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

Diabetes Care 2018;41:2162–2169 | https://doi.org/10.2337/dc18-0695

Mathilde Nativel,[1] Fabrice Schneider,[2,3] Pierre-Jean Saulnier,[3,4,5] Elise Gand,[6] Stéphanie Ragot,[3,4,5] Olivier Meilhac,[7,8] Philippe Rondeau,[7] Elena Burillo,[7] Maxime Cournot,[7,9] Louis Potier,[10,11,12] Gilberto Velho,[12] Michel Marre,[10,11,12] Ronan Roussel,[10,11,12] Vincent Rigalleau,[1,13,14] Kamel Mohammedi,[1,13,14] and Samy Hadjadj[3,5,15,16]

[1]*Département d'Endocrinologie, Diabétologie, Nutrition, Hôpital Haut-Lévêque, Pessac, Bordeaux, France*
[2]*Département de Chirurgie Vasculaire, CHU de Poitiers, Poitiers, France*
[3]*UFR de Médecine et Pharmacie, Université de Poitiers, Poitiers, France*
[4]*Centre d'Investigation Clinique, CHU de Poitiers, Poitiers, France*
[5]*CIC 1402, INSERM, Poitiers, France*
[6]*Pôle Dune, CHU de Poitiers, Poitiers, France*
[7]*UMR 1188 Diabète athérothrombose Thérapies Réunion Océan Indien (DéTROI), INSERM, Université de La Réunion, Saint Denis de La Réunion, France*
[8]*CHU de La Réunion, Saint Denis de La Réunion, France*
[9]*Service de cardiologie, Centre Hospitalier Gabriel Martin, Saint-Paul, France*
[10]*DHU FIRE, Département d'Endocrinologie, Diabétologie, Nutrition, Assistance Publique Hôpitaux de Paris, Bichat Hospital, Paris, France*
[11]*UFR de Médecine, Université Paris Diderot, Sorbonne Paris Cité, Paris, France*
[12]*UMRS 1138, Centre de Recherche des Cordeliers, INSERM, Paris, France*
[13]*Faculté de Médecine, Université de Bordeaux, Bordeaux, France*
[14]*Centre de Recherche INSERM-Université de Bordeaux U1219 "Bordeaux Population Health," Bordeaux, France*
[15]*Département d'Endocrinologie, Diabétologie, Nutrition, CHU de Poitiers, Poitiers, France*
[16]*Research Unit 1082, INSERM, Poitiers, France*

Corresponding author: Kamel Mohammedi, km.mmohammedi@gmail.com.

Received 29 March 2018 and accepted 1 June 2018.

This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc18-0695/-/DC1.

K.M. and S.H. share equal authorship.

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, Diabete de type 2 et Genetique) 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.

PATHOPHYSIOLOGY/COMPLICATIONS

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, Diabete de Type 2 et Genetique (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. Nondiabetic 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 m^2. 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

		Major LEAD		
	Overall	No	Yes	*P* value
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

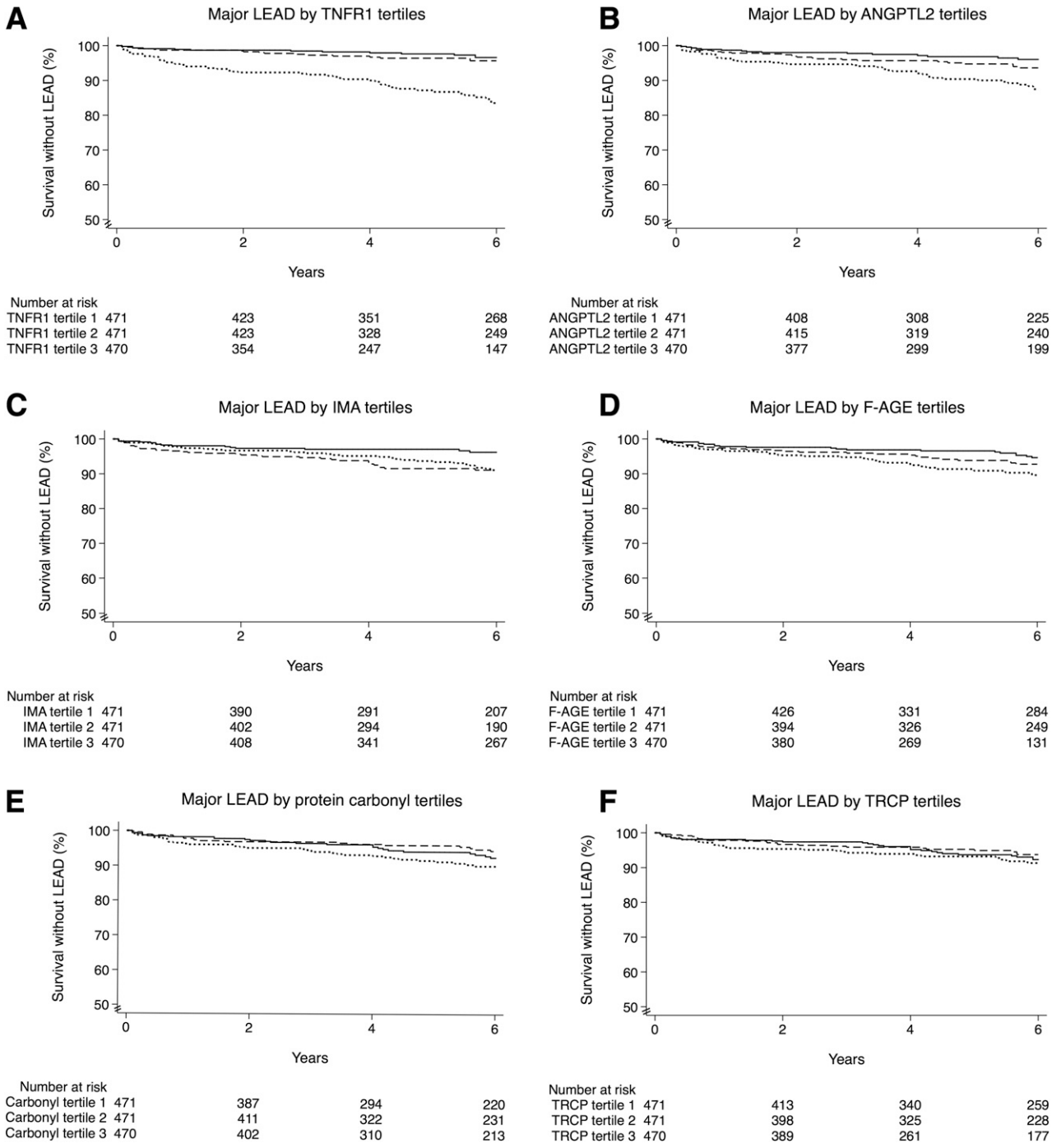

Figure 1—Major LEAD during follow-up by plasma concentrations of inflammatory and oxidative stress biomarkers at baseline. Survival without major LEAD in T3 (dotted line) and T2 (dashed line) compared with T1 (solid line) of TNFR1 ($P < 0.0001$) (*A*), ANGPTL2 ($P < 0.0001$) (*B*), IMA ($P = 0.002$)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

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).

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,

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.

Acknowledgments. The authors thank all the patients participating in the SURDIAGENE cohort and Elodie Migault (CIC 1402, INSERM, Poitiers, France). The staff of the diabetes department in Poitiers Hospital is acknowledged for help with data collection and monitoring. The authors also thank Sonia Brishoual (Biological Resources Center, BRC BB-0033-00068, Poitiers, France) for biological determinations, Eric Thorin and Nathalie Trescasses (Montreal Heart Institute) for ANGPTL2 determination, and Alexandre Pavy and Marie-Claire Pasquier (Information Technology Department, CHU de Poitiers, Poitiers, France).

Funding. The SURDIAGENE study was supported by grants from the French Ministry of Health (PHRC Poitiers 2004, PHRC Interregional 2008), Association Française des Diabétiques (Research grant 2003), and Groupement pour l'Étude des Maladies Métaboliques et Systémiques (GEMMS Poitiers, France). These organizations did not participate in the data analyses and the publication process.

Duality of Interest. Assays for TNFR1 were partially supported by EKF Diagnostics. F.S. reports personal fees from Bard Company; Gore Medical, Vascular Division; Medtronic; Maquet-Getinge Group; Boston Scientific; and Terumo outside the submitted work. L.P. reports grants and personal fees from Sanofi and personal fees from Eli Lilly, Novo Nordisk, and Servier outside the submitted work. K.M. reports personal fees and nonfinancial support from Novo Nordisk and Sanofi and nonfinancial support from VitalAire outside the submitted work. S.H. reports grants, personal fees, and nonfinancial support from AstraZeneca, Boehringer Ingelheim, Eli Lilly, Janssen, MSD, Novartis, Novo Nordisk, Sanofi, Servier, Takeda, and Valbiotis outside the submitted work. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. M.N., F.S., P.-J.S., E.G., S.R., O.M., P.R., E.B., M.C., L.P., G.V., M.M., R.R., V.R., K.M., and S.H. approved the final version of the manuscript. M.N., K.M., and S.H. designed the study and researched data. M.N. and S.H. reviewed the manuscript. F.S., P.-J.S., E.G., S.R., L.P., G.V., M.M., R.R., and V.R. contributed to the discussion and reviewed the manuscript. O.M., P.R., E.B., and M.C. researched data and reviewed the manuscript. K.M. drafted the manuscript. K.M. and S.H. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analyses.

References

1. Fowkes FG, Rudan D, Rudan I, et al. Comparison of global estimates of prevalence and risk factors for peripheral artery disease in 2000 and 2010: a systematic review and analysis. Lancet 2013;382:1329–1340

2. Joosten MM, Pai JK, Bertoia ML, et al. Associations between conventional cardiovascular risk factors and risk of peripheral artery disease in men. JAMA 2012;308:1660–1667

3. Shah AD, Langenberg C, Rapsomaniki E, et al. Type 2 diabetes and incidence of cardiovascular diseases: a cohort study in 1·9 million people. Lancet Diabetes Endocrinol 2015;3:105–113

4. Boyko EJ, Seelig AD, Ahroni JH. Limb- and person-level risk factors for lower-limb amputation in the Prospective Seattle Diabetic Foot Study. Diabetes Care 2018;41:891–898

5. Mohammedi K, Woodward M, Hirakawa Y, et al.; ADVANCE Collaborative Group. Presentations of major peripheral arterial disease and risk of major outcomes in patients with type 2

diabetes: results from the ADVANCE-ON study. Cardiovasc Diabetol 2016;15:129
6. Mohammedi K, Woodward M, Zoungas S, et al.; ADVANCE Collaborative Group. Absence of peripheral pulses and risk of major vascular outcomes in patients with type 2 diabetes. Diabetes Care 2016;39:2270–2277
7. Mohammedi K, Woodward M, Hirakawa Y, et al.; ADVANCE Collaborative Group. Microvascular and macrovascular disease and risk for major peripheral arterial disease in patients with type 2 diabetes. Diabetes Care 2016;39:1796–1803
8. Mahoney EM, Wang K, Cohen DJ, et al.; REACH Registry Investigators. One-year costs in patients with a history of or at risk for atherothrombosis in the United States. Circ Cardiovasc Qual Outcomes 2008;1:38–45
9. Marrett E, DiBonaventura Md, Zhang Q. Burden of peripheral arterial disease in Europe and the United States: a patient survey. Health Qual Life Outcomes 2013;11:175
10. Weber C, Noels H. Atherosclerosis: current pathogenesis and therapeutic options. Nat Med 2011;17:1410–1422
11. Stocker R, Keaney JF Jr. New insights on oxidative stress in the artery wall. J Thromb Haemost 2005;3:1825–1834
12. Dopheide JF, Obst V, Doppler C, et al. Phenotypic characterisation of pro-inflammatory monocytes and dendritic cells in peripheral arterial disease. Thromb Haemost 2012;108:1198–1207
13. Dopheide JF, Doppler C, Scheer M, et al. Critical limb ischaemia is characterised by an increased production of whole blood reactive oxygen species and expression of TREM-1 on neutrophils. Atherosclerosis 2013;229:396–403
14. Saulnier PJ, Gand E, Ragot S, et al.; SURDIAGENE Study Group. Association of serum concentration of TNFR1 with all-cause mortality in patients with type 2 diabetes and chronic kidney disease: follow-up of the SURDIAGENE Cohort. Diabetes Care 2014;37:1425–1431
15. Gellen B, Thorin-Trescases N, Sosner P, et al. ANGPTL2 is associated with an increased risk of cardiovascular events and death in diabetic patients. Diabetologia 2016;59:2321–2330
16. Saulnier PJ, Gand E, Velho G, et al.; SURDIAGENE Study Group. Association of circulating biomarkers (adrenomedullin, TNFR1, and NT-proBNP) with renal function decline in patients with type 2 diabetes: a French prospective cohort. Diabetes Care 2017;40:367–374
17. Cournot M, Burillo E, Saulnier PJ, et al. Circulating concentrations of redox biomarkers do not improve the prediction of adverse cardiovascular events in patients with type 2 diabetes mellitus. J Am Heart Assoc 2018;7:e007397
18. Hadjadj S, Fumeron F, Roussel R, et al.; DIABHYCAR Study Group; DIAB2NEPHROGENE Study Group; SURDIAGENE Study Group. Prognostic value of the insertion/deletion polymorphism of the ACE gene in type 2 diabetic subjects: results from the Non-insulin-dependent Diabetes, Hypertension, Microalbuminuria or Proteinuria, Cardiovascular Events, and Ramipril (DIABHYCAR), Diabete de type 2, Nephropathie et Genetique (DIAB2NEPHROGENE), and Survie, Diabete de type 2 et Genetique (SURDIAGENE) studies. Diabetes Care 2008;31:1847–1852
19. Pencina MJ, D'Agostino RB Sr., D'Agostino RB Jr., Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med 2008;27:157–172; discussion 207–112
20. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc 1999;94:496–509
21. Fiotti N, Giansante C, Ponte E, et al. Atherosclerosis and inflammation. Patterns of cytokine regulation in patients with peripheral arterial disease. Atherosclerosis 1999;145:51–60
22. Pande RL, Brown J, Buck S, et al. Association of monocyte tumor necrosis factor α expression and serum inflammatory biomarkers with walking impairment in peripheral artery disease. J Vasc Surg 2015;61:155–161
23. Murabito JM, Keyes MJ, Guo CY, et al. Cross-sectional relations of multiple inflammatory biomarkers to peripheral arterial disease: the Framingham Offspring Study. Atherosclerosis 2009;203:509–514
24. Carlsson AC, Östgren CJ, Nystrom FH, et al. Association of soluble tumor necrosis factor receptors 1 and 2 with nephropathy, cardiovascular events, and total mortality in type 2 diabetes. Cardiovasc Diabetol 2016;15:40
25. Brevetti G, Giugliano G, Brevetti L, Hiatt WR. Inflammation in peripheral artery disease. Circulation 2010;122:1862–1875
26. Ridker PM, Stampfer MJ, Rifai N. Novel risk factors for systemic atherosclerosis: a comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease. JAMA 2001;285:2481–2485
27. Pradhan AD, Shrivastava S, Cook NR, Rifai N, Creager MA, Ridker PM. Symptomatic peripheral arterial disease in women: nontraditional biomarkers of elevated risk. Circulation 2008;117:823–831
28. Cotran RS, Pober JS. Cytokine-endothelial interactions in inflammation, immunity, and vascular injury. J Am Soc Nephrol 1990;1:225–235
29. Marucha PT, Zeff RA, Kreutzer DL. Cytokine-induced IL-1 beta gene expression in the human polymorphonuclear leukocyte: transcriptional and post-transcriptional regulation by tumor necrosis factor and IL-1. J Immunol 1991;147:2603–2608
30. Feingold KR, Serio MK, Adi S, Moser AH, Grunfeld C. Tumor necrosis factor stimulates hepatic lipid synthesis and secretion. Endocrinology 1989;124:2336–2342
31. Kawakami M, Pekala PH, Lane MD, Cerami A. Lipoprotein lipase suppression in 3T3-L1 cells by an endotoxin-induced mediator from exudate cells. Proc Natl Acad Sci U S A 1982;79:912–916
32. Esposito K, Nappo F, Marfella R, et al. Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. Circulation 2002;106:2067–2072
33. Ohgushi M, Taniguchi A, Fukushima M, et al. Soluble tumor necrosis factor receptor 2 is independently associated with pulse wave velocity in nonobese Japanese patients with type 2 diabetes mellitus. Metabolism 2007;56:571–577
34. Suzuki E, Kashiwagi A, Nishio Y, et al. Increased arterial wall stiffness limits flow volume in the lower extremities in type 2 diabetic patients. Diabetes Care 2001;24:2107–2114
35. Shen XL, Lin CJ, Han LL, Lin L, Pan L, Pu XD. Assessment of ischemia-modified albumin levels for emergency room diagnosis of acute coronary syndrome. Int J Cardiol 2011;149:296–298
36. Refaai MA, Wright RW, Parvin CA, Gronowski AM, Scott MG, Eby CS. Ischemia-modified albumin increases after skeletal muscle ischemia during arthroscopic knee surgery. Clin Chim Acta 2006;366:264–268
37. Abboud H, Labreuche J, Meseguer E, et al. Ischemia-modified albumin in acute stroke. Cerebrovasc Dis 2007;23:216–220
38. Chan B, Dodsworth N, Woodrow J, Tucker A, Harris R. Site-specific N-terminal auto-degradation of human serum albumin. Eur J Biochem 1995;227:524–528
39. Bhardwaj A, Truong QA, Peacock WF, et al. A multicenter comparison of established and emerging cardiac biomarkers for the diagnostic evaluation of chest pain in the emergency department. Am Heart J 2011;162:276–282.e1
40. Horio E, Kadomatsu T, Miyata K, et al. Role of endothelial cell-derived Angptl2 in vascular inflammation leading to endothelial dysfunction and atherosclerosis progression. Arterioscler Thromb Vasc Biol 2014;34:790–800