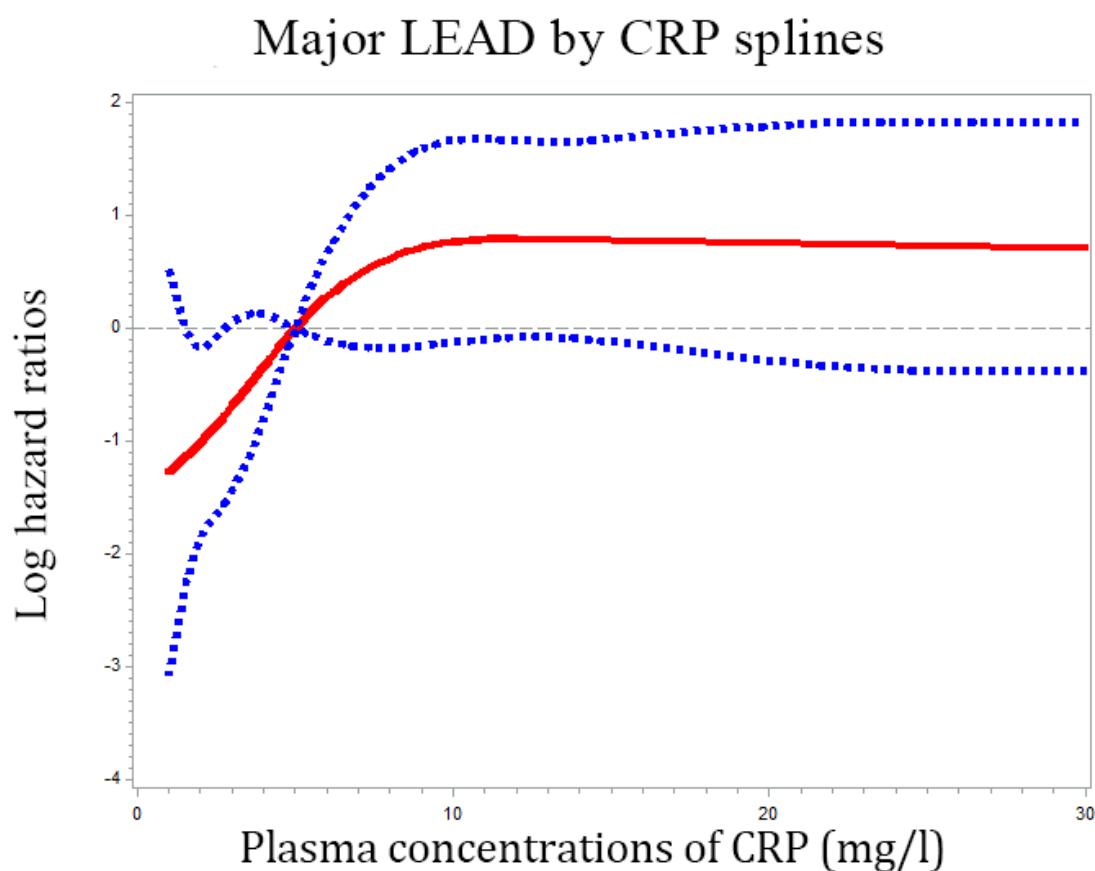
LEGENDS OF SUPPLEMENTAL FIGURE 3

Supplementary Figure 3. Hazard ratios for major LEAD during follow-up by splines of plasma concentrations of C-reactive protein at baseline

Multi-adjusted hazard ratios (solid red line) and 95% confidence intervals (dashed blue lines) for major LEAD during follow-up by baseline CRP splines, using knots at 1, 2, 13 and 30 mg/l, and reference at 5 mg/l.

Analyses were adjusted as in model 2: age, sex, BMI, duration of diabetes, HbA1c, systolic and diastolic blood pressure, urinary ACR, eGFR, diabetic retinopathy stages, plasma concentrations of HDL-cholesterol, total cholesterol and triglycerides, use of insulin therapy, antihypertensive, statin, fibrate, and antiplatelet drugs, and history of current smoking and macrovascular disease (p for non-linearity <0.0001).

Analyses performed in a subset of 291 participants from whom CRP measurements were available at baseline.



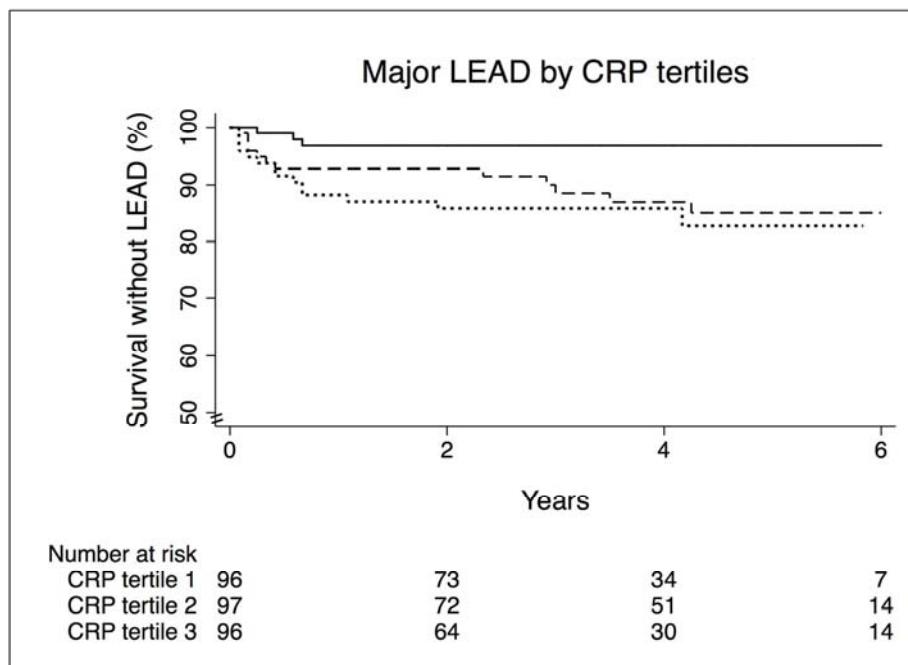
SUPPLEMENTARY DATA

LEGENDS OF SUPPLEMENTAL FIGURE 2

Supplementary Figure 4. Major LEAD during follow-up by tertiles of CRP plasma concentrations at baseline

Survival without major LEAD in the third (dotted line) and the second tertiles (dashed line) compared to the first one (solid line) of plasma concentrations of C-reactive protein at baseline ($p=0.02$).

Analyses performed in a subset of 291 participants from whom CRP measurements were available at baseline.



SUPPLEMENTARY DATA

Supplementary Table 1. Characteristics of participants according to tertiles of plasma concentrations of each biomarker at baseline

	TNFR1			ANGPTL2			IMA		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
Clinical parameters									
Male sex	290 (62)	248 (53)	284 (60)	283 (60)	263 (56)	276 (59)	284 (60)	262 (56)	276 (59)
Age (years)	61 (11)	64 (10)	69 (9)	60 (10)	65 (10)	69 (9)	64 (11)	65 (11)	65 (10)
Diabetes duration (years)	10 (5,18)	12 (5,18)	16 (10,25)	10 (4,16)	12 (6,22)	16 (10,24)	12 (5,19)	12 (6,20)	14 (8,23)
BMI (kg/m ²)	30 (5)	32 (6)	32 (7)	31 (6)	32 (7)	31 (6)	32 (7)	31 (6)	31 (6)
SBP (mmHg)	130 (17)	132 (16)	136 (19)	129 (16)	133 (18)	135 (18)	132 (17)	132 (17)	135 (19)
DBP (mmHg)	73 (11)	72 (11)	72 (11)	73 (11)	72 (12)	73 (11)	72 (11)	73 (11)	73 (11)
Biological parameters									
HbA1c (%)	7.8 (1.5)	7.8 (1.5)	7.8 (1.6)	7.7 (1.6)	7.8 (1.6)	7.8 (1.5)	7.8 (1.5)	7.8 (1.5)	7.8 (1.6)
HbA1c (mmol/mol)	62 (17)	62 (17)	61 (17)	61 (17)	62 (17)	62 (17)	62 (17)	61 (17)	62 (18)
ACR (mg/mmol)	2 (1,7)	2 (1,8)	9 (2,82)	2 (1,7)	2 (1,9)	9 (2,65)	3 (1,12)	3 (1,11)	4 (1,22)
eGFR (ml/min/1.73m ²)	87 (16)	79 (18)	54 (24)	88 (16)	76 (20)	56 (24)	76 (24)	74 (24)	70 (26)
Total cholesterol (mmol/l)	4.8 (1.0)	4.7 (1.2)	4.8 (1.2)	4.7 (1.0)	4.8 (1.1)	4.9 (1.2)	4.6 (1.1)	4.8 (1.1)	5.0 (1.2)

SUPPLEMENTARY DATA

HDL cholesterol (mmol/l)	1.3 (0.4)	1.2 (0.4)	1.1 (0.4)	1.2 (0.4)	1.2 (0.4)	1.2 (0.4)	1.2 (0.4)	1.2 (0.4)
Triglycerides (mmol/l)	1.4 (1.0, 2.2) 2.3 (2.3)	1.6 (1.1, 2.4)	1.7 (1.2, 2.2)	1.5 (1.0, 2.3)	1.6 (1.1, 2.4)	1.7 (1.2, 2.4)	1.6 (1.1, 2.3)	1.5 (1.1, 2.2)
Medical history								
Current smoking	65 (14)	54 (11)	33 (7)	76 (16)	40 (8)	36 (8)	55 (12)	48 (10)
Diabetic retinopathy	165 (35)	191 (41)	268 (57)	181 (38)	180 (38)	263 (56)	183 (39)	196 (42)
Macrovascular disease	137 (29)	160 (34)	210 (45)	136 (29)	155 (33)	216 (46)	163 (35)	170 (36)
Major LEAD	20 (4)	36 (8)	66 (14)	25 (5)	40 (8)	57 (12)	44 (9)	38 (8)
Lower-limb amputation	12 (3)	18 (4)	39 (8)	10 (2)	20 (4)	39 (8)	22 (5)	21 (4)
Peripheral revascularization	12 (3)	21 (4)	36 (8)	16 (3)	26 (6)	27 (6)	26 (6)	25 (5)
History of treatments								
Antihypertensive treatment	336 (71)	388 (82)	448 (95)	342 (73)	402 (85)	428 (91)	395 (84)	388 (82)
Statin	219 (47)	196 (42)	223 (47)	215 (46)	213 (45)	210 (45)	218 (46)	236 (50)
Fibrate	44 (9)	68 (14)	48 (10)	45 (10)	59 (13)	56 (12)	55 (12)	40 (8)
Antiplatelet drugs	173 (37)	190 (40)	230 (49)	170 (36)	202 (43)	221 (47)	192 (41)	200 (42)
Insulin therapy	242 (51)	275 (58)	329 (70)	234 (50)	280 (59)	332 (71)	269 (57)	266 (56)
								311 (66)

SUPPLEMENTARY DATA

Plasma concentrations of biomarkers		Clinical parameters					
TNFR1 (ng/ml)	1.4 1.6	(1.3, 1.8 (1.7,2)	2.8 3.5)	(2.4, 1.8)	1.6 2.2)	(1.4, 3.3)	(1.8 2.3) (1.6, 2.3) (1.6, 2.5)
ANGPTL2 (ng/ml)	12 (9,15)	15 (12,19)	21 (16,30)	10 (8,11)	15 (14,17)	24 (21,32)	15 (11,20) 15 (11,21) 16 (12,22)
IMA (AU)	0.5 0.7	(0.3, 0.6)	0.5 0.6)	(0.3, 0.6)	0.5 0.6)	(0.3, 0.6)	(0.3, 0.7) (0.2 0.3) (0.1, 0.3) (0.5 0.6) (0.5 0.6) (0.4, 0.6) (0.7 0.8)
F-AGE (10^{-3} AU)	106 122)	(88, 130)	110 149)	(92, 121)	(100, 125)	(87, 130)	(95, 144) (97, 130) (111 130) (96, 138) (116 138) (96, 128) (108 87)
Carbonyls (mmol/mg)	28 30)	(26, 28 (25,31)	29 (26,32)	28 (26,31)	28 (26,31)	29 (26,32)	28 (26,30) 28 (26,30) 29 (26,32)
TRCP (gallic acid equiv)	115 138)	(99, 141)	118 158)	102, 127	(107, 149)	118 141)	(102, 148) (122 148) (104, 110,157) (128 141) (117 141) (102, 141) (114 139) (97, 139)
		F-AGE		Carbonyl		TRCP	
		T1	T2	T3	T1	T2	T3
Male sex	271 (58)	270 (57)	281 (60)	277 (59)	266 (56)	279 (59)	266 (56) 281 (60) 275 (59)
Age (y)	63 (10)	64 (11)	66 (11)	63 (11)	65 (11)	66 (10)	64 (11) 65 (10) 66 (11)

SUPPLEMENTARY DATA

Diabetes duration (y)	12 (6, 21)	12 (6, 20)	13 (6, 21)	12 (6, 19)	13 (6, 21)	14 (7, 23)	12 (7, 20)	13 (6, 21)	13 (6, 21)
BMI (kg/m^2)	30 (35)	(27, 35)	31 (27, 35)	30 (27, 34)	31 (27, 34)	30 (27, 35)	30 (27, 35)	30 (27, 34)	30 (27, 35)
SBP (mmHg)	132 (18)	133 (17)	132 (18)	132 (17)	131 (18)	135 (18)	133 (18)	133 (18)	132 (18)
DBP (mmHg ^g)	73 (12)	73 (10)	72 (11)	73 (11)	72 (11)	72 (11)	73 (11)	72 (10)	72 (12)
Biological parameters									
HbA1c (%)	7.9 (1.6)	7.8 (1.5)	7.6 (1.5)	7.8 (1.6)	7.8 (1.5)	7.8 (1.5)	7.9 (1.6)	7.8 (1.5)	7.6 (1.5)
HbA1c (mmol/mol)	63 (17)	62 (17)	60 (16)	62 (17)	61 (17)	62 (17)	63 (18)	62 (17)	60 (16)
ACR (mg/mmol)	2 (1, 9)	3 (1, 10)	6 (2, 27)	3 (1, 10)	3 (1, 11)	4 (1, 23)	2 (1, 10)	3 (1, 13)	4 (1, 20)
eGFR (ml/min/1.73m ²)	81 (20)	75 (23)	64 (27)	76 (23)	73 (24)	70 (26)	77 (21)	74 (24)	69 (28)
Total cholesterol (mmol/l)	4.8 (1.1)	4.8 (1.2)	4.8 (1.2)	4.9 (1.2)	4.8 (1.1)	4.7 (1.1)	4.8 (1.1)	4.8 (1.1)	4.8 (1.2)
HDL cholesterol (mmol/l)	1.2 (0.4)	1.2 (0.4)	1.2 (0.4)	1.2 (0.4)	1.2 (0.4)	1.2 (0.4)	1.2 (0.4)	1.2 (0.4)	1.2 (0.4)

SUPPLEMENTARY DATA

Triglycerides (mmol/l)	1.4 (2.0)	1.1, 2.3	1.6 2.4	1.1, 2.5	1.8 2.4	(1.2, 2.4)	1.7 2.3	(1.2, 2.3)	1.5 2.2	(1.1, 2.2)	1.5 2.2	(1.1, 2.4)	1.6 2.4	(1.1, 2.4)	1.6 2.3	(1.2, 2.3)
Medical history																
Current smoking	51 (11)	52 (11)	49 (10)	51 (11)	47 (10)	54 (11)	51 (11)	51 (11)	54 (11)	53 (11)	53 (11)	48 (10)				
Diabetic retinopathy	233 (49)	198 (42)	193 (41)	188 (40)	205 (44)	231 (49)	202 (43)	203 (43)	202 (43)	203 (43)	203 (43)	219 (47)				
Macrovascular disease	145 (31)	157 (33)	205 (44)	146 (31)	179 (38)	182 (39)	148 (31)	175 (37)	175 (37)	175 (37)	175 (37)	184 (39)				
Major LEAD	33 (7)	32 (7)	57 (12)	42 (9)	35 (7)	45 (10)	36 (8)	45 (10)	36 (8)	45 (10)	45 (10)	41 (9)				
Lower extremity amputation	19 (4)	17 (4)	33 (7)	20 (4)	22 (5)	27 (6)	22 (5)	22 (5)	22 (5)	22 (5)	22 (5)	25 (5)				
Peripheral revascularization	20 (4)	16 (3)	33 (7)	26 (6)	21 (4)	22 (5)	19 (4)	22 (5)	19 (4)	28 (6)	28 (6)	22 (5)				
History of treatments																
Antihypertensive treatment	360 (76)	395 (84)	417 (89)	373 (79)	398 (85)	401 (85)	386 (82)	392 (83)	394 (84)							
Statin	190 (40)	215 (46)	233 (50)	209 (44)	224 (48)	205 (44)	191 (41)	229 (49)	218 (46)							

SUPPLEMENTARY DATA

	Plasma concentrations of biomarkers					
Fibrate	63 (13)	59 (13)	38 (8)	63 (13)	61 (13)	36 (8) 74 (16) 44 (9) 42 (9)
Antiplatelet drugs	171 (36)	189 (40)	233 (50)	182 (39)	197 (42)	214 (46) 198 (42) 195 (41) 200 (43)
Insulin therapy	292 (62)	286 (61)	268 (57)	258 (55)	286 (61)	302 (64) 284 (60) 279 (59) 283 (60)
TNFR1 (ng/ml)	1.7 (2.1)	1.5 (2.2)	1.8 (2.8)	2.1 (2.2)	1.7 (2.2)	1.8 (2.3) 1.9 (2.6) 1.8 (2.2) 1.8 (2.3) 1.8 (1.5, 2.7)
ANGPTL2 (ng/ml)	14 (10, 19)	15 (12, 20)	17 (12, 23)	14 (11, 19)	15 (11, 20)	16 (12, 23) 15 (11, 20) 15 (11, 20) 15 (11, 22)
IMA (AU)	0.5 (0.7)	0.5 (0.6)	0.5 (0.6)	0.5 (0.6)	0.5 (0.6)	0.5 (0.7) 0.5 (0.7) 0.5 (0.7) 0.5 (0.6) 0.5 (0.3, 0.6) 0.5 (0.3, 0.6) 0.5 (0.3, 0.6) 0.5 (0.3, 0.6)
F-AGE (10^{-3} AU)	85 (75, 93)	112 (106, 117)	143 (162)	112 (133, 132)	94 (94, 134)	111 (93, 132) 112 (91, 132) 109 (92, 126) 117 (98, 135) 111 (88, 135)
Protein carbonyl (mmol/mg)	28 (26, 31)	28 (26, 31)	28 (26, 31)	25 (23, 26)	28 (28, 29)	32 (31, 36) 28 (26, 30) 28 (26, 31) 29 (27, 31)
TRCP (gallic acid equivalents)	120 (97, 173)	115 (101, 141)	125 (108, 143)	115 (101, 136)	121 (102, 150)	122 (106, 151) 96 (86, 103) 120 (114, 127) 170 (145, 289)

Data presented as numbers (%), mean (SD), or median (25th, 75th percentiles) for variables with skewed distribution: duration of diabetes, urinary albumin to creatinine ratio (ACR), triglycerides, tumor necrosis factor receptor 1 (TNFR1), angiopoietin-like 2 protein (ANGPTL2), ischemia-modified albumin (IMA), fluorescent advanced glycation end products (F-AGE), and total reductive capacity of plasma (TRCP).

LEAD, lower-extremity artery disease; SBP and DBP, systolic and diastolic blood pressure; eGFR, estimated glomerular filtration rate

SUPPLEMENTARY DATA

Supplementary Table 2. Major lower-extremity artery disease during follow-up according to plasma concentrations of inflammatory and oxidative stress biomarkers at baseline with correction for competing risk of cardiovascular death

		Subhazard ratio (95% CI)		P
TNFR1	2 nd versus 1 st tertile	1.07 (0.60 - 1.94)	0.81	
	3 rd versus 1 st tertile	2.07 (1.12 - 3.83)	0.02	
ANGPTL2	2 nd versus 1 st tertile	1.30 (0.72 - 2.33)	0.38	
	3 rd versus 1 st tertile	1.54 (0.85 - 2.80)	0.16	
IMA	2 nd versus 1 st tertile	2.45 (1.37 - 4.38)	0.002	
	3 rd versus 1 st tertile	2.12 (1.22 - 3.69)	0.008	
F-AGE	2 nd versus 1 st tertile	1.00 (0.61 - 1.63)	0.99	
	3 rd versus 1 st tertile	1.05 (0.63 - 1.76)	0.85	
Protein carbonyl	2 nd versus 1 st tertile	0.74 (0.44 - 1.22)	0.23	
	3 rd versus 1 st tertile	1.18 (0.75 - 1.85)	0.48	
TRCP	2 nd versus 1 st tertile	0.88 (0.54 - 1.45)	0.62	
	3 rd versus 1 st tertile	1.03 (0.62 - 1.70)	0.92	

Subdistribution hazard ratios and 95% confidence intervals (CI) for 2nd and 3rd tertiles compared to the 1st one, adjusting as in model 2: age, sex, BMI, duration of diabetes, HbA1c, systolic and diastolic blood pressure, urinary albumin-to-creatinine ratio, estimated glomerular filtration rate, diabetic retinopathy stages, plasma concentrations of HDL-cholesterol, total cholesterol and triglycerides, use of insulin therapy, antihypertensive, statin, fibrate and antiplatelet drugs, and history of current smoking and macrovascular disease. P<0.05 was significant. TNFR1, tumor necrosis factor receptor 1; ANGPTL2, angiopoietin-like 2 protein; IMA, ischemia-modified albumin; F-AGE, fluorescent advanced glycation end products; TRCP, total reductive capacity of plasma.

SUPPLEMENTARY DATA

Supplementary Table 3. Risk for major lower-extremity artery disease during follow-up according to plasma concentrations of inflammatory and oxidative stress biomarkers in different subsets of participants

	Participants free of lower-extremity artery disease at baseline	Participants free of macrovascular disease at baseline				Participants free of chronic kidney disease at baseline			
		Major LEAD		Major LEAD		Major LEAD		Major LEAD	
		No, n (%)	Yes, n	No, n (%)	Yes, n	No, n (%)	Yes, n	No, n (%)	Yes, n
TNFRI	All	1217	73 (6)	841	64 (7)	957	59 (6)		
	T1	415	15 (3)	Ref.	-	293	9 (3)	Ref.	-
								1.2 (0.5-	1.6 (0.7-
	T2	416	14 (3)	1.1 (0.5-2.3)	.88	291	11 (4)	3.2)	.65
								3.7 (1.5-	3.1 (1.4-
	T3	386	44 (10)	2.7 (1.3-5.6)	.009	257	44 (15)	9.1)	.004
								307	31 (9)
	ANGPTL2	T1	414	16 (4)	Ref.	-	291	11 (4)	Ref.
								-	324
								15 (4)	Ref.
	T2	414	16 (4)	0.9 (0.4-2.0)	.85	281	21 (7)	3.8)	.17
								318	21 (6)
									2.2)
									.79
									1.2 (0.6-
	T3	389	41 (9)	1.8 (0.9-3.7)	.09	269	32 (11)	3.6)	.27
								315	23 (7)
	IMA	T1	418	12 (3)	Ref.	-	289	13 (4)	Ref.
								-	333
								6 (2)	Ref.
									-

SUPPLEMENTARY DATA

						1.5 (0.7-	5.1 (2.1-
T2	406	24 (6)	1.9 (0.9-3.8)	.08	280	23 (8)	3.1)
						.24	312
							27 (8)
							12.5)
						1.6 (0.8-	3.3 (1.3-
T3	393	37 (9)	2.0 (1.0-3.9)	.04	272	28 (9)	3.2)
						.19	312
							26 (8)
							8.1)
						.01	
F-AGE	T1	408	22 (5)	Ref.	-	280	22 (7)
						Ref.	-
							Ref.
							-
						0.7 (0.3-	0.8 (0.4-
T2	399	31 (7)	1.4 (0.8-2.6)	.23	283	19 (6)	1.3)
						.27	324
							15 (4)
							1.6)
							.58
						1.0 (0.5-	1.4 (0.7-
T3	410	20 (5)	1.0 (0.5-1.9)	.91	278	23 (8)	2.0)
						.94	316
							22 (7)
							2.6)
						.31	
Carbonyls	T1	409	21 (5)	Ref.	-	282	22 (7)
						Ref.	-
							Ref.
							-
						0.6 (0.3-	0.4 (0.2-
T2	411	19 (5)	1.0 (0.5-1.9)	.98	286	15 (5)	1.3)
						.22	327
							12 (4)
							0.9)
							.02
						1.1 (0.6-	1.0 (0.5-
T3	397	33 (7)	1.3 (0.7-2.4)	.35	273	27 (9)	2.1)
						.64	315
							23 (7)
							1.8)
						.96	
TRCP	T1	404	26 (6)	Ref.	-	282	20 (7)
						Ref.	-
							Ref.
							-
						1.1 (0.5-	1.3 (0.7-
T2	410	20 (5)	0.7 (0.4-1.3)	.33	284	18 (6)	2.0)
						.87	316
							23 (7)
							2.5)
						.34	
T3	403	27 (6)	0.9 (0.5-1.6)	.73	275	26 (9)	1.6 (0.8-
						.16	322
							16 (5)
							1.1 (0.5-
							.85

SUPPLEMENTARY DATA

3.0) 2.1)

Hazard ratio (HR) and 95% confidence intervals (CI) for the 2nd and 3rd tertiles compared to the 1st one. Analyses were adjusted as in model 2: age, sex, BMI, duration of diabetes, HbA1c, systolic and diastolic blood pressure, urinary albumin-to-creatinine ratio, estimated glomerular filtration rate, diabetic retinopathy stages, plasma concentrations of HDL-cholesterol, total cholesterol and triglycerides, use of insulin therapy, antihypertensive, statin, fibrate, and antiplatelet drugs, and history of current smoking and macrovascular disease (except for the subset of participants free of macrovascular disease at baseline). P<0.05 was significant.

TNFR1, tumor necrosis factor receptor 1; ANGPTL2, angiopoietin-like 2 protein; IMA, ischemia-modified albumin; F-AGE, fluorescent advanced glycation end products; TRCP, total reductive capacity of plasma.

SUPPLEMENTARY DATA

Supplementary Table 4. Alternative primary endpoint* during follow-up according to plasma concentrations of inflammatory and oxidative stress biomarkers at baseline

		Major lower-extremity artery disease		HRs (95% CI)	P
		No, n	Yes, n (%)		
	All	1300	100 (7.1)		
TNFR1	1 st tertile	447	20 (4.3)	Ref.	
	2 nd tertile	444	23 (4.9)	1.21 (0.65 - 2.24)	0.55
	3 rd tertile	409	57 (12.2)	2.04 (1.08 - 3.87)	0.03
ANGPTL2	1 st tertile	447	20 (4.3)	Ref.	
	2 nd tertile	437	30 (6.4)	1.25 (0.69 - 2.25)	0.45
	3 rd tertile	416	50 (10.7)	1.29 (0.70 - 2.39)	0.42
IMA	1 st tertile	452	15 (3.2)	Ref.	
	2 nd tertile	428	39 (8.3)	2.52 (1.37 - 4.62)	0.03
	3 rd tertile	420	46 (9.9)	2.12 (1.16 - 3.88)	0.01
F-AGE	1 st tertile	437	30 (6.4)	Ref.	
	2 nd tertile	432	35 (7.5)	1.08 (0.65 - 1.81)	0.76
	3 rd tertile	431	35 (7.5)	1.03 (0.60 - 1.77)	0.92
Protein carbonyls	1 st tertile	437	30 (6.4)	Ref.	
	2 nd tertile	440	27 (5.8)	0.75 (0.43 - 1.29)	0.30
	3 rd tertile	423	43 (9.2)	1.15 (0.70 - 1.88)	0.59
TRCP	1 st tertile	434	33 (7.1)	Ref.	
	2 nd tertile	438	29 (6.2)	0.80 (0.48 - 1.33)	0.39
	3 rd tertile	428	38 (8.1)	1.09 (0.66 - 1.79)	0.75

*Defined as the first occurrence during follow-up of one of the following: (i) minor lower-limb amputation with peripheral revascularization; (ii) major lower-limb amputation; or (iii) requirement of peripheral revascularization procedure. Hazard ratios (HRs) and 95% confidence intervals (CI) for the 2nd and 3rd teriles compared to the 1st one. Analyses were adjusted as in model 2: age, sex, BMI, duration of diabetes, HbA1c, systolic and diastolic blood pressure, urinary albumin-to-creatinine ratio, estimated glomerular filtration rate, diabetic retinopathy stages, plasma concentrations of HDL-cholesterol, total cholesterol and triglycerides, use of insulin therapy, antihypertensive, statin, fibrate, and antiplatelet drugs, and history of current smoking and macrovascular disease. P<0.05 was significant. TNFR1, tumor necrosis factor Receptor 1; ANGPTL2, angiopoietin-like 2 protein; IMA, ischemia-modified albumin; F-AGE, fluorescent advanced glycation end products; TRCP, total reductive capacity of plasma.

SUPPLEMENTARY DATA

Supplementary Table 5. Characteristics of participants according to the availability of CRP measurements at baseline

SUPPLEMENTARY DATA

		Availability of CRP measurements		
	No	Yes	P	
N (%)	1121 (79.4)	291 (20.6)		
Clinical parameters				
Male sex	651 (58.1)	171 (58.8)	0.84	
Age (years)	64.4 (10.5)	65.8 (11.2)	0.05	
Duration of diabetes (years)	12 (6, 20)	13 (6, 22)	0.16	
Body mass index (kg/m ²)	31.1 (6.0)	32.0 (7.4)	0.04	
Systolic blood pressure (mmHg)	133 (17)	130 (18)	0.02	
Diastolic blood pressure (mmHg)	72 (11)	72 (11)	0.52	
Biological parameters				
HbA1c (%)	7.7 (1.4)	8.1 (1.8)	<0.0001	
HbA1c (mmol/mol)	61 (16)	65 (20)		
Urinary albumin to creatinine ratio (mg/mmol)	3 (1, 15)	3 (1, 13)	0.69	
eGFR (ml/min/1.73m ²)	73 (24)	72 (27)	0.52	
Serum total cholesterol (mmol/l)	4.88 (1.14)	4.46 (1.14)	<0.0001	
Serum HDL cholesterol (mmol/l)	0.47 (0.16)	0.44 (0.15)	0.01	
Serum triglycerides (mmol/l)	1.57 (1.12, 2.35)	1.55 (1.12, 2.17)	0.37	

SUPPLEMENTARY DATA

CRP (mg/l)	-	5.2 (2.1 - 13.3)	-
Medical history			
Current smoking	119 (10.6)	33 (11.3)	0.57
Diabetic retinopathy	483 (43.1)	141 (48.4)	0.11
Macrovascular disease	400 (35.7)	107 (36.8)	0.73
Major lower-extremity artery disease	90 (8.0)	32 (11.0)	0.13
Lower-limb amputation	49 (4.3)	20 (6.9)	0.08
Peripheral revascularization	49 (4.4)	20 (6.8)	0.09
History of treatments			
Use of antihypertensive treatment	917 (81.8)	255 (87.6)	0.02
Use of statin	492 (43.9)	146 (50.2)	0.05
Use of fibrate	148 (13.2)	12 (4.1)	<0.0001
Use of antiplatelet drugs	442 (39.4)	151 (51.9)	0.0001
Use of insulin therapy	665 (59.3)	181 (62.2)	0.38

Data presented as numbers (%), mean (SD), or median (25th, 75th percentiles) for variables with skewed distribution: duration of diabetes, urinary albumin to creatinine ratio, triglycerides. Comparisons of qualitative and quantitative parameters were performed using Chi-square and ANOVA tests, respectively. Wilcoxon test was used for comparisons of variables with skewed distribution. p<0.05 was significant. CRP, C-reactive protein.

SUPPLEMENTARY DATA

Supplementary Table 6. Risks for peripheral revascularisation and lower-limb amputation during follow-up according to plasma concentrations of inflammatory and oxidative stress biomarkers at baseline

		Peripheral revascularisation			Peripheral revascularisation vs. not			Lower-limb amputation vs. not	
		No, n	Yes, n (%)	HRs (95%CI)	P	No, n	Yes, n (%)	HRs (95%CI)	P
All	1333	79 (5.6)				1354	58 (4.1)		
TNFR1	1 st tertile	456	15 (3.2)	Ref.		460	11 (2.3)	Ref.	
	2 nd tertile	450	21 (4.5)	1.47 (0.74 - 2.91) 0.27		462	9 (1.9)	0.87 (0.35 - 2.21)	
	3 rd tertile	427	43 (9.1)	2.11 (1.03 - 4.32) 0.04		432	38 (8.1)	2.42 (1.03 - 5.68)	
ANGPTL2	1 st tertile	455	16 (3.4)	Ref.		464	7 (1.5)	Ref.	
	2 nd tertile	445	26 (5.5)	1.33 (0.69 - 2.56) 0.39		457	14 (3.0)	1.86 (0.70 - 4.96)	
	3 rd tertile	433	37 (7.9)	1.28 (0.64 - 2.57) 0.49		433	37 (7.9)	3.67 (1.43 - 9.43)	
IMA	1 st tertile	460	11 (2.3)	Ref.		460	11 (2.3)	Ref.	
	2 nd tertile	439	32 (6.8)	3.07 (1.54 - 6.14) 0.001		449	22 (4.7)	1.99 (0.95 - 4.18)	
	3 rd tertile	434	36 (7.7)	2.35 (1.18 - 4.70) 0.01		445	25 (5.3)	1.52 (0.72 - 3.20)	
F-AGE	1 st tertile	448	23 (4.9)	Ref.		454	17 (3.6)	Ref.	
	2 nd tertile	443	28 (5.9)	1.24 (0.70 - 2.20) 0.46		455	16 (3.4)	1.00 (0.47 - 2.09)	

SUPPLEMENTARY DATA

	3 rd tertile	442	28 (6.0)	1.19 (0.65 - 2.19)	0.57	445	25 (5.3)	1.68 (0.82 - 3.41)	0.15
Protein carbonyls	1 st tertile	446	25 (5.3)	Ref.		455	16 (3.4)	Ref.	
	2 nd tertile	449	22 (4.7)	0.67 (0.37 - 1.21)	0.18	458	13 (2.8)	0.73 (0.33 - 1.59)	0.43
	3 rd tertile	438	32 (6.8)	0.98 (0.57 - 1.71)	0.96	441	29 (6.2)	1.61 (0.83 - 3.12)	0.16
TRCP	1 st tertile	443	28 (5.9)	Ref.		449	22 (4.7)	Ref.	
	2 nd tertile	449	22 (4.7)	0.78 (0.44 - 1.38)	0.39	452	19 (4.0)	0.79 (0.41 - 1.52)	0.49
	3 rd tertile	441	29 (6.2)	1.09 (0.63 - 1.88)	0.77	453	17 (3.6)	0.69 (0.34 - 1.40)	0.31

Hazard ratios (HRs) and 95% confidence intervals (CI) for the 2nd and 3rd tertiles compared to the 1st one. Analyses were adjusted as in model 2: age, sex, BMI, duration of diabetes, HbA1c, systolic and diastolic blood pressure, urinary albumin-to-creatinine ratio, estimated glomerular filtration rate, diabetic retinopathy stages, plasma concentrations of HDL-cholesterol, total cholesterol and triglycerides, use of insulin therapy, antihypertensive, statin, fibrate, and antiplatelet drugs, and history of current smoking and macrovascular disease. P<0.05 was significant.

TNFR1, tumor necrosis factor Receptor 1; ANGPTL2, angiopoietin-like 2 protein; IMA, ischemia-modified albumin; F-AGE, fluorescent advanced glycation end products; TRCP, total reductive capacity of plasma.

PARTIE III : DISCUSSION GENERALE ET PERSPECTIVES

Nous avons observé dans ce travail une association entre les concentrations plasmatiques de TNFR1 et d'IMA à l'inclusion et le risque de développer une AOMI majeure durant le suivi. Cette association était indépendante des principaux facteurs confondants notamment les marqueurs glycémiques (durée du diabète et HbA1c), de la fonction rénale (excrétion urinaire d'albumine et débit de filtration glomérulaire), facteurs de risque de maladie cardiovasculaire traditionnels, ainsi que le décès du à une maladie cardiovasculaire considéré comme un risque compétitif. Nos résultats étaient également constants après considération de certains sous-groupes particuliers de patients naïfs d'AOMI, de maladie cardiovasculaire, ou de maladie rénale à l'inclusion. De plus, ajouté aux autres facteurs de risque, le dosage du TNFR1 apportait des informations pronostiques supplémentaires en améliorant la prédiction et la classification du risque d'AOMI. Notre étude montre aussi une association indépendante entre les concentrations plasmatiques de l'IMA et un risque augmenté d'AOMI et revascularisation périphérique, mais non significative pour l'amputation du membre inférieur. L'association restait significative chez les patients sans AOMI ni maladie rénale chronique à l'inclusion. En revanche, les concentrations plasmatiques d'IMA n'amélioraient pas la classification du risque d'AOMI.

Le TNF alpha, cytokine produite par les macrophages, les cellules dendritiques, les lymphocytes T et NK, a une action apoptotique sur les cellules infectées, stimule l'action cytotoxique des macrophages et induit la libération de molécules cytotoxiques de défense immunitaires (radicaux libres, NO). Il amplifie également la réponse inflammatoire en favorisant la synthèse de cytokines (IL-1, IL-6, IL-8) et de facteurs de croissance. Plus spécifiquement au niveau endothérial il augmente la perméabilité vasculaire, induit une activité procoagulante et l'expression de molécules d'adhésion et de chimiokines. Il semble donc

impliqué dans la pathogénèse de la plaque d'athérome. Il a deux récepteurs solubles, le TNFR1, activé par la forme soluble du TNF alpha et le TNFR2, activé par la forme membranaire. Le récepteur 1 au TNF initie la plupart des fonctions biologiques du TNF alpha dont son action apoptotique et proinflammatoire. La concentration augmentée plasmatique des récepteurs aux cytokines, dont les récepteurs 1 et 2 du TNF, a été corrélée à l'activité des maladies néoplasiques, autoimmunes et inflammatoires. Des travaux ont montré une concentration en TNF alpha augmentée chez les diabétiques, imputable d'après l'étude d'Esposito et al. à l'hyperglycémie chronique, favorisant l'inflammation engendrée par un mécanisme oxydatif (17). Néanmoins, selon notre connaissance aucune étude n'a rapporté d'association entre TNFR1 et AOMI chez les patients diabétiques. Dans la population générale, le TNFR1 était associé à un risque augmenté d'AOMI (définie par un IPS<0,9, une claudication intermittente, une amputation au membre inférieur) dans la cohorte Framingham Offspring (18). Ohgushi et al. a rapporté une association forte indépendante entre une concentration en TNRF2 et la courbe de l'onde de pouls, marqueur de rigidité artérielle et de mortalité cardiovasculaire, chez des sujets japonais diabétiques de type 2 non obèses, mais il n'a pas observé cependant d'association significative avec le TNRF1 (19, 20). Dans la population diabétique, les concentrations plasmatiques de TNFR1 étaient associées à un risque augmenté de maladie rénale chronique, d'évènement cardiovasculaire et de mortalité totale (21, 22).

Le stress oxydatif semble également jouer un rôle dans le développement d'AOMI. Dopheide et al. a montré des concentrations augmentées en produits du stress oxydatif chez des patients souffrant de claudication intermittente ou d'ischémie critique versus un groupe contrôle (23).

Nous avons également observé dans notre présente étude une association entre des concentrations plasmatiques élevées d'IMA, marqueur du stress oxydatif, et risque de développer un évènement vasculaire périphérique. En situation d'ischémie, secondairement aux changements biologiques (acidose, stress oxydant, troubles hydro-éléctrolytiques,

hypoxémie), l’acide terminal de l’albumine, site de liaison au cobalt et au cuivre, est modifié (24). D’autres études antérieures ont rapporté des associations de l’IMA avec l’ischémie myocardique, musculaire et cérébrale, mais pas encore étudiée dans l’AOMI (25, 26).

La cohorte monocentrique française SURDIAGENE constitue une source de recherche clinique, biologique et génétique dans les complications vasculaires chez des diabétiques de type 2. Néanmoins, nos résultats ne permettent pas de tirer des conclusions étiologiques, mais renforcent l’hypothèse du rôle de l’inflammation et du stress oxydatif dans la pathogénèse de l’AOMI.

Il est aujourd’hui certain que l’HTA, le tabac, le diabète, l’hypercholestérolémie jouent un rôle majeur dans la pathogénie de l’athérosclérose. L’hypothèse de l’inflammation n’émerge qu’au XX^e siècle, et c’est R. Ross qui définit enfin l’athérosclérose comme une maladie inflammatoire, une pathologie évolutive secondaire à l’association d’une dysfonction endothéliale et une réaction inflammatoire chez des sujets à risque cardiovasculaire (27). Les facteurs de risque cardiovasculaires habituels, particulièrement tabac et diabète, favorisent le stress oxydatif et majorent les processus inflammatoires (28). Ridker et al. en 1998 dans une étude prospective chez des hommes en bonne santé a montré qu’une CRP augmentée majorait le risque d’AOMI de façon significative, indépendamment des autres facteurs de risque cardiovasculaire (29). Il est intéressant de noter que nous avons également observé dans notre présent travail une association forte et indépendante entre la CRP et les événements vasculaires périphériques dans un sous-groupe de participants ayant eu un dosage de CRP à l’inclusion. Des résultats similaires ont été rapportés dans d’autres études, notamment pour d’autres marqueurs tels la molécule d’adhésion intercellulaire 1, l’IL6 et la lipoprotéine (30). Des déterminants génétiques semblent également favoriser en partie le développement d’une AOMI, particulièrement en présence d’un polymorphisme des gènes codant pour des protéines

de l'inflammation comme la molécule d'adhésion intercellulaire 1, l'IL6, l'E. Selectine et les métalloprotéases 1 et 3 (31).

Considérées ensemble, ces données n'encouragent pas seulement l'utilisation de biomarqueurs d'inflammation pour le diagnostic d'AOMI, mais également le développement d'agents anti-inflammatoires pour traiter l'athérosclérose et les maladies cardiovasculaires. A ce titre, l'étude CANTOS (*Canakinumab antiinflammatory thrombosis outcome study*) a récemment démontré l'efficacité d'un traitement par CANAKINUMAB, anticorps monoclonal dirigé contre l'IL1 bêta (molécule impliquée dans la cascade de signalisation de l'IL6) chez 10 061 patients (dont 40% de diabétiques) avec un antécédent d'infarctus du myocarde et une CRP ultra-sensible ≥ 2 mg/l (32). Le traitement par CANAKINUMAB diminuait significativement, en comparaison au placebo, le risque d'événement cardiovasculaire majeur (infarctus du myocarde non fatal, AVC non fatal ou mortalité cardiovasculaire). En revanche, ce traitement était associé à un risque élevé d'infection sévère. Les résultats de cette étude sont extrêmement encourageants et ouvrent encore le défi de comment améliorer l'inflammation de bas grade de la maladie athéromateuse sans augmenter le risque d'évènements indésirables notamment infectieux.

PARTIE IV : CONCLUSION

L’artériopathie oblitérante des membres inférieurs est une complication vasculaire sévère et péjorative concernant les pronostics fonctionnel, cardiovasculaire et vital des patients diabétiques. Malgré la gravité et le poids socioéconomique de cette pathologie, de nombreux aspects, notamment les outils de dépistage et diagnostic, n’ont pas été suffisamment étudiés. Ce travail de thèse s’est attaché à évaluer un lien entre les concentrations plasmatiques de marqueurs de l’inflammation et du stress oxydatif et le risque de développer une AOMI dans une cohorte prospective de diabétique de type 2.

La présente étude montre une association indépendante entre les concentrations plasmatiques en TNRF1 et IMA, marqueurs de l’inflammation et du stress oxydatif, et un risque augmenté de développer une AOMI chez les patients diabétiques de type 2. Également le dosage du TNRF1 semble apporter des informations additionnelles dans la prédition de survenue d’une AOMI, suggérant un intérêt à l’intégrer dans la stratégie d’évaluation du risque de survenue d’AOMI chez les patients diabétiques.

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RESUME EN FRANÇAIS

Valeurs pronostiques de biomarqueurs d'inflammation et de stress oxydatif dans la prédition du risque de développer une artériopathie oblitérante des membres inférieurs majeure chez des sujets diabétiques de type 2

Objectif : L'inflammation et le stress oxydatif jouent un rôle important dans la physiopathologie de l'artériopathie oblitérante des membres inférieurs (AOMI). Nous avons évalué l'intérêt des concentrations plasmatiques de certains marqueurs biologiques d'inflammation et du stress oxydatif dans la prédition d'AOMI chez des patients diabétiques de type 2.

Matériel et méthode : Nous avons dosé dans les concentrations plasmatiques du récepteur au TNF-1 (TNRF-1), angiopoietin like-2, ischemia-modified albumin (IMA), produits avancés de glycation, carbonyles, et la capacité totale plasmatique d'oxydo-réduction, dans la cohorte SURDIAGENE (SURvie, DIAbete de type 2 et GENEtique). L'AOMI majeure était définie par la survenue durant le suivi d'une amputation d'un membre inférieur (mineur ou majeur) ou le recours à une procédure de revascularisation périphérique.

Résultats : Parmi 1412 patients, 112 (7,9%) sujets ont développé une AOMI au cours d'une durée médiane de suivi (25^{ème} et 75^{ème} percentiles) de 5,6 [3,0 ; 8,6] années. Nous avons observé une association indépendant entre des concentrations plasmatiques élevées en TNRF-1 (HR [IC 95%] pour le deuxième tertile comparé au premier : 1,12 [0,62– 2,03], p=0,71; troisième comparé au premier tertile : 2,16 [1,19–3,92], p=0,01) ou IMA (2,42 [1,38–4,23], p=0,002; 2,04 [1,17–3,57], p=0,01) et risque d'AOMI. Les concentrations plasmatiques de TNRF-1 améliore la prédition du risque d'AOMI majeure.

Conclusion : Ce travail est la première observation d'association indépendante entre des concentrations plasmatiques élevées en TNRF-1 et IMA et un risque augmenté d'AOMI majeure chez des diabétiques de type 2. TNRF1 a également une valeur pronostique pour le risque de développer une AOMI majeure, constituant donc un biomarqueur intéressant.

Discipline : Diabétologie, Endocrinologie et Maladies Métaboliques

Mots clés : Artériopathie oblitérante des membres inférieurs ; inflammation ; stress oxydatif ; diabète de type 2

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SERMENT D'HIPPOCRATE

Au moment d'être admise à exercer la médecine, je promets et je jure d'être fidèle aux lois de l'honneur et de la probité.

Mon premier souci sera de rétablir, de préserver ou de promouvoir la santé dans tous ses éléments, physiques et mentaux, individuels et sociaux.

Je respecterai toutes les personnes, leur autonomie et leur volonté, sans aucune discrimination selon leur état ou leurs convictions. J'interviendrais pour les protéger si elles sont affaiblies, vulnérables ou menacées dans leur intégrité ou leur dignité. Même sous la contrainte, je ne ferai pas usage de mes connaissances contre les lois de l'humanité.

J'informerai les patients des décisions envisagées, de leurs raisons et de leurs conséquences.

Je ne tromperai jamais leur confiance et n'exploiterai pas le pouvoir hérité des circonstances pour forcer les consciences.

Je donnerai mes soins à l'indigent et à quiconque me le demandera. Je ne me laisserai pas influencer par la soif du gain ou la recherche de la gloire.

Admis dans l'intimité des personnes, je tairai les secrets qui me seront confiés. Reçu à l'intérieur des maisons, je respecterai les secrets des foyers et ma conduite ne servira pas à corrompre les mœurs.

Je ferai tout pour soulager les souffrances. Je ne prolongerai pas abusivement les agonies. Je ne provoquerai jamais la mort délibérément.

Je préserverais l'indépendance nécessaire à l'accomplissement de ma mission. Je n'entreprendrai rien qui dépasse mes compétences. Je les entretiendrai et les perfectionnerai pour assurer au mieux les services qui me seront demandés.

J'apporterai mon aide à mes confrères ainsi qu'à leurs familles dans l'adversité.

*Que les hommes et mes confrères m'accordent leur estime si je suis fidèle à mes promesses ;
que je sois déshonorée et méprisée si j'y manque.*

RESUME EN FRANÇAIS

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