Relationship Between Diabetic Retinopathy Stages and Risk of Major Lower-Extremity Arterial Disease in Patients With Type 2 Diabetes

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OBJECTIVE

We evaluated the association between diabetic retinopathy stages and lower-extremity arterial disease (LEAD), its prognostic value, and the influence of potential contributors to this relationship in a prospective cohort of patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Diabetic retinopathy was staged at baseline as absent, nonproliferative, or proliferative. A Cox regression model was fitted in order to compute the hazard ratio (HR) (95% CI) for major LEAD (lower-limb amputation or revascularization) during follow-up by baseline retinopathy stages. The retinopathy-LEAD association was assessed in subgroups by age, sex, diabetes duration, HbA_{1c}, systolic blood pressure, diabetic kidney disease, smoking, and macrovascular disease at baseline. The performance of retinopathy in stratifying LEAD risk was assessed by using the C statistic, integrated discrimination improvement (IDI), and net reclassification improvement (NRI).

RESULTS

Among 1,320 participants without a history of LEAD at baseline, 94 (7.1%) developed a major LEAD during a 7.1-year median follow-up (incidence rate 9.6 per 1,000 person-years [95% CI 7.8–11.7]). The LEAD incidence rate (per 1,000 person-years) increased as retinopathy worsened: it was 5.5 (95% CI 3.9–7.8) in participants in whom retinopathy was absent, 14.6 (11.1–19.3) in those with nonproliferative retinopathy, and 20.1 (11.1–36.3) in those with proliferative retinopathy. Nonproliferative retinopathy (adjusted HR 2.31 [95% CI 1.43–3.81], $P = 0.0006$) and proliferative retinopathy (3.14 [1.40–6.15], $P = 0.007$) remained associated with major LEAD. No heterogeneity was observed across subgroups. Retinopathy enhanced the C statistic (+0.023 [95% CI 0.003–0.044], $P = 0.02$), IDI (0.209 [0.130–0.321], $P < 0.001$), and NRI (0.562 [0.382–0.799], $P < 0.001$) values for risk of LEAD, beyond traditional risk factors.

CONCLUSIONS

An independent dose-response relationship was identified between diabetic retinopathy stages and major LEAD. Retinopathy yielded incremental prognostic information for stratifying risk of LEAD, suggesting its usefulness as a predictor of LEAD.

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Lower-extremity arterial disease (LEAD) is a cardinal manifestation of systemic atherosclerosis and affects over 200 million people worldwide, including 40 million living in Europe (1). LEAD is a common complication in patients with diabetes, among whom it is two to four times more frequent than in the general population (2,3). It leads to worse prognosis with major adverse limb events, including the need for revascularization, limb loss, or both (4,5). LEAD is also associated with high rates of cardiovascular and noncardiovascular diseases and death, reduced quality of life, and high medical costs (1,6–9). LEAD is usually considered as a large-artery atherosclerosis–related disease, but its link with microvascular disease has been suggested in recent studies (10,11).

Diabetic retinopathy remains one of the leading causes of moderate and severe vision loss and of blindness worldwide, despite substantial improvement in visual outcomes achieved in the past three decades thanks to prompt screening and therapeutic advances (12,13). In 2010, diabetic retinopathy was responsible for 3.7 million cases of visual impairment and more than 833,000 cases of blindness globally (14). Diabetic retinopathy also has been highlighted as a predictor for cardiovascular disease in adults with type 2 diabetes (15–19), but only a few studies have investigated the relationship between diabetic retinopathy and LEAD in patients with diabetes (20,21). Uncertainties persist regarding whether the excess risk of LEAD reported in patients with severe retinopathy might occur earlier during the course of diabetic retinopathy. Also, whether this association could be mediated by traditional contributors, including diabetes duration, diabetic kidney disease (DKD), and cardiovascular risk factors and condition, remains unknown. The purpose of this study was to assess the relationship between diabetic retinopathy stages and the risk of major LEAD and to test the influence of a range of risk factors and conditions in this association in a prospective cohort of patients with type 2 diabetes. We also evaluated the prognostic performance of diabetic retinopathy to identify patients with type 2 diabetes who are at high risk of LEAD.

RESEARCH DESIGN AND METHODS

Participants

Survie, Diabete de Type 2 et Genetique (SURDIAGENE) is a single-center, population-based, prospective cohort study conducted at Poitiers University Hospital (22). It has been designed to investigate genetic and environmental determinants of complications in participants with type 2 diabetes who have had the diagnosis for at least 2 years. Participants were enrolled between 2002 and 2012 and have been followed every 2 years between 2007 and 31 December 2015. The main exclusion criteria were the presence of nondiabetic kidney disease or short follow-up (<1 year). The Poitiers University Hospital Ethics Committee (Committee for the Protection of Persons Ouest 3) approved the SURDIAGENE study protocol, and all participants gave written informed consent before enrollment. In the investigation presented here, 22 participants were excluded because of missing or incomplete data regarding retinopathy stage. Also, 126 participants with a history of LEAD at baseline were excluded from the main analyses, including the primary outcome evaluation and its subsequent analyses (Fig. 1).

Clinical and Biological Parameters at Baseline

Urinary albumin concentration was measured by nephelometry (Roche Diagnostics GmbH, Mannheim, Germany), and serum concentrations of creatinine were measured with the colorimetric method; both were run on an automated analyzer (Kone Optima; Thermo Clinical Labsystems, Vantaa, Finland). Glomerular filtration rate was estimated (eGFR) by using the Chronic Kidney Disease–Epidemiology Collaboration equation. DKD was defined as sustained (at least two determinations) albumin-to-creatinine ratio (ACR) >30 mg/mmol, eGFR <60/min/1.73 m^2, or both. The history of macrovascular disease was defined as the presence at baseline of at least one of the following conditions: myocardial infarction, stable angina, stroke, transient ischemic attack, coronary or carotid artery revascularization.

Diabetic Retinopathy at Baseline

Screening for and Diagnosis of Diabetic Retinopathy

Each participant had a retinal examination after pupillary dilatation by a trained ophthalmologist or was screened for diabetic retinopathy through the use of three-field (nasal, temporal, and central) retinal photography. Participants were consecutively referred to an ophthalmologist for further investigation if diabetic retinopathy was observed or photographs were uninterpretable. Hence, 225 participants (17%) had only retinal photographs at baseline; in 3% the photos could not be interpreted. A total of 1,188 participants (90%) had an eye fundus examination, which included retinal angiography in 132 subjects (10%). Inclusion criteria and clinical conditions, including diabetic retinopathy status, were approved at baseline by a validation committee.

Classification of Diabetic Retinopathy

Diabetic retinopathy was staged for the worse eye on the basis of an international diabetic retinopathy classification scale: absent (no abnormalities), mild nonproliferative retinopathy (microaneurysms only), moderate nonproliferative retinopathy (microaneurysms plus other lesions including dot and blot hemorrhages, hard exudates, or cotton wool spots), or severe nonproliferative retinopathy (moderate nonproliferative retinopathy plus any of the following abnormalities: intraretinal hemorrhages [≥20 in each quadrant], venous beading [in two quadrants], intraretinal microvascular abnormalities [in one quadrant]), and proliferative retinopathy (severe nonproliferative retinopathy plus at least one of either neovascularization or vitreous/preretinal hemorrhage) (23). For statistical analyses, participants were categorized into three retinopathy groups: absent, nonproliferative retinopathy (mild, moderate, and severe were considered together), and proliferative retinopathy.

Diabetic macular edema was defined as a localized or diffuse thickening of the macular area usually associated with retinal exudates, cysts, and microaneurysms.

Outcomes

The primary outcome, a new case of major LEAD, was defined as the first occurrence of either nontraumatic lower-limb amputation (transmetatarsal, transtibial, or transfemoral) or a required lower-limb revascularization procedure (angioplasty or surgery) during follow-up in participants without a history of LEAD at baseline. Minor (transmetatarsal), major (transtibial or transfemoral), or any lower-limb amputation and requirement for lower-limb artery revascularization were also considered separately as secondary outcomes. Lower-limb amputation was assessed in participants without a history of amputation at baseline, and lower-limb revascularization was evaluated in those with no history of such a procedure at baseline (Fig. 1). An independent adjudication committee validated each end point.

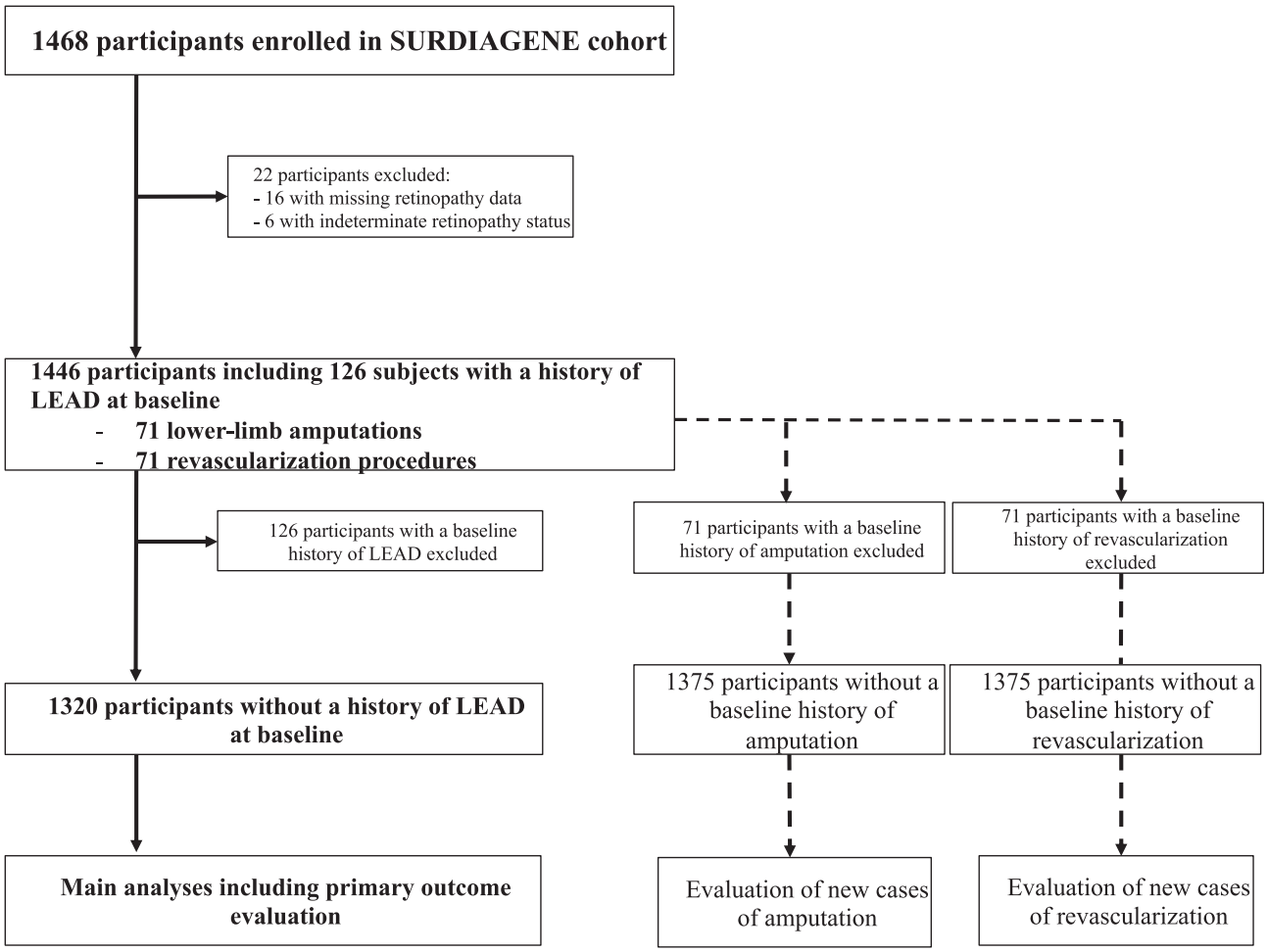

Figure 1—Flowchart of the SURDIAGENE prospective cohort.

Reasons for Lower-Limb Amputation

We also examined patients' medical files to determine the potential causes of lower-limb amputation at the time the end point was reached: neuropathy (as reported by the investigator), LEAD (abolition of peripheral pulses, intermittent claudication, lower-limb artery stenosis >50% with hemodynamic effects identified during ultrasound examination), foot infection (skin, soft tissue, bone, or joint), or a combination of these.

Statistical Analyses

Categorical variables are presented as the number (percentage) of participants. Continuous variables are presented as the mean and SD or, for those with skewed distribution, as the median (25th percentile, 75th percentile). Baseline characteristics of participants were compared between groups by using the χ^2, ANOVA, Wilcoxon, or Kruskal-Wallis test.

Kaplan-Meier curves were plotted in order to illustrate the proportion of patients who experienced outcomes during follow-up by diabetic retinopathy stage at baseline; the curves were compared by using the log-rank test. Cox proportional hazards regression models were fitted in order to calculate hazard ratios (HRs) and related 95% CIs for outcomes during follow-up by diabetic retinopathy stage at baseline (nonproliferative, proliferative, or any retinopathy vs. no retinopathy [absent]). Models were adjusted for age plus each potential confounding variable that was nominally associated ($P < 0.10$) with major LEAD in the univariate analyses: sex, duration of diabetes, BMI, systolic blood pressure, urinary ACR, eGFR, HDL and LDL cholesterol, history of tobacco smoking (never, former, current), history of macrovascular disease, and use of antihypertensive treatments, statins, metformin, or insulin (model 1). Also, we used the Fine and Gray method to estimate the subdistribution HRs for major LEAD by diabetic retinopathy stage, while accounting for the competing risk of all-cause death further to adjusting as in model 1 (24). The proportional hazards assumption was checked by using the Schoenfeld residuals method ($P = 0.97$). Analyses were performed of the whole cohort and across different subgroups (quantitative variables were categorized as ≤ and > median) by sex, age, duration of diabetes, HbA_{1c}, systolic blood pressure, history of tobacco smoking (never, former, current), DKD, and macrovascular disease at baseline. We tested any heterogeneity in the retinopathy-LEAD association throughout the subgroups using multiplicative interaction.

The Harrell C statistic, integrated discrimination improvement (IDI), and net reclassification improvement (NRI) indexes were computed in order to compare discrimination and classification of major LEAD, as assessed by using survival methodology, between two prognostic models: model 1 and model 1 plus diabetic retinopathy (25,26).

We tested as a sensitivity analysis the risk of major LEAD by diabetic retinopathy stage using alternative categories of diabetic retinopathy: absent, simple (mild or moderate nonproliferative), and severe (severe nonproliferative or proliferative) retinopathy.

Statistics were calculated by using Stata software version 15 (StataCorp; www.stata.com), SAS software version 9.4 (SAS Institute; www.sas.com), and JMP software version 14 (SAS Institute; www.jmp.com). Two-sided *P* values <0.05 were considered significant.

RESULTS

Characteristics of Participants by Retinopathy Stage at Baseline

Among 1,320 participants without a history of LEAD at baseline, 44% were women and 11% were current smokers. Their mean age was 64 years (SD 11 years) at baseline. Their mean duration of diabetes was 14 years (SD 10 years), and their mean HbA_{1c} was 7.8% (SD 1.6%). Retinopathy stages were established at baseline: 762 participants (58%) with no retinopathy, 475 (36%) with nonproliferative retinopathy, and 83 (6%) with proliferative retinopathy. History of diabetic macular edema was present at baseline in 135 participants (10%). Characteristics of participants according to diabetic retinopathy stages are shown in Supplementary Table 1. Participants who had a history of diabetic retinopathy were older than those without the condition and had a longer duration of diabetes; higher systolic and diastolic blood pressure, HbA_{1c}, ACR, and LDL cholesterol; lower eGFR and triglyceride levels; and more prevalent macular edema and DKD. They also were more likely to use insulin therapy and less likely to use a fibrate and metformin at baseline.

Primary Outcome by Diabetic Retinopathy Stage at Baseline

Among participants, 94 (7.1%) developed a major LEAD during a median follow-up of 7.1 years (25th, 75th percentiles, 4.4, 10.7 years), corresponding to 9,601 person-years and an incidence rate of 9.6 per 1,000 person-years (95% CI 7.8–11.7). Characteristics of participants at baseline by incidence of major LEAD during follow-up are displayed in Table 1. Briefly, participants who experienced a major LEAD during follow-up were more frequently male and had a longer duration of diabetes; higher systolic blood pressure and ACR; and lower BMI, eGFR, and HDL cholesterol at baseline than those who did not experience a major LEAD. They were

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

		Major LEAD		
	Overall (n = 1,320)	No (n = 1,226)	Yes (n = 94)	*P*
Clinical parameters				
Female sex	580 (44)	562 (46)	18 (19)	<0.0001
Age (years)	64 ± 11	64 ± 11	65 ± 10	0.33
Duration of diabetes (years)	14 ± 10	14 ± 10	16 ± 9	0.02
BMI (kg/m^2)	31 ± 6	32 ± 6	30 ± 5	0.01
Heart rate (bpm)	71 ± 14	71 ± 14	70 ± 14	0.65
Systolic blood pressure (mmHg)	132 ± 17	132 ± 17	137 ± 18	0.01
Diastolic blood pressure (mmHg)	72 ± 11	73 ± 11	71 ± 12	0.27
Biological parameters				
HbA_{1c} (%)	7.8 ± 1.6	7.8 ± 1.5	7.9 ± 1.6	0.45
HbA_{1c} (mmol/mol)	62 ± 17	62 ± 17	63 ± 17	
Urinary ACR (mg/mmol)	3 (1, 12)	3 (1, 11)	9 (2, 64)	<0.0001
eGFR (mL/min/1.73 m^2)	74 ± 25	74 ± 24	65 ± 29	0.0005
Total cholesterol (mg/dL)	186 ± 45	185 ± 45	190 ± 50	0.26
HDL cholesterol (mg/dL)	47 ± 16	47 ± 16	43 ± 14	0.03
LDL cholesterol (mg/dL)	106 ± 37	106 ± 37	113 ± 40	0.07
Triglycerides (mg/dL)	137 (97, 203)	137 (97, 202)	147 (96, 204)	0.58
Medical history				
Diabetic macular edema	135 (10)	119 (10)	16 (17)	0.07
DKD	449 (34)	396 (32)	53 (56)	<0.0001
Tobacco smoking				
Never	703 (53)	672 (55)	31 (33)	<0.0001
Former	478 (36)	433 (35)	45 (48)	
Current	139 (11)	121 (10)	18 (19)	
Cigarette packs smoked per year	25 (10, 40)	25 (10, 40)	30 (15, 42)	0.51
Macrovascular disease	451 (34)	411 (33)	40 (42)	0.07
Medication history				
Antihypertensive drugs	1,078 (82)	992 (81)	86 (91)	0.008
Statin	585 (44)	533 (44)	52 (55)	0.03
Fibrate	157 (12)	150 (12)	7 (7)	0.19
Antiplatelet or anticoagulant drugs	526 (40)	483 (39)	43 (46)	0.23
Metformin	647 (49)	612 (50)	35 (37)	0.02
Insulin therapy	786 (59)	719 (59)	67 (71)	0.02

Data are *n* (%), mean ± SD, or median (25th percentile, 75th percentile) for variables with skewed distribution (urinary ACR, triglycerides, and number of cigarette packs per year). Comparisons of qualitative and quantitative parameters were performed using χ^2 and ANOVA tests, respectively. Wilcoxon test was used for comparisons of variables with skewed distribution. Analyses were performed in participants without a baseline history of LEAD. $P < 0.05$ was considered as significant.

less likely to never have smoked and were more likely to be former or current smokers at baseline. They were more likely to use statins, antihypertensive drugs, and insulin and less likely to use metformin.

The cumulative incidence of LEAD increased as diabetic retinopathy worsened: 4.1% in those with no retinopathy, 11.0% in those with nonproliferative retinopathy, and 13.2% in those with proliferative retinopathy ($P < 0.0001$, log-rank test) (Fig. 2). The incidence rate per 1,000 patient-years (95% CI) was 5.5 (3.9–7.8) in participants with no retinopathy, 14.6 (11.1–19.3) in those with nonproliferative retinopathy, and 20.1 (11.1–36.3) in those with proliferative retinopathy. The Cox regression model confirmed the increasing risk of major LEAD associated with the severity of diabetic retinopathy (Table 2). This association remained significant after adjustment for key confounding variables (HR for nonproliferative vs. absent retinopathy, 2.31 [95% CI 1.43–3.81], $P = 0.0006$; for proliferative vs. absent retinopathy, 3.14 [1.40–6.15], $P = 0.007$) or when considering all-cause mortality as a competing risk (subdistribution HR for nonproliferative vs. absent retinopathy, 2.13 [95% CI 1.29–3.51], $P = 0.003$; for proliferative vs. absent retinopathy, 2.62 [1.25–5.49], $P = 0.01$). Similar results were obtained when diabetic retinopathy was staged as absent, simple, or severe (Supplementary Table 2). We did not find any significant heterogeneity across the different subgroups (all P for interaction >0.05) (Fig. 3). The history of macular edema was associated with an increased risk of major LEAD in the unadjusted model (HR 1.86 [95% CI 1.05–3.11], $P = 0.03$), but this association did not persist after adjustment as in model 1 (1.05 [0.55–1.89], $P = 0.88$).

Prognostic Performance of Diabetic Retinopathy for Major LEAD Risk Stratification

Diabetic retinopathy significantly enhanced the C statistic index for risk of major LEAD (change +0.023 [95% CI 0.003–0.044], $P = 0.02$) when it was added to model 1, which comprised a comprehensive set of usual risk factors and conditions (Table 3). Diabetic retinopathy also improved IDI (0.209 [0.130–0.321], $P < 0.001$) and NRI (0.562 [0.382–0.799], $P < 0.001$) measures for 5-year LEAD risk (Table 3).

Secondary Outcomes by Diabetic Retinopathy Stage at Baseline

Among 1,375 participants without a history of limb loss at baseline, 52 subjects (3.8%) had a lower limb amputated during follow-up (incidence rate, 4.9 per 1,000 person-years [95% CI 3.7–6.5]). Every patient who lost a limb during follow-up had at least one factor indicating evidence for LEAD at the end point (77% experienced abolition of peripheral pulses, 56% had intermittent claudication, 94%

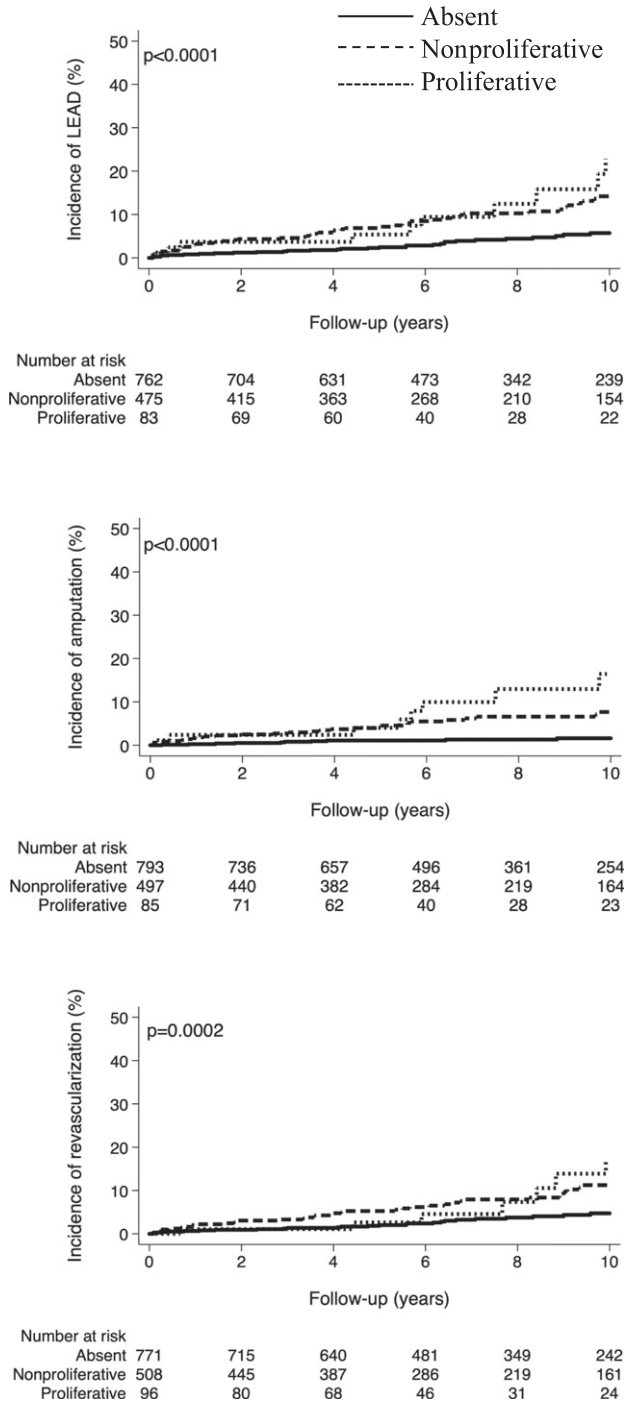

Figure 2—Major LEAD by retinopathy stage. Cumulative incidence of major LEAD (top), lower-limb amputation (middle), and requirement of a revascularization procedure (bottom) during follow-up in participants in whom retinopathy was absent (solid line) or who had nonproliferative retinopathy (dashed line) or proliferative retinopathy (dotted line) at baseline. Analyses included participants without a history of LEAD (for primary outcome), amputation (for lower-limb amputation secondary outcome), or revascularization (for lower-limb revascularization secondary outcome) at baseline.

Table 2—Primary and secondary outcomes by diabetic retinopathy stage at baseline

	Outcomes		Unadjusted model		Adjusted model*	
	No, *n*	Yes, *n* (%)	HR (95% CI)	*P*†	HR (95% CI)	*P*†
Major LEAD						
Diabetic retinopathy at baseline						
Absent	731	31 (4.1)	Reference	—	Reference	—
Nonproliferative	423	52 (11.0)	2.75 (1.77–4.34)	<0.0001	2.31 (1.43–3.81)	0.0006
Proliferative	72	11 (13.2)	3.64 (1.75–7.03)	0.001	3.14 (1.40–6.15)	0.007
Nonproliferative or proliferative	495	63 (11.3)	2.87 (1.88–4.47)	<0.0001	2.41 (1.51–3.93)	0.0002
Lower-limb amputation						
Diabetic retinopathy at baseline						
Absent	782	11 (1.4)	Reference	—	Reference	—
Nonproliferative	465	32 (6.4)	4.71 (2.45–9.80)	<0.0001	4.58 (2.21–10.27)	<0.0001
Proliferative	76	9 (10.6)	8.32 (3.35–20.12)	<0.0001	8.45 (2.99–23.30)	0.0001
Nonproliferative or proliferative	541	41 (7.0)	5.21 (2.77–10.66)	<0.0001	4.97 (2.44–11.02)	<0.0001
Lower-limb revascularization						
Diabetic retinopathy at baseline						
Absent	745	26 (3.4)	Reference	—	Reference	—
Nonproliferative	466	42 (8.3)	2.53 (1.56–4.18)	0.0002	1.92 (1.13–3.29)	0.01
Proliferative	88	8 (8.3)	2.85 (1.21–6.03)	0.02	2.21 (0.89–4.97)	0.08
Nonproliferative or proliferative	554	50 (8.3)	2.58 (1.62–4.20)	<0.0001	1.95 (1.17–3.32)	0.01

HRs (95% CIs) were estimated by using Cox proportional hazards regression models for outcomes in participants with nonproliferative, proliferative, or any retinopathy versus those with no history of retinopathy (reference) at baseline. *Adjusting for age, sex, duration of diabetes, BMI, systolic blood pressure, eGFR, urinary ACR, plasma concentrations of HDL and LDL cholesterol, history of tobacco smoking (never, former, current), history of macrovascular disease, and use of antihypertensive, statin, metformin, and insulin therapies. Analyses included participants without a history of LEAD (for primary outcome), amputation (for lower-limb amputation secondary outcome), or revascularization (for lower-limb revascularization secondary outcome) at baseline. †*P* values <0.05 are significant.

had lower-limb arterial stenosis >50% with hemodynamic effects observed on ultrasound examination). Peripheral diabetic neuropathy was reported in 75% of amputees at the time of amputation, whereas foot infection was reported in 50%.

A lower limb was amputated in 11 participants (1.4%) with no retinopathy, 32 (6.4%) with nonproliferative retinopathy, and 9 (10.6%) with proliferative retinopathy ($P < 0.0001$, log-rank test) (Fig. 2). Participants with nonproliferative (HR, 4.58 [95% CI 2.21–10.27], $P < 0.0001$) or proliferative (8.45 [2.99–23.30], $P = 0.0001$) retinopathy had a higher risk of lower-limb amputation during follow-up than did those without a history of retinopathy (Table 2). Comparable results were found when minor and major amputations were considered separately (Supplementary Table 3).

Lower-limb revascularization procedures were performed during follow-up in 76 participants (5.5%) among those without a history of peripheral revascularization at baseline. The incidence rate was 7.6 per 1,000 person-years (95% CI 6.1–9.5). Peripheral revascularization procedures were achieved in 26 participants (3.4%) with no retinopathy, 42 (8.3%) with nonproliferative retinopathy, and 8 (8.3%) with proliferative retinopathy ($P = 0.0002$, log-rank test) (Fig. 2). This association remained significant for any retinopathy (HR 1.95 [95% CI 1.17–3.32], $P = 0.01$) or nonproliferative retinopathy (1.92 [1.13–3.29], $P = 0.01$), but not for proliferative retinopathy (2.21 [0.89–4.97], $P = 0.08$) (Table 2).

CONCLUSIONS

In this study, we investigated the association between nonproliferative and proliferative retinopathy and the occurrence of major LEAD in patients with type 2 diabetes without a history of LEAD at baseline. We also evaluated the potential role of diabetes, kidney disease, and cardiovascular risk factors and conditions in this association. We identified strong and consistent associations between diabetic retinopathy stages and 7-year risk of major LEAD. We showed that these associations were independent of a broad array of putative confounders and were not influenced by the competing risk of all-cause death.

Few studies have investigated prospectively the relationship between diabetic retinopathy and risk of LEAD in patients with type 2 diabetes, and they focused particularly on severe diabetic retinopathy (20,21). In the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified-Release Controlled Evaluation (ADVANCE) study, a history of microvascular disease, including severe retinopathy (defined as retinal photocoagulation therapy, proliferative retinopathy, macular edema, or blindness), at baseline was associated with a higher risk of major LEAD in patients with type 2 diabetes (20). Pongrac Barlovic et al. (21) also investigated the link between severe diabetic retinopathy (defined as such when laser photocoagulation was required) and the risk of major cardiovascular disease in the Finnish Diabetic Nephropathy (FinnDiane) cohort. They identified an independent association between severe retinopathy and LEAD in people with long-standing type 1 diabetes. In our present study, we were able to extend those results in order to highlight some important issues. We found a dose-response relationship between diabetic retinopathy stages and major LEAD. The incidence rates of major LEAD increased as diabetic retinopathy worsened (5.5, 14.6, and 20.1 per 1,000 patient-years in participants with absent, nonproliferative, and proliferative retinopathy, respectively), and the relative risk was twofold higher in patients with retinopathy since the nonproliferative retinopathy stage. A similar trend was observed when diabetic retinopathy was staged as absent, simple, or severe. We did not, however, observe an independent association between

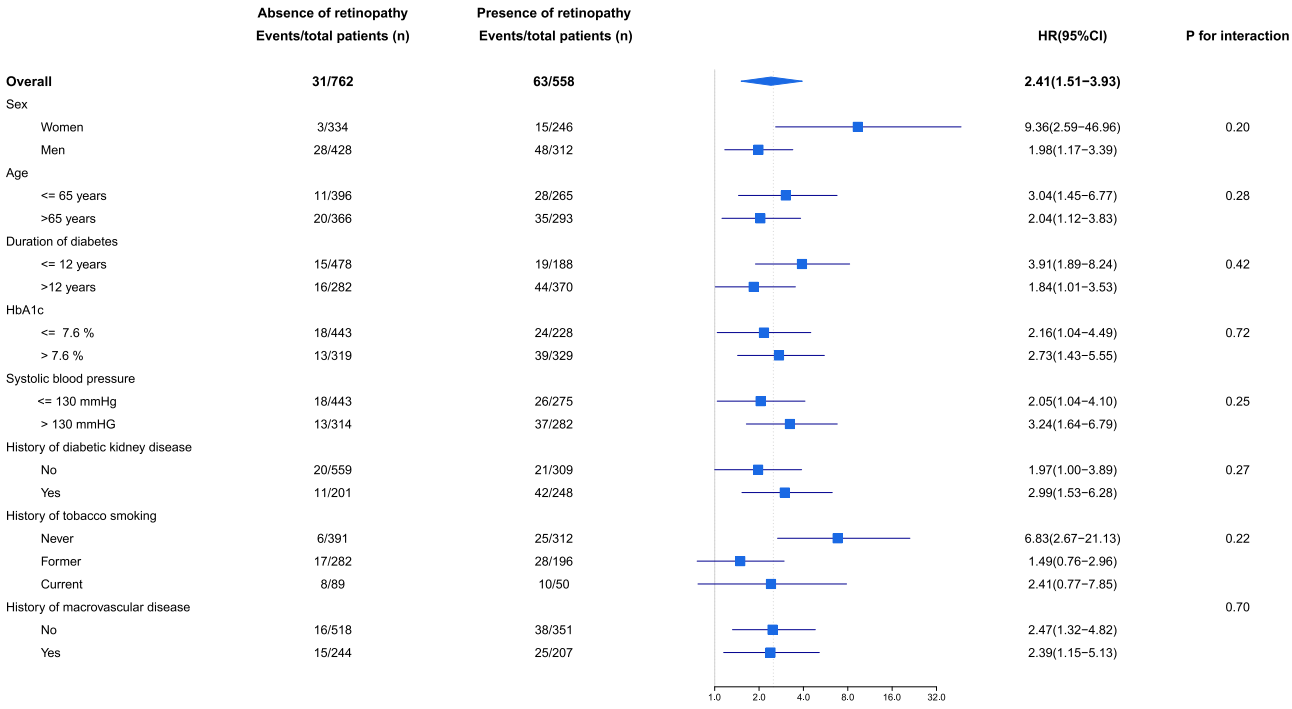

Figure 3—Major LEAD by diabetic retinopathy. Analyses of heterogeneity across subgroups. HRs with 95% CIs were estimated by using Cox proportional hazards regression models for major LEAD in participants with any (nonproliferative or proliferative) retinopathy versus those with no history of retinopathy in various subgroups. Analyses adjusted for age, sex, duration of diabetes, BMI, systolic blood pressure, eGFR, urinary ACR, plasma concentrations of HDL and LDL cholesterol, history of tobacco smoking (never, former, current), history of macrovascular disease, and use of antihypertensive, statin, metformin, and insulin therapies. Each variable used for subgroup composition was deleted from the adjusted model as appropriate (e.g., sex was deleted from the adjusted model for sex subgroup analysis). $P > 0.05$ reflects an absence of heterogeneity between subgroup components. Analyses were performed in participants without a history of LEAD at baseline.

diabetic macular edema at baseline and a risk of LEAD during follow-up. This could be explained by the low prevalence of macular edema at baseline in our cohort. Further investigations are needed in order to determine the relationship between LEAD and macular edema, as accurately diagnosed by using modern retinal imaging systems (e.g., optical coherence tomography technologies).

Of note, the retinopathy-LEAD association was reliable across various subgroups by sex, age, duration of diabetes, HbA_{1c}, blood pressure, history of DKD, tobacco smoking, or macrovascular disease at baseline. The higher risk of major LEAD related to diabetic retinopathy was remarkable in women, although the incidence was three times lower than that in men. We also observed a substantial increase in the risk of major LEAD in patients with diabetic retinopathy, even those with a shorter duration of diabetes, suggesting an early interaction between these two conditions. A previous study reported impaired microvascular function in the retina (retinal arteriolar dilation response to flickering light) early during the course of type 2 diabetes and in prediabetes (27). We observed a similar retinopathy-LEAD association in patients with and without a history of DKD at baseline. Likewise, a significant retinopathy-LEAD association was identified in the participants with type 1 diabetes without a history of DKD who were included in the FinnDiane cohort (21). In addition to reduced eGFR ($<$60 mL/min/1.73 m^2, as used to define chronic kidney disease in the FinnDiane study), we further considered a sustained increase in ACR ($>$30 mg/mmol) in our definition, which may allow the findings to be more reliable. Although a well-recognized link exists between diabetic retinopathy and cardiovascular disease and its risk factors (18,19,28), it is unlikely that this link could explain our results, as they remained significant after multivariable adjustment and after analyses of subgroups stratified by cardiovascular risk factors and disease. Of note, the

Table 3—Prognostic performance of diabetic retinopathy in stratifying risk of major LEAD

Risk of major LEAD	Measures	P†
C statistic (95% CI) for basic model	0.766 (0.719–0.814)	
Change in C statistic (95% CI) for basic model + diabetic retinopathy*	0.023 (0.003–0.044)	0.02
IDI (95% CI)	0.209 (0.130–0.321)	<0.001
Categorical NRI (95% CI)	0.562 (0.382–0.799)	<0.001

The basic model (model 1) included age, sex, duration of diabetes, BMI, systolic blood pressure, eGFR, urinary ACR, plasma concentrations of HDL and LDL cholesterol, history of tobacco smoking (never, former, current), history of macrovascular disease, and use of antihypertensive, statin, metformin, and insulin therapies at baseline. IDI and categorical (5 and 10% risk thresholds) NRI tests were performed for 5-year risk of major LEAD associated with basic model plus baseline retinopathy vs. basic model alone. Analyses included participants without a baseline history of LEAD. *Diabetic retinopathy was defined as a history of any (nonproliferative or proliferative) retinopathy at baseline. †P values $<$0.05 are significant.

retinopathy-LEAD association seemed to be higher among participants who have never smoked, although no evidence was observed for a significant interaction across smoking categories.

Our study highlights—to our knowledge for the first time—the prognostic performance of diabetic retinopathy in stratifying risk of major LEAD in people with type 2 diabetes. The history of diabetic retinopathy enhanced the C statistic index with a substantial increment beyond a widespread range of potential prognostic determinants. Furthermore, IDI and NRI measures for 5-year risk of LEAD significantly improved when diabetic retinopathy burden was added to model 1. In the same vein, a recent study reported enhancement in the C statistic related to retinal abnormalities to predict critical limb ischemia in the general population cohort of the Atherosclerosis Risk in Communities (ARIC) study (11). This improvement was particularly remarkable in participants with diabetes (13%) in the ARIC study cohort. Taken together, these findings support the use of diabetic retinopathy as a valuable prognostic determinant to identify people with type 2 diabetes who are at high risk of adverse events related to lower-limb arteries.

The pathophysiological mechanisms by which diabetic retinopathy might predispose a person to a high risk of LEAD have not been fully investigated. The design of our study does not allow us to make any conclusion on a possible causal relationship, nor any etiological conclusion, but our results confirm and extend data supporting the involvement of microvascular dysfunction in the occurrence of LEAD. Although LEAD has been recognized as a traditional presentation of atherosclerosis affecting large vessels, recent data have provided evidence also linking LEAD to microvascular dysfunction (11,29). One study showed microvascular histological changes including thickening of the capillary basement membrane and a decrease in capillary density in neuroischemic diabetic foot (29). Diabetic retinopathy and LEAD may share some pathophysiologic processes, including low-grade inflammation, oxidative stress, and endothelial dysfunction (30–37). Microvascular dysfunction occurs in LEAD, as do macrovascular changes, and together these affect the structure and function of endothelial cells, leading to decreased nitric oxide production, precapillary arteriole collapse, increased free radical production, inappropriate platelet activation, and leukocyte adhesion—all of which lead to microthrombosis within the capillaries and impaired oxygen exchange (38,39). Neovascularization arising from the vasa vasorum may also be a shared process in LEAD and microvascular disease (39). Further investigations are warranted in order to understand the mechanisms explaining the independent association between diabetic retinopathy and LEAD.

The key strength of our work is the collection of a comprehensive range of demographic, clinical, and biological features within a prospective cohort of patients with type 2 diabetes who were followed for a median duration of 7 years (25th, 75th percentiles, 4, 10). Also, we investigated prespecified end points (limb loss or requirement of a revascularization procedure), which were adjudged within an independent adjudication committee process. There are limitations of our study to acknowledge. We did not evaluate early LEAD stages because we did not have accurate and comprehensive information concerning intermittent claudication, peripheral pulse palpation, and ankle-brachial index at baseline. The other main concern is the lack of information regarding peripheral neuropathy at baseline in our study. All amputees, however, had strong evidence for LEAD at the time of amputation, especially lower-limb arterial stenosis $>$50% with hemodynamic effects observed on ultrasound examination (94% of participants). At the same time, peripheral diabetic neuropathy was reported in 75% of amputees, and foot infection was reported in 50%, supporting limb loss as a dramatic consequence of several concomitant complications. Furthermore, diabetic retinopathy was also significantly associated with a required revascularization procedure, considered individually, although this association was mainly driven by nonproliferative retinopathy and was substantially weaker than what we found with amputation. Another limitation is related to diabetic retinopathy assessment, which we performed according to recommended standards of care, but we did not apply a quality control procedure, and retinopathy was not evaluated based on a prespecified research protocol. Finally, we studied a single-center French inpatient cohort predominantly composed of White Europeans, and our conclusions may not apply to people of other ethnic backgrounds nor be systematically generalizable to all people with type 2 diabetes.

In summary, we report a dose-response relationship between diabetic retinopathy stage and 7-year risk of major LEAD in patients with type 2 diabetes. This association was independent of key confounders, without heterogeneity across various subgroups. Diabetic retinopathy yielded incremental prognostic information for the risk of major LEAD, suggesting its potential use as a valuable predictor when stratifying risk of major LEAD in this population. Our findings encourage extensive monitoring in patients with diabetic retinopathy, irrespective of its stage, in order to prevent the development of major adverse limb events.

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