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Retinal microvascular findings and risk of incident peripheral artery disease: An analysis from the Atherosclerosis Risk in Communities (ARIC) Study

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Abstract

Background and aims—Lower-extremity peripheral artery disease (PAD) is usually considered large artery disease. Interestingly, retinal microvascular findings were shown to predict PAD progression in diabetes. However, it is unknown whether retinal microvascular parameters are associated with incident PAD and its severe form, critical limb ischemia (CLI), in a community-based cohort.

Methods—Among 9,371 ARIC participants (aged 49–72 years) free of a history of PAD, we quantified the associations of several retinal measures by retinal photography during 1993–1995 with PAD risk using Cox models. Incident PAD was defined as the first hospitalization with PAD

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Author contributions

CY and KM conceived and designed the study. CY analyzed the data under the supervision of KM. CY and KM wrote the first draft manuscript. All authors reviewed manuscript and provided critical scientific input. All authors approved of final manuscript. KM has responsibility for final content.

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The authors do not have any conflict of interest to declare for this paper.

Sincerely,

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diagnosis or leg revascularization (considered CLI if an additional diagnosis of ulcer, gangrene, or amputation).

Results—During a median follow-up of 18.8 years, 303 participants developed PAD (including 91 CLI cases). Although generalized retinal arteriolar narrowing was not associated with PAD, most measures of retinopathy demonstrated strong associations with PAD beyond potential confounders including diabetes, with adjusted hazard ratios (HR) of 3.26 (95% CI 2.18–4.90) for blot-shaped hemorrhages, 3.11 (1.83–5.29) for hard exudates, and 2.18 (1.62–2.95) for any retinopathy. Adjusted HRs were significantly greater for CLI (ranging from 3.2 to 5.9) than for PAD (all p -values <0.05). Retinopathy measures showed particularly strong associations in participants with diabetes (p -value for interaction [vs. those without diabetes] <0.001).

Conclusions—Several retinopathy measures were strongly associated with PAD, especially with CLI and in diabetes. Our results support the contribution of microvascular abnormalities to the development and progression of PAD and would have implications on its preventive and therapeutic approaches.

Graphical Abstract

Keywords

Peripheral artery disease; critical limb ischemia; retinopathy; microvascular disease

Introduction

Lower-extremity peripheral artery disease (PAD) affects 8 to 10 million adults in the U.S. and more than 200 million individuals worldwide [1, 2]. PAD is associated with major clinical outcomes including mortality, coronary heart disease, stroke, heart failure, and lower-extremity amputation [3–5]. PAD is often considered as a large artery disease due to the presence of atherosclerosis in large arteries in many PAD patients [6]. On the other hand, for the development of critical limb ischemia (CLI), a very severe form of PAD, microvascular disease is indicated to play an important role by impairing collateral formation and wound healing [7, 8]. Indeed, a few small studies ($n \approx 100$) of diabetic patients have identified retinopathy as predictors of PAD severity and progression [9, 10]. However,

to our knowledge, the association of objectively assessed retinal measures with the development of PAD has not been systematically evaluated in a large community-based cohort. Therefore, our objective was to quantify the associations of retinal findings, reflecting *in vivo* visualized information on the microvasculature [11], with incident PAD and CLI in the Atherosclerosis Risk in Communities (ARIC) Study. We *a priori* hypothesized that retinal findings would be particularly strongly associated with incident CLI [12].

Materials and methods

Study design and study population

The ARIC Study is an ongoing, prospective cohort study that included 15,792 individuals aged 45–64 years at recruitment (1987–1989, visit 1) from four U.S. communities (Forsyth County, North Carolina; Jackson, Mississippi; suburban Minneapolis, Minnesota; and Washington County, Maryland) [13]. ARIC Study conducted follow-up visits in 1990 to 1992, 1993 to 1995, 1996 to 1998, 2011 to 2013, and 2016 to 2017. The current analysis used data from participants at visit 3 (1993–1995), when retinal photographs were taken. Of the 12,887 individuals who participated in this visit, we excluded 38 whose race was neither black or white, 563 with a prior history of PAD (defined as intermittent claudication, ankle-brachial index < 0.9, or a history of leg revascularization at visit 1 or any PAD events between visit 1 and visit 3 as detailed below), 701 with missing information on any of the covariates, 2,193 with no retinal photographs or ungradable photographs, and 21 with retinal arteriolar or venous occlusion [14], leaving a sample size of 9,371 for final analysis. Compared to these 9,371 participants in the current study, 2,193 participants excluded for missing or ungradable retinal data were more likely to be older, black, had higher systolic blood pressure, and higher prevalence of stroke, heart failure, and diabetes (Supplementary Table 1).

Data collection at baseline

Trained interviewers collected information using standardized questionnaires on demographic, life-style, and detailed medical information at visit 3, unless otherwise specified. Education information was collected at visit 1, and was categorized into three groups: basic (less than high school), intermediate (high school graduate or vocational school), and advanced (college, graduate school, or professional school). Smoking and alcohol drinking status were self-reported and categorized as current, former, or never. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Sitting blood pressure represented the mean of the last two of the three measurements using a random zero sphygmomanometer after a 5-minute rest. Diabetes was defined as fasting glucose level ≥ 126 mg/dL (≥ 7.0 mmol/L), non-fasting glucose level ≥ 200 mg/dL (≥ 11.1 mmol/L), self-reported physician diagnosis, or use of anti-diabetic medications. Though information on type 1 vs.2 diabetes was not available in the ARIC Study, given its property as a community-based cohort, most diabetic participants were likely to be type 2 [15]. A history of coronary heart disease, stroke, or heart failure was defined as prevalent cases at visit 1 and subsequent clinical events identified between visit 1 and visit 3 [16, 17]. Medications were determined through self-reported usage in the prior

two weeks and inspection of medication containers. Blood samples were obtained from participants who were asked to fast for 12 hours and stored at -80°C according to standardized protocols. Plasma total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were measured using automated enzymatic procedures, and low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation [18].

Assessment of retinal findings

The procedures to obtain retinal photographs and the grading of retinal microvascular signs in the ARIC Study were described in detail previously [19, 20]. Briefly, after a five-minute dark adaptation, a 45° retinal photograph was taken at a randomly selected eye of each individual with a non-mydriatic, auto-focus fundus Canon CR-45UAF camera (Canon USA Inc., Lake Success, NY), and the photograph was centered on the region of optic disc and macula. The retinal photographs were evaluated by trained graders at the Atherosclerosis Risk in Communities Study Retinal Reading Center in the Department of Ophthalmology, University of Wisconsin, Madison, Wisconsin [21]. For qualitative assessment of focal signs of arteriolar changes and phenotypes indicative of retinopathy, photographs were viewed with a monocular $8\times$ stand viewer (Agfa-Gevaert, Mortsels, Belgium) on a 6200°K color rating fluorescent light box, with a grid printed on transparent film overlaid on the retinal photograph to divide the retina into standard regions. For quantitative assessment of retinal vessels, photographs were digitized with a high-resolution scanner, and a computer software Integrative Vessel Analysis (IVAN) was used to measure the calibers of all arterioles and venules coursing through an area half to one disc diameter from the optic disc margin [19]. The graders were masked to participants' clinical characteristics, and evaluated the photographs for the presence of retinal microvascular abnormalities using a standardized protocol [19, 20]. The grading rules used in ARIC are shown in Supplementary Appendix.

Using the method described by Parr and colleagues [19, 22, 23], the results from image analysis were summarized quantitatively as central retinal arteriole equivalent (CRAE) and central retinal venule equivalent (CRVE), after accounting for branching patterns, representing average arteriolar and venular diameter, respectively. Additionally, the following retinal microvascular abnormalities were recorded qualitatively: arteriovenous nicking, focal arteriolar narrowing, blot-shaped hemorrhages, flame-shaped hemorrhages, microaneurysms, soft exudates, hard exudates, macular edema, intraretinal microvascular abnormalities, venous beading, new vessels at the disc or elsewhere, vitreous and/or preretinal hemorrhage, fibrous proliferation, papillary swelling, and laser photocoagulation scars. Each abnormality was graded as definite, probable, or none in each of the four quadrants of the retina, and a retinal microvascular abnormality was defined as present if graded definite or probable in any of the four quadrants [14]. Any retinopathy was considered to be present if any of the above abnormalities, except for arteriovenous nicking and focal arteriolar narrowing, were detected [14]. Among participants with diabetes, we applied the Early Treatment Diabetic Retinopathy Study (ETDRS) severity scale for diabetic retinopathy [24]. Specifically, we categorized diabetic participants into no retinopathy, mild non-proliferative diabetic retinopathy (NPDR), moderate to severe NPDR, and proliferative diabetic retinopathy (PDR), corresponding to 10, 14–35, 43–53, and 61 on the ETDRS

scale, respectively [25]. Since there were few individuals in PDR category (n=9), we combined moderate to severe NPDR and PDR categories together.

We analyzed each individual retinal finding separately except when the prevalence of relevant finding was <1% in the study sample [14]. These individual retinal findings were grouped into two categories based on severity [26]: findings related to microvascular wall/diameter (CRAE, CRVE, arteriovenous nicking, and focal arteriolar narrowing), and any phenotypes indicative of retinopathy (blot-shaped/flame-shaped/any hemorrhages, hard/soft exudates, and microaneurysms).

CRAE and CRVE were analyzed as continuous variables (per 1 standard deviation decrement for CRAE, representing arteriolar narrowing; and per 1 standard deviation increment for CRVE, representing venular widening) and as quartiles (the 4th quartile, the least degree of narrowing, was the reference for CRAE; and the 1st quartile, the least degree of widening, was the reference for CRVE). Other retinal findings were analyzed as dichotomous variables (i.e., presence vs. absence). The retinal photograph gradings had moderate to high intra- and intergrader concordance [19]. Specifically, for quantitative parameters (CRAE and CRVE), the intra- and intergrader correlation coefficient ranged from 0.69 to 0.89. For qualitative parameters, the intra- and intergrader kappa statistics ranged from 0.29 to 0.61 for microvascular wall findings and from 0.58 to 0.99 for retinopathy findings.

Ascertainment of incident peripheral artery disease (PAD)

Based on previous literature [27, 28], clinical PAD was identified by hospitalizations with the following International Classification of Diseases Ninth Revision (ICD-9) discharge codes: 440.20, 440.21, 440.22, 440.23, 440.24, 440.29, 440.3, 440.4, 38.18, 39.25, 39.29, or 39.50 (Supplementary Table 2). Of PAD cases, individuals with 440.22, 440.23 or 440.24, and those with any of the code above with concurrent ICD-9 codes of ulcer (707.1), gangrene (785.4), and lower extremity amputation (84.1x) were considered as critical limb ischemia (CLI), a severe form of PAD. The first hospitalization with diagnosis of PAD or CLI was considered as an incident event. Follow-up of individuals ended on the date of death, date of last contact, or December 31, 2013 (i.e., administrative censoring), whichever came first.

Statistical analysis

We first summarized the baseline characteristics of participants by incident PAD status, and assessed the difference between groups using a Student's t-test or chi-square test. Subsequently, we used Cox proportional hazards model to quantify the association of each retinal finding and risk of incident PAD, in turn. To assess the potential impact of confounders, we constructed two models: Model 1 adjusted for age, gender, race, and center, and Model 2 additionally adjusted for education level, BMI, smoking status, alcohol drinking status, LDL cholesterol level, HDL cholesterol level, systolic blood pressure, use of anti-hypertensive medications, use of cholesterol-lowering medications, diabetes mellitus, a history of coronary heart disease, stroke, and heart failure. To formally test the difference in the strength of association for each of the retinal findings with incident PAD without CLI

vs. CLI in Model 2, we used seemingly unrelated estimation [29] that computes a simultaneous sandwich/robust covariance matrix for the two models with different outcome variables. We also compared the strength of association with other atherosclerotic outcomes, coronary heart disease and stroke, in Model 2.

Since some clinical conditions, e.g., diabetes and hypertension, are tightly related to retinopathy, we performed subgroup analyses stratified by age (> vs. ≤ 59 years, the median age), gender, race, smoking status (ever vs. never), presence/absence of diabetes, hypertension status (hypertension defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or taking any anti-hypertensive medication), and a history of cardiovascular disease (defined as having a history of coronary heart disease, stroke, or heart failure). Potential effect modification was tested using likelihood ratio test comparing models with and without the multiplicative two-way interaction term. For sensitivity analysis, we further assessed the potential confounding by the severity of diabetes through further adjusting for duration of diabetes (≥ vs. < 6 years) and hemoglobin A1c (HbA1c) level measured at visit 2, in addition to covariates in Model 2.

In addition, since we observed quite strong associations between retinopathy and CLI, as subsequently shown, we calculated the E-value for hazard ratio (HR) with CLI as an outcome in Model 2. The E-value has been proposed as a parameter indicating the minimum strength of association, on the risk ratio scale, that an unmeasured confounder would need to have with both the exposure and outcome, conditional on measured covariates, to fully explain away a specific exposure-outcome association [30].

Finally, we assessed the incremental value in risk discrimination for PAD for the significant retinal measures, employing Model 2 (including known risk factors of atherosclerosis) as the base model. We used Harrell's c-statistic as a measure of risk discrimination. We also evaluated category-based net reclassification improvement (NRI) to evaluate reclassification. Since there are no established risk categories for PAD, we arbitrarily selected cutoffs of 20-year risk of 10% and 20% for PAD and 3.5% and 7.0% for CLI.

Statistical significance level was set at 0.05 for all hypothesis tests. All analyses were conducted in Stata version 13.0 (StataCorp LP, College Station, TX).

Results

Baseline characteristics

The mean age of participants at baseline was 59.5 years (SD 5.6 years), with 55.7% (n=5,217) females and 21.0% (n=1,966) blacks. The three most common retinal microvascular abnormalities were focal arteriolar narrowing (14.8%), arteriovenous nicking (14.5%), and any retinopathy (7.1%).

Among the 9,371 individuals free of PAD at baseline, 303 individuals were diagnosed with PAD during hospitalization over a median follow-up of 18.8 years (crude incidence rate: 1.9 cases/1000 person-years), of whom 91 had CLI (0.6 cases/1000 person-years). Compared with participants who did not develop PAD, those with incident PAD were more likely to be

older, male, black, and have generally poorer cardiovascular risk profiles (e.g., higher systolic blood pressure and LDL cholesterol level, lower HDL cholesterol level, and higher prevalence of current smokers, diabetes, coronary heart disease, stroke, and heart failure) (Table 1). Although CRAE was similar between the two groups, CRVE tended to be greater at baseline in those who developed incident PAD compared to those who did not. All retinal microvascular abnormalities were more common in those who developed PAD than those who did not, although the difference in focal arteriolar narrowing was not statistically significant.

Measures of microvascular wall/diameter with PAD and CLI

We generally did not observe independent associations between parameters related to microvascular wall/diameter and incident PAD or CLI (Table 2). Although greater CRVE was associated with increased risk of PAD (HR 1.31, 95% confidence interval [CI] 1.16–1.46 per 1 SD increment) in Model 1, this association was no longer significant after adjusting for known atherosclerotic risk factors (HR 1.08 [0.96–1.22] in Model 2). Lower CRAE, focal arteriolar narrowing, and arteriovenous nicking did not show significant associations with PAD in either Model 1 or Model 2. Similar patterns were observed for CLI.

Measures of retinopathy with PAD and CLI

In contrast to the generally null associations seen for measures of microvascular wall/diameter, measures indicative of retinopathy demonstrated significant and strong positive associations with incident PAD (Table 3). In Model 1, participants with retinal hemorrhages (blot-shaped, flame-shaped, and any), exudates (hard and soft), microaneurysms, or any retinopathy had 3–7 times higher risk of PAD compared to their counterparts without these retinopathy findings. The associations remained independent and strong for most measures of retinopathy in Model 2, with adjusted HRs ranging from 2 to 3. In Model 2, blot-shaped hemorrhages and hard exudates demonstrated particularly strong associations with incident PAD (HR 3.26 [2.18–4.90], and 3.11 [1.83–5.29], respectively).

These associations were even more remarkable with incident CLI. Specifically, in Model 1, those with any of these measures indicative of retinopathy had 6–15 times higher risk of incident CLI than those without retinopathy. The associations remained strong for most measures of retinopathy in Model 2, with adjusted HRs ranging from 3 to 6. Again, hard exudates and blot-shaped hemorrhages demonstrated particularly strong and independent associations (HR 5.92 [3.00–11.70] and 5.32 [3.06–9.26], respectively). Based on seemingly unrelated estimation (Supplementary Table 3), all measures of retinopathy were more strongly associated with CLI than PAD without CLI in Model 2 (all p -values for difference in log HR <0.05).

When we contrasted the strength of association between any retinopathy and other major atherosclerotic cardiovascular diseases, CLI demonstrated the strongest association, followed by overall PAD, stroke, and coronary heart disease (Figure 1). The difference in the strength of association with CLI vs. stroke and coronary heart disease was statistically significant (both p -values <0.001). The HR for overall PAD was greater than that for

coronary heart disease and stroke, and the differences were also statistically significant (both p-values <0.001).

Possibility of an unmeasured confounder

The E-value for most retinopathy parameters ranged from 5–11 (Supplementary table 4), which means that an unmeasured confounder, if any, have to be associated with both of a retinopathy parameter and CLI by risk ratio of 5–11 to make observed HR for retinopathy and CLI to null. Even for a more conservative scenario to make the associations of those retinopathy parameters with CLI not statistically significant (point estimates of HR could be >1), an unmeasured confounder should have to be associated with both a retinopathy parameter and CLI by risk ratio of 3–5.

Subgroup analysis

As shown in Figure 2, the associations of any retinopathy with PAD/CLI were qualitatively consistent in demographic and clinical subgroups. One exception was for diabetes status, with increased PAD risk seen only in participants with diabetes (p-value for interaction <0.001). This pattern was consistently seen for other individual retinopathy parameters (Supplementary Table 5). The associations between retinopathy findings and PAD/CLI among participants with diabetes were attenuated after further adjusting for duration of diabetes, HbA1c at visit 2, or for both, but remained strong and significant (Supplementary Table 6). The sensitivity analysis with the ETDRS severity scale confirmed significant associations of mild and moderate/severe NPRD and PDR with PAD, especially CLI (Supplementary Table 7).

Retinal measures and prediction of PAD and CLI

The discrimination of the base model, including standard risk factors, was good, with a Harrell's c-statistic of 0.828 [0.805–0.850] for PAD and 0.868 [0.827–0.909] for CLI (Table 4). For PAD, adding microaneurysms to the model significantly improved the c-statistic by 0.006 [0.001–0.012]. Any retinopathy and blot-shaped hemorrhages showed a similar level of discrimination improvement (c-statistic ~0.005) but were not statistically significant. For CLI, blot-shaped hemorrhages significantly improved risk discrimination by 0.010 [0.001–0.019]. Any retinopathy and any hemorrhages demonstrated a similar improvement but were not statistically significant. Adding a retinopathy measure to standard risk factors did not yield a meaningful NRI for PAD or CLI (Table 5 and Supplementary Table 8).

When we restricted to participants with diabetes at baseline, for PAD, microaneurysms and any retinopathy significantly improved the discrimination by c-statistic of 0.032 [0.004–0.059] and 0.035 [0.007–0.063], respectively. For CLI, none of the retinopathy measures significantly improved risk discrimination although the magnitude of improvement in c-statistics were similar to PAD. However, some of retinopathy measures demonstrated significant NRIs for the risk of PAD and CLI in participants with diabetes. Specifically, the overall NRI was 0.128 [0.016–0.239] for PAD and 0.180 [0.046–0.315] for CLI by adding any retinopathy (p-values all <0.05); similar significant overall NRIs were observed for the addition of blot-shaped hemorrhages or any hemorrhages.

Discussion

In this bi-racial, community-based cohort study, measures indicative of retinopathy such as blot-shaped hemorrhages, exudates, and microaneurysms were independently and strongly associated with increased future risk of PAD. The associations were particularly evident for CLI. In contrast, there were no material associations between retinal vessel calibers and incident PAD. The associations of retinopathy with PAD risk were generally consistent across different demographic and clinical subgroups, but appeared to be particularly strong in persons with diabetes. Among persons with diabetes, the associations remained consistent after further adjusting for the duration of diabetes and HbA1c levels. Furthermore, some measures of retinopathy improved risk discrimination for future risk of PAD and CLI beyond standard atherosclerotic risk factors in the entire study sample although the incremental margin was small. Of importance, the risk prediction improvement was considerable among persons with diabetes.

Our findings associating retinopathy with increased PAD risk are consistent with previous studies that showed associations of retinopathy measures with other atherosclerotic diseases, such as stroke and coronary heart disease, in the general population [14, 31, 32] and in populations with diabetes [10, 33, 34]. However, the strengths of associations of retinopathy measures with CLI in our study were quite remarkable. The adjusted HRs in our study ranged from 6 to 15 in demographically adjusted models and 3 to 6 even after accounting for a number of potential confounders, whereas HRs for retinopathy in studies of other atherosclerotic diseases ranged from 2 to 3 [35]. Indeed, in our study population, the strength of associations of retinopathy with CLI was much stronger than that with coronary heart disease and stroke (Figure 1). Importantly, to explain away the observed retinopathy-CLI association in our study with an unmeasured confounder, if any, such a confounder needs to be associated with both retinopathy and CLI by risk ratio of 5–10 (Supplementary Table 4).

On the other hand, unlike previous reports for coronary heart disease and stroke [36–38], measures of microvascular diameters (i.e., CRAE, CRVE, and focal narrowing) did not demonstrate associations with PAD in our study. Reasons for this are not clear. However, since measures of microvascular diameter are generally considered early or mild stages of microvascular abnormality [39], our findings suggest that severe microvascular abnormalities may be particularly important in the development of PAD.

Our findings overall seem to support our hypothesis regarding the involvement of microvascular damage (especially when severe) in the pathophysiology of PAD and CLI. Although a small cross-sectional study of 127 PAD patients previously reported retinopathy as a strong correlate of the presence of CLI [9], our study extends existing knowledge in several aspects, such as confirmation of temporality with a prospective design, comparison of several objectively assessed retinal measures, stratified analysis based on a large sample size, and evaluation of improvement in risk prediction.

There are several plausible mechanisms linking microvascular disease to incident PAD and particularly CLI. Microcirculatory changes are tightly related to decreased nitric oxide

production, precapillary arteriole collapse, microthrombosis in capillaries, and impaired oxygen exchange [7], all of which may impede the establishment of compensatory collateral flow and the process of wound healing [7, 8]. Besides, deficiency in vasa vasorum in leg arteries and the subsequent neovascularization have been shown to potentially contribute to the development of CLI [40, 41]. Although these microcirculatory changes are likely to be relevant to many vascular beds, a body of evidence suggests that microvascular abnormalities may be particularly important for PAD due to relative importance of collateral formation in its progression and clinical manifestation [42]. Nonetheless, future studies are warranted to investigate pathophysiological pathways linking microvascular disease to the development of CLI.

It may not be surprising that we observed stronger associations between retinopathy measures and incident PAD in those with diabetes than those without, since diabetes is a leading cause of retinopathy [43] and most participants with signs of retinopathy had diabetes in our study. Nonetheless, our results indicate that retinal abnormality is especially prognostic in persons with diabetes. Indeed, previous studies showed that retinopathy was an independent risk factor for all-cause mortality and fatal and nonfatal cardiovascular events in persons with diabetes [33], and our study extends this observation to PAD as well. Our observation of the stronger association of blot-shaped hemorrhages (a phenotype of diabetic retinopathy) vs. flame-shaped hemorrhages (hypertension as a major cause) with PAD further supports the particular prognostic value of retinal measures in diabetes [43]. Although retinopathy status may merely reflect the duration and severity of diabetes [44] and hyperglycemia was associated with increased risk of incident PAD [45], we found that the association of retinopathy with PAD was independent of diabetes duration and HbA1c.

Our study results may have pathophysiological and clinical implications. Although PAD is often considered a large artery disease, our results suggest the involvement of microvascular disease in the development of PAD, particularly CLI. This perspective is important since the current major therapeutic options for PAD are largely extended from treatment of macrovascular disease (e.g., statins and aspirin) [46] and may not be that effective for preventing CLI. Of note, the skeletal muscle microvasculature is recognized to play a central role in functional impairment in PAD patients [47]. Thus, taken recent literature and our results together, it seems reasonable to seek for therapeutic options targeting microvascular abnormalities for the management of PAD. Also, our results suggest that identifying retinopathy may also help identify individuals at high risk of PAD and CLI. Risk prediction for CLI may be particularly important since once the diagnosis of CLI is made the prognosis is extremely poor, with over a half of cases either dying or needing amputation within a year [48], and thus CLI prevention is critical. Although the improvement of risk prediction by adding retinopathy may look small, importantly, eye examinations are currently recommended for patients with diabetes [49], and thus information on retinopathy should be readily available in a number of diabetic patients. Since the adherence to annual foot care recommended by clinical guidelines for diabetic patients is poor [50], diabetic patients with retinopathy should be even more strongly encouraged to undergo periodic foot care and monitoring.

There are a few limitations to acknowledge in this study. First, PAD and CLI cases were identified from hospitalization records based on ICD-9 codes, which is prone to misclassification. Also, this approach is not likely to capture mild or asymptomatic PAD cases. Nonetheless, our main hypothesis was whether retinal measures were particularly strongly associated with CLI, the severe form of PAD. Second, retinal examinations were performed in a single eye, potentially missing retinopathy in the unstudied eye in some participants. However, such misclassification usually biases estimates towards null. Third, reflecting the availability in the middle of 1990s, retinal examination was based on fundus photography. Thus, studies with contemporary techniques such as retinal optical coherence tomography angiography may provide further insights regarding the association of retinal abnormalities with PAD and CLI. Fourth, due to low prevalence in our community-based cohort, we could not evaluate some individual retinopathy findings (e.g., intraretinal microvascular abnormalities). Fifth, our study included whites and blacks, and thus replication studies in other racial groups would be needed. Finally, there may be residual confounding due to the nature of an observational study.

Conclusions

In summary, our study found measures of retinopathy, including retinal hemorrhages, exudates, and microaneurysms, were strongly associated with increased incidence of PAD, particularly its severe form, CLI. The associations were especially evident among those with diabetes. These findings support the involvement of microvascular disease in the development of PAD and CLI and would have implications on their preventive and therapeutic approaches.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights:

1. Retinopathy measures (e.g., hemorrhage and exudates) were robustly associated to incident PAD.
2. The associations were particularly evident for critical limb ischemia (CLI).
3. Retinopathy measures improved risk prediction of PAD and CLI, especially in persons with diabetes.

Figure 1. Forest plot of hazard ratio (HR) for any retinopathy for different atherosclerotic outcomes in multivariate-adjusted model (Model 2).

Model 2 adjusted for age, gender, race, ARIC visit center, education, BMI, LDL, HDL cholesterol, cholesterol-lowering medication, systolic blood pressure, anti-hypertensive medication, diabetes, history of CHD/stroke/HF, smoking status, and drinking status. p-values for the difference in log HR for any retinopathy comparing CLI vs. CHD, and CLI vs. stroke were all <0.001 based on seemingly unrelated estimation.

(A) PAD

Figure 2A.

Forest plot of subgroup analysis on hazard ratio (95% CI) of PAD for any retinopathy based on Model 2.

(B) CLI

Figure 2B.

Forest plot of subgroup analysis on hazard ratio (95% CI) of CLI for any retinopathy based on Model 2.

Table 1.

Baseline characteristics of participants by incident PAD status.

Variables	Total	Incident PAD	No incident PAD	p-value
N (%)	9371 (100%)	303 (3%)	9068 (97%)	
Age, y (SD)	59.5 (5.6)	60.9 (5.6)	59.4 (5.6)	<0.001
Male, %	44.3	60.1	43.8	<0.001
Black, %	21.0	24.4	20.9	0.135
Education level				<0.001
Basic, %	18.2	28.7	17.8	
Intermediate, %	42.6	37.6	42.8	
Advanced, %	39.2	33.7	39.4	
Body mass index, kg/m ² (SD)	28.4 (5.5)	29.2 (5.4)	28.4 (5.5)	0.016
Systolic blood pressure, mmHg (SD)	123.6 (18.5)	130.0 (20.8)	123.4 (18.4)	<0.001
Diastolic blood pressure, mmHg (SD)	71.9 (10.3)	70.9 (11.2)	71.9 (10.3)	0.095
Anti-hypertensive medication, %	35.9	58.1	35.1	<0.001
Low-density lipoprotein cholesterol, mg/dl (SD)	127.1 (34.3)	129.4 (33.6)	127.0 (34.3)	0.232
High-density lipoprotein cholesterol, mg/dl (SD)	52.6 (18.1)	43.9 (15.3)	52.9 (18.1)	<0.001
Cholesterol lowering medication, %	9.3	17.2	9.0	<0.001
Prevalent coronary heart disease, %	6.3	24.4	5.7	<0.001
Prevalent stroke, %	1.6	4.3	1.5	<0.001
Prevalent heart failure, %	4.4	9.2	4.3	<0.001
Diabetes, %	13.4	38.9	12.6	<0.001
Smoking status				<0.001
Never smokers, %	41.7	26.4	42.2	
Former smokers, %	41.3	39.6	41.4	
Current smokers, %	16.9	34.0	16.4	
Alcohol use				0.197
Never, %	23.8	20.8	23.9	
Former, %	21.7	25.4	21.5	
Current, %	54.6	53.8	54.6	
Central retinal artery equivalent (CRAE), μ m (SD)	162.3 (16.5)	163.5 (17.0)	162.2 (16.5)	0.176
Central retinal venous equivalent (CRVE), μ m (SD)	193.0 (16.6)	197.0 (18.4)	192.9 (16.5)	<0.001
Focal arterior narrowing, %	14.8	16.8	14.7	0.301
Arteriovenous nicking, %	14.5	18.8	14.4	0.032
Microaneurysms, %	3.6	14.2	3.2	<0.001
Soft exudates, %	1.5	6.3	1.3	<0.001
Hard exudates, %	1.1	5.3	0.9	<0.001
Flame-shaped hemorrhages, %	1.0	2.6	0.9	0.003
Blot-shaped hemorrhages, %	2.0	9.9	1.7	<0.001
Any hemorrhages, %	3.1	12.2	2.8	<0.001
Any retinopathy, %	7.1	19.5	6.6	<0.001

Numbers were shown as mean (SD) for continuous variables and percentage for categorical variables.

Table 2.

Hazard ratio (95% CI) for retinal wall/diameter related findings in demographically-adjusted, and multivariate-adjusted models. (n=9371)

Retinal findings	Model 1 PAD	Model 1 CLI	Model 2 PAD	Model 2 CLI
CRAE (4 th quartile as ref.)				
CRAE 3 rd quartile	0.91 (0.67,1.23)	0.86 (0.51,1.46)	1.07 (0.78,1.46)	1.00 (0.58,1.71)
CRAE 2 nd quartile	0.65 (0.47,0.91)	0.56 (0.31,1.03)	0.72 (0.51,1.01)	0.57 (0.31,1.06)
CRAE 1 st quartile	0.85 (0.62,1.16)	0.63 (0.35,1.14)	0.98 (0.70,1.36)	0.67 (0.36,1.25)
CRVE (1 st quartile as ref.)				
CRVE 2 nd quartile	0.74 (0.51,1.06)	0.55 (0.28,1.10)	0.71 (0.50,1.02)	0.57 (0.29,1.14)
CRVE 3 rd quartile	0.98 (0.70,1.37)	0.89 (0.49,1.62)	0.80 (0.57,1.13)	0.75 (0.40,1.39)
CRVE 4 th quartile	1.69 (1.24,2.29)	1.32 (0.76,2.31)	1.15 (0.83,1.59)	0.94 (0.52,1.69)
CRAE (per 1 SD decrement)	0.88 (0.79,0.99)	0.86 (0.69,1.06)	0.92 (0.81,1.04)	0.86 (0.69,1.08)
CRVE (per 1 SD increment)	1.31 (1.16,1.46)	1.24 (1.01,1.53)	1.08 (0.96,1.22)	1.03 (0.84,1.28)
Arterio-venous nicking	1.31 (0.98,1.75)	1.11 (0.64,1.93)	1.09 (0.81,1.45)	0.93 (0.53,1.63)
Focal arterial narrowing	1.08 (0.80,1.46)	0.94 (0.52,1.69)	1.00 (0.73,1.37)	0.73 (0.40,1.34)
Arterio-venous ratio (per 1 SD increment)	0.91 (0.81,1.03)	0.99 (0.81,1.23)	1.03 (0.91,1.16)	1.15 (0.93,1.43)

CRAE = central retinal artery equivalent, CRVE = central retinal venous equivalent,

PAD = peripheral artery disease, CLI = critical limb ischemia.

Model 1 adjusted for age, gender, race, and center.

Model 2 additionally adjusted for education, BMI, LDL, HDL cholesterol, cholesterol-lowering medication, systolic blood pressure, anti-hypertensive medication, diabetes, history of CHD/stroke/HF, smoking status, and drinking status.

Table 3.

Hazard ratio (95% CI) for retinal findings indicative of retinopathy in demographically-adjusted, and multivariate-adjusted models. (n=9371)

Retinal findings	Model 1 PAD	Model 1 CLI	Model 2 PAD	Model 2 CLI
Any retinopathy	3.47 (2.60,4.63)	6.64 (4.26,10.34)	2.18 (1.62,2.95)	3.40 (2.14,5.42)
Hard exudates	6.86 (4.13,11.39)	14.55 (7.66,27.67)	3.11 (1.83,5.29)	5.92 (3.00,11.70)
Soft exudates	5.09 (3.17,8.17)	8.21 (4.30,15.70)	2.54 (1.55,4.14)	3.16 (1.62,6.17)
Microaneurysms	5.06 (3.64,7.03)	10.00 (6.22,16.08)	2.80 (1.98,3.97)	4.27 (2.58,7.09)
Any hemorrhages	5.35 (3.77,7.59)	11.19 (6.89,18.18)	2.60 (1.80,3.76)	4.40 (2.61,7.40)
Blot-shaped hemorrhages	6.84 (4.67,10.04)	14.78 (8.87,24.61)	3.26 (2.18,4.90)	5.32 (3.06,9.26)
Flame-shaped hemorrhages	3.20 (1.58,6.48)	5.88 (2.37,14.60)	1.29 (0.62,2.65)	1.98 (0.77,5.09)

PAD = peripheral artery disease, CLI = critical limb ischemia.

Model 1 adjusted for age, gender, race, and center.

Model 2 additionally adjusted for education, BMI, LDL, HDL cholesterol, cholesterol-lowering medication, systolic blood pressure, anti-hypertensive medication, diabetes, history of CHD/stroke/HF, smoking status, and drinking status.

Table 4.

Harrell's c-statistic for PAD and CLI over entire follow-up for multivariate-adjusted model plus measures indicative of retinopathy vs. multivariate-adjusted model.

	Overall (n=9371)	Among those with diabetes (n=1260)
Model	c-statistic	c-statistic
PAD		
Base model ^a	0.828 (0.805,0.850)	0.745 (0.700,0.791)
+ any retinopathy	0.005 (−0.000,0.009)	0.035 (0.007,0.063)
+ hard exudates	0.002 (−0.001,0.005)	0.015 (−0.004,0.034)
+ soft exudates	0.002 (−0.001,0.005)	0.010 (−0.007,0.027)
+ microaneurysms	0.006 (0.001,0.012)	0.032 (0.004,0.059)
+ any hemorrhages	0.003 (−0.001,0.006)	0.025 (−0.001,0.050)
+ blot-shaped hemorrhages	0.004 (−0.000,0.008)	0.023 (−0.002,0.048)
+ flame-shaped hemorrhages	−0.000 (−0.001,0.000)	0.002 (−0.002,0.006)
CLI		
Base model ^a	0.868 (0.827,0.909)	0.795 (0.734,0.857)
+ any retinopathy	0.011 (−0.001,0.022)	0.038 (−0.001,0.077)
+ hard exudates	0.006 (−0.001,0.012)	0.015 (−0.013,0.044)
+ soft exudates	−0.001 (−0.003,0.002)	0.001 (−0.017,0.020)
+ microaneurysms	0.011 (−0.000,0.023)	0.035 (−0.003,0.072)
+ any hemorrhages	0.009 (−0.000,0.018)	0.026 (−0.010,0.062)
+ blot-shaped hemorrhages	0.010 (0.001,0.019)	0.029 (−0.008,0.066)
+ flame-shaped hemorrhages	0.001 (−0.001,0.003)	0.002 (−0.005,0.009)

PAD = peripheral artery disease, CLI = critical limb ischemia.

^a c-statistic (95% CI).

Model 2 adjusted for age, gender, race, center, education, BMI, LDL, HDL cholesterol, cholesterol-lowering medication, systolic blood pressure, anti-hypertensive medication, diabetes, history of CHD/stroke/HF, smoking status, and drinking status.

Table 5.

Net improvement on reclassification of 20-year individual risk of PAD and CLI based on Model 2 with the addition of measures indicative of retinopathy.

Retinal finding marker	Overall (n=9371)	Among those with diabetes (n=1260)
	Overall NRI	Overall NRI
PAD ^a		
Any retinopathy	0.016 (−0.021,0.052)	0.128 (0.016,0.239)
Hard exudates	0.005 (−0.024,0.033)	0.054 (−0.015,0.124)
Soft exudates	0.008 (−0.020,0.036)	0.035 (−0.042,0.113)
Microaneurysms	0.011 (−0.025,0.048)	0.128 (0.022,0.233)
Any hemorrhages	0.020 (−0.013,0.052)	0.147 (0.051,0.244)
Blot-shaped hemorrhages	0.016 (−0.017,0.050)	0.109 (0.016,0.202)
Flame-shaped hemorrhages	−0.007 (−0.016,0.003)	0.014 (−0.005,0.032)
CLI ^b		
Any retinopathy	−0.058 (−0.155,0.040)	0.180 (0.046,0.315)
Hard exudates	−0.014 (−0.090,0.061)	0.057 (−0.037,0.152)
Soft exudates	−0.061 (−0.123,0.000)	−0.017 (−0.103,0.069)
Microaneurysms	−0.062 (−0.157,0.033)	0.118 (−0.016,0.251)
Any hemorrhages	−0.051 (−0.148,0.047)	0.154 (0.021,0.288)
Blot-shaped hemorrhages	−0.060 (−0.155,0.035)	0.131 (0.003,0.259)
Flame-shaped hemorrhages	0.025 (−0.006,0.056)	0.050 (−0.004,0.104)

NRI = net reclassification improvement, PAD = peripheral artery disease, CLI = critical limb ischemia.

^aBased on risk cutoffs of 10% and 20% for PAD.

^bBased on risk cutoffs of 3.5% and 7.0% for CLI.

Model 2 adjusted for age, gender, race, center, education, BMI, LDL, HDL cholesterol, cholesterol-lowering medication, systolic blood pressure, anti-hypertensive medication, diabetes, history of CHD/stroke/HF, smoking status, and drinking status.