Inflammatory Cytokines Associated With Failure of Lower-Extremity Endovascular Revascularization (LER): A Prospective Study of a Population With Diabetes

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OBJECTIVE

Peripheral artery disease (PAD) is one of the most relevant complications of diabetes. Although several pharmacological and revascularization approaches are available for treating patients with diabetes and PAD, an endovascular approach is often associated with postprocedural complications that can increase the risk for acute limb ischemia or amputation. However, no definitive molecular associations have been described that could explain the difference in outcomes after endovascular treatment in patients with diabetes, PAD, and chronic limb-threatening ischemia (CLTI).

RESEARCH DESIGN AND METHODS

We evaluated the relationship between the levels of the main cytokines associated with diabetic atherosclerosis and the outcomes after endovascular procedures in patients with diabetes, PAD, and CLTI.

RESULTS

A total of 299 patients with below-the-knee occlusive disease who were undergoing an angioplasty procedure were enrolled. The levels of key cytokines—osteoprotegerin (OPG), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP)—were measured, and major adverse limb events (MALE) and major adverse cardiovascular events (MACE) were assessed 1, 3, 6, and 12 months after the procedure. There was a linear trend from the lowest to the highest quartile for each cytokine at baseline and incident MALE. A linear association was also observed between increasing levels of each cytokine and incident MACE. Receiver operating characteristics models were constructed using clinical and laboratory risk factors, and the inclusion of cytokines significantly improved the prediction of incident events.

CONCLUSIONS

We demonstrated that elevated OPG, TNF-α, IL-6, and CRP levels at baseline correlate with worse vascular outcomes in patients with diabetes, PAD, and CLTI undergoing an endovascular procedure.

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Diabetes will affect about 366 million people by 2030 (1). Among the complications of diabetes, peripheral artery disease (PAD) is one of the most relevant (2). Patients with symptomatic PAD of the lower limbs can have claudication upon exertion, but at advanced stages, chronic critical leg ischemia can manifest as ischemic pain at rest and cutaneous ulcers leading to limb amputation (3). Diabetes is associated with aggressive below-the-knee PAD and chronic limb-threatening ischemia (CLTI) (4,5). Furthermore, patients with diabetes and PAD have a higher risk of major amputation than patients without diabetes, with rates ~10 times higher (1). Several pathological mechanisms have been proposed in diabetic PAD (6).

In patients with diabetes, tight glycemic control has not reduced major cardiovascular events (MACE), but good metabolic control might be associated with better outcomes in patients undergoing endovascular procedures (7). Endovascular therapy includes balloon angioplasty with or without stenting and atherectomy (8). The success of endovascular treatment depends on many factors, such as the presence of comorbidities, associated risk factors, concomitant medical treatments, and the localization and extension of atherosclerotic lesions (9). However, patients with similar clinical features and endovascular approaches might experience completely different procedural outcomes (10).

Several mechanisms have been proposed to explain the different outcomes of similar endovascular interventions. For instance, in a retrospective analysis high fasting blood glucose (FBG) during an endovascular procedure was associated with a loss of primary patency (7). Among the proinflammatory cytokines, C-reactive protein (CRP) was associated with restenosis after percutaneous transluminal angioplasty of the arteries of the lower limbs (11). Higher CRP levels were also measured in patients with diabetes who underwent major amputation after percutaneous transluminal angioplasty of the lower limb (12). Furthermore, interleukin (IL)-6, tumor necrosis factor (TNF)-α, and CRP were associated with 1-year mortality in subjects with CLTI (13). Given these data, we hypothesize that the different outcomes after endovascular treatment might depend, at least in part, on the basal profile of the cytokines involved in the inflammatory and atherosclerotic processes.

This study describes the relationship between the level of the main cytokines involved in diabetic atherosclerosis and the correlation of inflammatory biomarkers at baseline with outcomes after endovascular procedures in patients with diabetes, PAD, and CLTI. In particular, we studied TNF-α, IL-6, CRP, and osteoprotegerin (OPG) levels.

RESEARCH DESIGN AND METHODS

Study Design

The study was approved by the ethics committee of Fondazione Policlinico Universitario A. Gemelli Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) and adhered to the principles of the Declaration of Helsinki. All the individuals agreed to participate in the study and gave informed consent. This clinical protocol was designed as a prospective nonrandomized study to verify the relationship between inflammatory cytokine levels and the incidence of restenosis or occlusion after endovascular revascularization performed in patients with diabetes, PAD, and CLTI.

Clinical Assessment and Endovascular Procedures

We analyzed consecutive patients with diabetes, PAD, and CLTI admitted to the Department of Vascular Diseases of Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy, from 1 October 2015 to 30 January 2018.

Of 378 individuals evaluated, 299 had CLTI below the knee and were enrolled and followed for the entire duration of the study. Inclusion criteria were age 40 years or older, diagnosis of type 2 diabetes received at least 1 year earlier, an ankle/arm pressure index lower than 0.80, substantial peripheral artery stenosis (>50%) documented by duplex ultrasound, presence of PAD with a Rutherford category of 4 or 5, presence of CLTI deserving of endovascular treatment, and no infection present or within the previous month. When foot ulcers were present, additional criteria were no local signs of infection and no need for topical or systemic antibiotic therapy. In particular, the Wound, Ischemia, and foot Infection (WIfI) classification system was used to stratify patients with diabetic foot ulcers, as previously described (14,15). When indicated, radiological examinations were performed to exclude osteomyelitis. Other exclusion criteria included ongoing use of systemic steroids or a history of use in the previous 3 months, suspected or known pregnancy, absolute contraindication to endovascular treatment, lower-limb endovascular treatment or lower-limb bypass surgery within the past 3 months, diabetic peripheral neuropathy, need for ongoing oral anticoagulant therapy, life expectancy <6 months, known liver disease with a functional status according to Child-Pugh classification B or above, congenital or acquired thrombophilia (e.g., activated protein C resistance, antiphospholipid antibodies syndrome), and active autoimmune disease. Subjects who met all inclusion criteria were included in the study.

The following data were also collected: history of hypertension, dyslipidemia, obesity, tobacco use, renal insufficiency (defined as an estimated glomerular filtration rate <60 mL/min), and atherosclerotic lesion description (location, severity). PAD was diagnosed in accordance with the criteria established by the Ad Hoc Committee on Reporting Standards of the Society for Vascular Surgery and the International Society for Cardiovascular Surgery (16). All patients underwent bilateral high-resolution B-mode ultrasonography (Acuson 128XP/10 color Doppler ultrasound system; Acuson, Mountain View, CA). In patients with an ankle-brachial pressure index ≥1.40 (suspected medial calcification), duplex ultrasound was also performed to exclude hemodynamically substantial stenosis of the peripheral arteries (8,17,18). CLTI was defined as persistent pain at rest and an ankle systolic pressure <50 mmHg, and/or ulceration, gangrene, or nonhealing wounds on the foot (19).

All patients underwent an evaluation in order to rule out a peripheral diabetic neuropathy. Vibration perception threshold was assessed using a biothesiometer, according to the procedure described by Young et al. (20). All subjects had a definite diagnosis of peripheral neuropathy, with a Neuropathy Disability Score >5 (21) and a pathological conduction velocity. Autonomic neuropathy was diagnosed according to the standardized procedure of Ewing and Clarke (22), including four cardiovascular autonomic tests.

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For all patients the lipid profile was evaluated and aggressive lipid-lowering therapy was recommended with a goal of LDL cholesterol <1.8 mmol/L (<70 mg/dL).

At the time of enrollment all patients were on aspirin therapy, and after the endovascular procedure they took dual antiplatelet therapy (aspirin and clopidogrel) for 1 month.

Endovascular Revascularization Strategies and Follow-up Evaluation

Balloon angioplasty, a stenting procedure, or both were performed according to standard techniques as previously described (23). Dilatation of a stenotic vessel was considered successful if the residual stenosis of the lumen diameter was less than 30%. We assessed complications of the intervention according to definitions from the Society of Interventional Radiology (24) and found no major complications. Only eight minor complications (three hematomas at the puncture site and five pseudoaneurysms) occurred, and no additional procedures were necessary. Of the 323 patients who underwent the procedure, 24 (7.4%) did not have a successful procedure and were excluded from the study. Technical success of the procedure was defined as anatomical and clinical improvements, as previously described (24). For the follow-up assessment, 299 patients were also evaluated 1, 3, 6, and 12 months after the procedure.

Blood Sampling Procedures and Biochemical Assays

For every patient, serum creatinine, glycated hemoglobin (HbA_{1c}), FBG, cholesterol, triglycerides, LDL, and white blood cell count were determined. Blood sampling was performed at baseline after an overnight fast and again just before the endovascular procedure. Blood samples were stored at −80°C until assayed, and they were centrifuged to separate and treat the serum. We determined CRP levels in these blood samples using a high-sensitivity ELISA kit (Biocheck Laboratories, Toledo, OH). We used a monoclonal mouse antihuman OPG antibody as a capture antibody and a biotinylated polyclonal goat antihuman OPG antibody for detection. The intra- and interassay coefficients of variation were 3.8% and 10.2%, respectively. The sensitivity, defined as the mean ± 3 SD of the zero standard, was calculated to be 0.15 pmol/mL. Using a Quantikine ELISA Kit (R&D Systems, Minneapolis, MN), we assessed IL-6 and TNF-α levels. In each patient, the assay was performed twice, and the results were averaged.

Definition of Outcomes

Every patient was evaluated at 1, 3, 6, and 12 months, and incident outcomes were assessed. The major adverse limb events (MALE) outcome was defined as a composite of acute limb ischemia, major vascular amputations (above the ankle), and limb-threatening ischemia leading to urgent revascularization. The MACE outcome was defined as composite of myocardial infarction, stroke, and cardiovascular death.

Statistical Analysis

Data were summarized as means (SDs) for continuous variables and counts (percentages) for categorical variables. We analyzed simple associations between cytokines and other clinical and laboratory parameters using simple linear regression with each cytokine as a dependent variable. To allow comparisons across cytokines, we report results as standardized β values.

We assessed the association between each cytokine and the risk of developing the outcomes of interest (incident MALE and MACE) by means of logistic regression models in which the outcome of interest was entered as the dependent variable and each cytokine was entered as a continuous independent variable. We used such models to obtain univariate and multivariate-adjusted odds ratios and 95% CIs for the outcome of interest associated with a 1-unit change of each cytokine. The multivariate-adjusted models included age, sex, BMI, high blood pressure, diabetes duration, smoking status (current, former, never), Rutherford staging, previous cardiovascular and cerebrovascular events, treatment (oral antidiabetic agents, insulin), total cholesterol, LDL cholesterol, triglycerides, FBG, and HbA_{1c}. We also stratified patients into quartiles according to serum levels of each cytokine, and we calculated the proportion of incident events occurring in each quartile. We then performed a statistical test for trend by including quartiles as a continuous variable in logistic regression models adjusted for the covariates listed above. Because patients in some quartiles did not experience any incident events, it was not possible to calculate odds ratios associated with the individual quartiles.

We assessed whether knowledge of the cytokine values would lead to improved prognostic prediction regarding the outcomes of interest by constructing receiver operating characteristic (ROC) curves for a model including only traditional risk factors (age, sex, BMI, high blood pressure, diabetes duration, smoking status, Rutherford staging, previous cardiovascular and cerebrovascular events, treatment, total cholesterol, LDL cholesterol, triglycerides, FBG, HbA_{1c}) and for a model including all the aforementioned risk factors plus all four cytokines as continuous variables. We then compared the areas under the ROC curves using the roccomp function in Stata software; this function tests the equality of two ROC areas obtained by applying two or more test modalities to the same sample or to independent samples (25). All statistical analyses were performed separately for each of the two outcomes of interest.

For all analyses, a two-tailed P value <0.05 was considered to be statistically significant. All analyses were performed with Stata software version 15.1 (StataCorp).

RESULTS

Baseline Characteristics of the Study Population

A selected population of 299 patients with diabetes and below-the-knee CLTI was enrolled in the study; their baseline characteristics are reported in Table 1. The mean (SD) age of the study population was 73.0 (5.1) years, and 51.2% were men.

Univariate associations between each cytokine and selected clinical and laboratory parameters are reported in Table 2. All cytokines were directly associated with age, previous coronary artery and cerebrovascular disease, and current smoking.

Cytokine Values and Risk of MALE at 12 Months

During 12 months of follow-up, 133 patients (44.5%) experienced a MALE. The association between each cytokine at baseline and the risk of MALE is reported in Table 3 and Fig. 1*A*. We observed a significant and independent linear trend from the lowest to the highest quartile

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Table 1—Demographic characteristics of the study cohort (n = 299) at baseline

Age, years	73.0 (5.1)
Male sex	153 (51.2%)
Diabetes duration, years	11.3 (1.9)
BMI, kg/m^2	30.2 (1.8)
Taking oral antidiabetic agents	55 (18.4%)
Taking insulin	195 (65.2%)
High blood pressure	141 (47.2%)
Smoking status	
Never smoked	71 (23.7%)
Past smoker	110 (36.8%)
Current smoker	118 (39.5%)
Ankle systolic blood pressure, mmHg	41.9 (4.1)
Ankle-brachial pressure index	0.34 (0.05)
Rutherford staging	
Stage 4	142 (47.5%)
Stage 5	157 (52.5%)
WIfI classification	
010	89 (29.8%)
020	53 (17.7%)
110	102 (34.1%)
120	55 (18.4%)
Previous coronary artery disease	140 (46.8%)
Previous cerebrovascular disease	64 (21.4%)
Total cholesterol, mg/dL	215.6 (19.1)
LDL cholesterol, mg/dL	110.0 (14.3)
Triglycerides, mg/dL	207.5 (11.1)
Glucose, mg/dL	137.7 (26.5)
HbA_{1c}, %	8.3 (2.2)
HbA_{1c}, mmol/mol	67.0 (1.1)
Total white blood cells ($\times 10^9$/L)	6.78 (2.8)
OPG, pmol/L	5.9 (2.3)
TNF-α, pg/mL	46.6 (18.8)
IL-6, pg/mL	36.3 (10.5)
CRP, mg/L	5.4 (2.5)

Data are reported as the mean (SD) for continuous variables and number (percentage) for categorical variables. WIfI, wound, ischemia, foot infection.

for each cytokine and the risk of MALE, as opposed to none in the lowest quartiles. Interestingly, these outcome differences were independent of glycemic control. In fact, FBG and HbA_{1c} did not correlate with any of the outcomes considered. Associations between each cytokine and MALE were similar for participants with and those without nonhealing ulcer (Supplementary Table 1).

Cytokine Values and Risk of MACE at 12 Months

There were 82 MACE (27.4%) during 12 months of follow-up. As for MALE, we found a significant linear association between the lowest and highest quartiles of each cytokine and risk of MACE; in particular, we observed a significant linear association between increasing levels of each cytokine and incident MACE among patients in the highest quartiles of OPG, TNF-α, IL-6, and CRP over 12 months of follow-up. The trend remained statistically significant even after adjusting for other risk factors (Table 4 and Fig. 1*B*). Associations between each cytokine and MACE were similar for participants with and those without nonhealing ulcer (Supplementary Table 2).

Improvement in the Prediction of Events After Adding Cytokine Values to Established Clinical and Laboratory Risk Factors

The baseline ROC model included age, sex, BMI, high blood pressure, diabetes duration, smoking status, Rutherford staging, previous cardiovascular and cerebrovascular events, treatment, total cholesterol, LDL cholesterol, triglycerides, FBG, and HbA_{1c}. The comparison of ROC curves between the baseline model with only clinical and laboratory predictors and the model including all four cytokines is reported in Fig. 1*C* for MALE and Fig. 1*D* for MACE. In both cases, including cytokines significantly improved the prediction of incident events: for MALE, the area under the ROC curve was 0.85 (95% CI 0.81, 0.90) for the baseline model and 1.00 (95% CI 1.00, 1.00) for the model with cytokines ($P < 0.001$); for MACE, the area under the ROC curve was 0.76 (95% CI 0.70, 0.82) for the baseline model and 0.91 (95% CI 0.87, 0.94) for the model with cytokines ($P < 0.001$).

CONCLUSIONS

In this study, we assessed the relationship between the levels of the main inflammatory cytokines involved in diabetic atherosclerosis and cardiovascular and limb outcomes after endovascular revascularization in patients with diabetes, PAD, and CLTI. Although inflammation certainly plays a role in the complications of diabetes, and diabetes itself creates a chronic, low-grade inflammatory status per se (6), a relationship was not demonstrated between markers of inflammation and incidence of MALE or MACE after an endovascular procedure. There are convincing data that show that the inflammatory state just preceding coronary angioplasty is related to the medium- and long-term results and to the incidence of new stenosis (26). There are also data regarding markers of inflammation and endovascular procedure outcomes in patients with PAD. In a case-control study of 14 patients—some of whom had and some of whom did not have diabetes—the authors showed no single plasma protein to be correlated with 1-year outcomes (27). However, the effect of the procedure itself on the levels of inflammatory markers has not yet been clarified. Moreover, it is possible that the inflammatory proteins are an expression of the pathological process that occurs in the arterial wall, but at the same time, these circulating cytokines could be dependent on the stress induced by the placement and ballooning of the stent, especially in the case of prolonged maneuvers and of long arterial stenosis (26,28,29). Similarly, Bleda et al. (30) analyzed the possible association between inflammatory markers before endovascular intervention and during 1-year follow-up and its variation during the study period. They found a significant

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Table 2—Univariate associations between cytokines and other parameters

	OPG	TNF-α	IL-6	CRP
Age				
Standardized β	0.20	0.22	0.21	0.19
P value	<0.001	<0.001	<0.001	0.001
Male sex				
Standardized β	0.09	0.11	0.08	0.08
P value	0.12	0.06	0.18	0.16
Coronary artery disease				
Standardized β	0.22	0.19	0.21	0.20
P value	<0.001	0.001	<0.001	<0.001
Cardiovascular disease				
Standardized β	0.41	0.30	0.31	0.37
P value	<0.001	<0.001	<0.001	<0.001
Former smoker				
Standardized β	0.22	0.12	0.12	0.20
P value	0.002	0.08	0.11	0.006
Current smoker				
Standardized β	0.26	0.30	0.28	0.31
P value	<0.001	<0.001	<0.001	<0.001
FBG				
Standardized β	0	0.02	0.05	0
P value	0.97	0.67	0.43	0.95
HbA_{1c}				
Standardized β	0	0	−0.03	0.02
P value	0.95	0.93	0.63	0.78

relationship between basal levels of inflammatory markers and the incidence of reintervention, cardiovascular events, and death. Furthermore, Stone et al. (31) found that elevated preprocedural CRP levels are associated with MALE and late cardiovascular events after lower-extremity endovascular interventions. Here, we demonstrated a correlation between OPG, TNF-α, IL-6, and CRP levels at baseline before the procedure and endovascular outcomes in patients with diabetes and PAD who undergo endovascular revascularization. In particular, we found a significant linear trend between increasing levels of each cytokine and the risk of MALE in this subset of patients with diabetes. Moreover, there was a significant association between increasing levels of OPG, TNF-α, IL-6, and CRP and the risk of MACE. The use of the ROC curves

Table 3—Association between cytokines and MALE

	OPG	TNF-α	IL-6	CRP
First quartile				
Range	1.3–4.0	12.3–28.4	12.2–27.3	1.0–3.1
Numbers of events/patients (%)	0/74 (0)	0/74 (0)	0/73 (0)	0/74 (0)
Second quartile				
Range	4.1–5.8	28.4–49.8	27.3–38.8	3.1–5.3
Numbers of events/patients (%)	6/75 (8.0)	0/75 (0)	0/76 (0)	0/64 (0)
Third quartile				
Range	5.9–7.5	50.3–64.4	38.9–45.7	5.4–7.5
Numbers of events/patients (%)	55/75 (73.3)	58/74 (78.4)	60/75 (80.0)	58/83 (69.9)
Fourth quartile				
Range	7.5–12.0	64.4–74.5	45.7–56.5	7.5–10.6
Numbers of events/patients (%)	72/75 (96.0)	75/76 (98.7)	73/75 (97.3)	75/78 (96.2)
P value for trend				
Univariate	<0.001	<0.001	<0.001	<0.001
Multivariate	<0.001	<0.001	<0.001	<0.001

Multivariate models were adjusted for age, sex, BMI, high blood pressure, diabetes duration, smoking status, Rutherford staging, previous cardiovascular and cerebrovascular events, treatment, total cholesterol, LDL cholesterol, triglycerides, FBG, and HbA_{1c}.

definitively demonstrated the predictive and independent power of these cytokines in foreseeing MALE or MACE after endovascular treatment. Our findings were similar for patients with and patients without nonhealing ulcer, although the limited sample size of the subgroups limited more detailed analyses.

Many possible explanations underlie the evidence from this study. In fact, OPG is a well-known marker of atherosclerosis in patients with diabetes (32–35) because of both its role in calcium metabolism and its direct interaction with the vascular endothelium. Moreover, there is increasing evidence that TNF-α plays a pivotal role in many pathophysiological steps of diabetic atherosclerosis (36). Furthermore, IL-6 inhibits nitric oxide production in endothelial cells, with consequent oxidative stress, and this effect is directly dependent on TNF-α activity. In addition, the indirect data obtained from the patients treated with the IL-6 inhibitor tocilizumab confirm that reducing the activity of IL-6 has positive effects on endothelial function and aortic stiffness (37). Finally, alongside the detrimental activities that CRP shares with TNF-α and IL-6, CRP has a multifaceted set of peculiar effects that induce and worsen the vascular dysfunction in patients with diabetes. Indeed, CRP enhances foam cell formation of atherosclerotic plaque and promotes platelet adhesion (38). Given previous data and the results of our study, it is possible to hypothesize that the presence of elevated OPG, TNF-α, IL-6, and CRP values before an endovascular procedure identifies a subset of patients who have more aggressive disease and are more susceptible to a worse outcome after the procedure.

One unmet clinical need in managing patients with diabetes who have PAD is identifying of biomarkers that may predict the outcomes of endovascular treatment. Biomarkers are available to identify diabetic pathology and PAD, such as CRP, IL-6, β_2-microglobulin, TNF-α, and serum amyloid A (39), but none are available for use in patients with diabetes, PAD, and CLTI. In this study, we identified a panel of easily accessible and reproducible laboratory parameters that are able to predict with reasonable certainty the possibility that the treated patient will face the complication.

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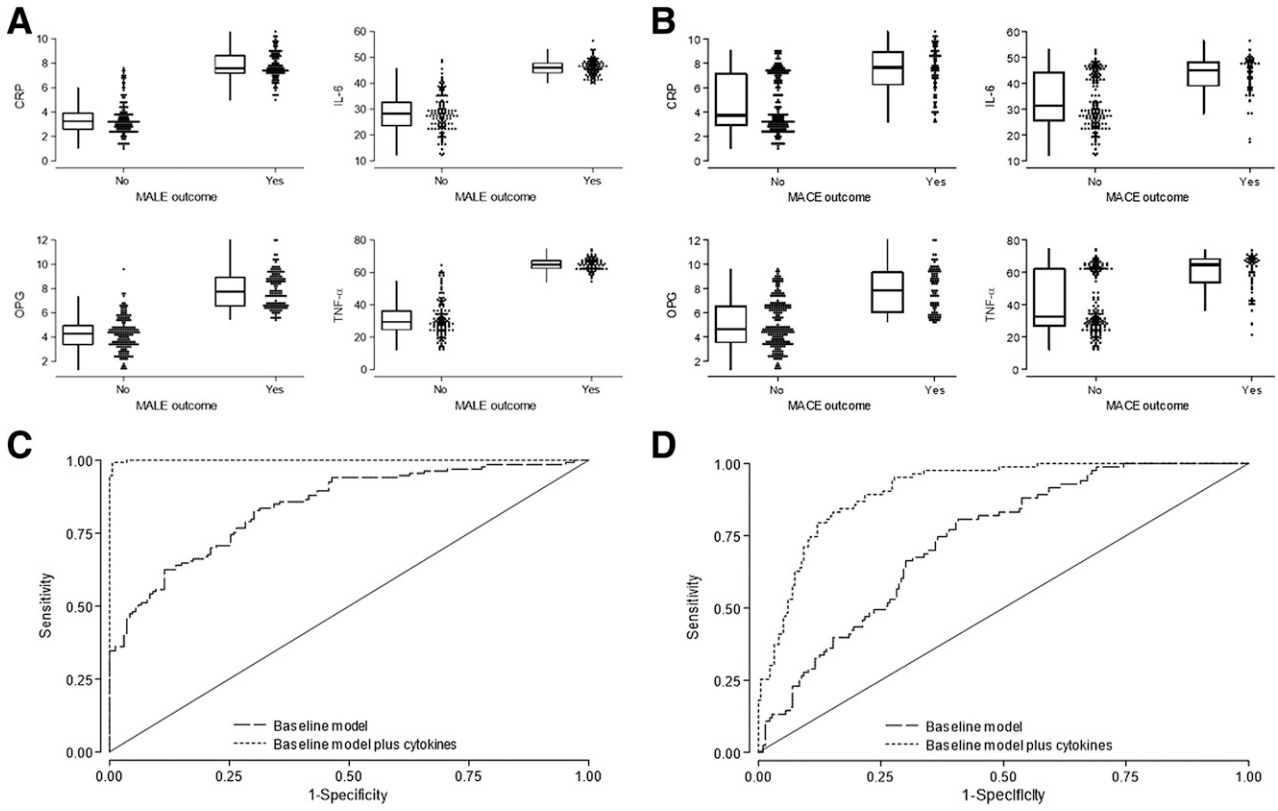

Figure 1—*A* and *B*: Box plots and raw data points for levels of CRP, IL-6, OPG, and TNF-α in patients with and patients without MALE (*A*) and those with and those without MACE (*B*). On the box plots, horizontal line represents the median, the length of the box represents the interquartile range, and the vertical lines extend to values within 1.5 times the interquartile range. *C* and *D*: ROC curves comparing the performance of a model with or without cytokines in predicting MALE (*C*) and MACE (*D*). The true-positive rate (sensitivity) is plotted as a function of the false-positive rate (1 − specificity).

A limitation of this single-center study is the relatively small number of patients included. Although both univariate and multivariable analyses suggest that FBG has no effect on any of the outcomes examined, it might be that statistically significant differences could not be detected because of the small sample size. Furthermore, given the number of patients it was not possible to carry out a meaningful analysis on the location of the stenosis and the outcomes. It is therefore possible that even a specific below-the-knee location correlates with a specific outcome. A further limitation of the study is the lack of a formal protocol (bone scans, MRI, or X-rays) to exclude infection. Moreover, even if patients with clear signs of infection were not included in the study, we cannot exclude the possibility that the presence of more severe ulcers correlates with a worse therapeutic outcome. However, the purpose of this study was to consider the typical patient with diabetes, PAD, and CLTI and to use easily accessible and reproducible biomarkers. Furthermore, infectious conditions do not influence all of these parameters, such as OPG.

Table 4—Association between cytokines and MACE

	OPG	TNF-α	IL-6	CRP
First quartile				
Range	1.3–3.7	12.3–27.9	12.2–26.4	1.0–3.1
Numbers of events/patients (%)	0/74 (0)	3/74 (4.1)	2/66 (3.0)	0/74 (0)
Second quartile				
Range	3.9–5.6	28.4–42.3	26.5–36.5	3.1–4.5
Numbers of events/patients (%)	16/75 (21.3)	14/75 (18.7)	10/72 (13.9)	17/74 (23.0)
Third quartile				
Range	5.6–7.5	42.3–63.6	37.1–45.5	4.8–7.5
Numbers of events/patients (%)	21/75 (28.0)	23/74 (31.1)	30/80 (37.5)	18/73 (24.7)
Fourth quartile				
Range	7.5–12.0	63.9–74.5	45.6–56.5	7.5–10.6
Numbers of events/patients (%)	45/75 (60.0)	42/76 (55.3)	41/81 (50.6)	47/78 (60.3)
P value for trend				
Univariate	<0.001	<0.001	<0.001	<0.001
Multivariate	<0.001	<0.001	<0.001	<0.001

Multivariate models were adjusted for age, sex, BMI, high blood pressure, diabetes duration, smoking status, Rutherford staging, previous cardiovascular and cerebrovascular events, treatment, total cholesterol, LDL cholesterol, triglycerides, FBG, and HbA_{1c}.

In conclusion, this study has shown that elevated OPG, TNF-α, IL-6, and CRP levels correlate with worse vascular outcomes in patients with diabetes and PAD who undergo an endovascular procedure. Although these data need further confirmations obtained on a more consistent number of evaluated patients, they represent a step forward in understanding and

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managing such a widespread, severe, and disabling disease.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.
Author Contributions. F.B. and P.M.F. designed the study, analyzed data, and reviewed the manuscript. F.B., P.M.F., W.R.H., F.A., E.N., A.L.C., A.S., D.P., R.L., and A.F. read and approved the final manuscript. W.R.H. drafted the manuscript. F.A. and E.N. carried out the immunoassays. A.S. and D.P. designed the study and performed statistical analyses. R.L. and A.F. conceived, designed, and coordinated the study and drafted the manuscript. A.F. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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