Circulation

ORIGINAL RESEARCH ARTICLE

Microvascular Disease, Peripheral Artery Disease, and Amputation

Joshua A. Beckman, MD
Meredith S. Duncan, MA
Scott M. Damrauer, MD
Quinn S. Wells, MD
Joey V. Barnett, PhD
David H. Wasserman, PhD
Roger J. Bedimo, MD
Adeel A. Butt, MD
Vincent C. Marconi, MD
Jason J. Sico, MD
Hilary A. Tindle, MD
Marc P. Bonaca, MD
Aaron W. Aday, MD
Matthew S. Freiberg, MD

BACKGROUND: The mechanism of adverse limb events associated with peripheral artery disease remains incompletely understood. We investigated whether microvascular disease is associated with amputation in a large cohort of veterans to determine whether microvascular disease diagnosed in any location increases the risk of amputation alone and in concert with peripheral artery disease.

METHODS: Participants in the Veterans Aging Cohort Study were recruited from April 1, 2003 through December 31, 2014. We excluded participants with known prior lower limb amputation. Using time-updated Cox proportional hazards regression, we analyzed the effect of prevalent microvascular disease (retinopathy, neuropathy, and nephropathy) and peripheral artery disease status on the risk of incident amputation events after adjusting for demographics and cardiovascular risk factors.

RESULTS: Among 125 674 veterans without evidence of prior amputation at baseline, the rate of incident amputation over a median of 9.3 years of follow-up was 1.16 per 1000 person-years, yielding a total of 1185 amputations. In time-updated multivariable-adjusted analyses, compared with those without peripheral artery disease or microvascular disease, microvascular disease alone was associated with a 3.7-fold (95% CI, 3.0–4.6) increased risk of amputation; peripheral artery disease alone conferred a 13.9-fold (95% CI, 11.3–17.1) elevated risk of amputation; and the combination of peripheral artery disease and microvascular disease was associated with a 22.7-fold (95% CI, 18.3–28.1) increased risk of amputation.

CONCLUSIONS: Independent of traditional risk factors, the presence of microvascular disease increases the risk of amputation alone and synergistically increases risk in patients with peripheral artery disease. Further research is needed to understand the mechanisms by which this occurs.

Key Words: amputation ■ cardiovascular diseases ■ peripheral artery disease ■ risk factors ■ veterans

Sources of Funding, see page 457

https://www.ahajournals.org/journal/circ

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Clinical Perspective

What Is New?

- The presence of microvascular disease (MVD) increases the risk of amputation significantly in the absence of peripheral artery disease (PAD).
- As many as 1 in 6 below-knee amputations may result from MVD without PAD.
- MVD potentiates the amputation risk in persons with PAD to more than 20-fold compared with persons with neither PAD nor MVD.

What Are the Clinical Implications?

- Patients with MVD, with or without diabetes mellitus, are at increased risk of amputation.
- The combination of MVD and PAD identifies a population at particularly high risk for amputation.
- Clinicians should routinely solicit clinical complaints and physical signs of lower extremity injury or lesion to ensure the addition of therapies to minimize amputation in patients with MVD and PAD and particularly the combination of both.

Peripheral artery disease (PAD) is a cardinal manifestation of atherosclerosis and is associated with an increased risk of cardiovascular events, such as myocardial infarction and stroke, and with adverse limb events, including the need for revascularization, amputation, or both. Although the severity of lower extremity artery occlusive disease in PAD, as measured by an ankle brachial index, strongly associates with cardiovascular events like myocardial infarction,[1] it does not relate as strongly to the development of limb complications.[1,2] These observations suggest the existence of other important contributors to the development of limb symptoms beyond large artery atherosclerosis.

Abnormalities in the microvasculature (vessels with a diameter of ≈100 μm) represent a putative candidate mechanism for the development of adverse limb events in individuals with peripheral artery disease. Although the term microcirculation has been applied to both small (pedal) artery disease and microvascular disease (MVD),[3] the microvasculature stands apart from the small pedal arteries (1–2 mm in diameter) of the foot. Pedal vessels are, in fact, 10-fold larger than the vessels that are compromised in MVD.

MVD often occurs alongside atherosclerosis but is not a direct manifestation of atherosclerotic disease. MVD is commonly ascribed to diabetes mellitus because of the significantly increased risk of microvascular dysfunction compared with patients without diabetes mellitus.[4] However, the aggregate population burden of retinopathy,[5] neuropathy,[6] and nephropathy[7] is much greater in the general population, creating a larger group at potential risk for MVD than just in persons with diabetes mellitus. To better characterize the impact of MVD on adverse limb events, we examined the association between PAD, MVD, and amputation in an older, predominantly male population of veterans. We chose amputation as our end point because of its definitive nature and clarity in the electronic health record. Although there is likely impact of MVD on the development of critical limb ischemia, ulceration, and infection, amputation is a well-defined objective end point that neither varies in definition between clinicians nor requires nuanced adjudication.

METHODS

The US Department of Veterans Affairs (VA) does not allow identifiable data sets to be used by non-VA investigators. However, after meeting all requirements per VA regulations, a deidentified data set can be provided to qualified investigators that would allow for replication of our results.

Study Sample

The VACS (Veterans Aging Cohort Study) is a prospective longitudinal cohort of HIV-infected and uninfected veterans matched 1:2 for sex, age, clinical site, race/ethnicity, and calendar year of enrollment. VACS participants are identified using a validated algorithm deployed in the VA national electronic medical record system.[8] Information regarding demographic and clinical data are extracted from the VA electronic medical record, Health Factor data set, and the VA Corporate Data Warehouse. Vital status is determined using the VA vital status file, the Veterans Health Administration Medical Statistical Analysis Systems inpatient data sets, the Social Security Administration death master file, and the Beneficiary Identification and Records Locator Subsystem. The institutional review boards from Vanderbilt University Medical Center, West Haven VA Medical Center, and Yale University approved this study.

We included all VACS participants in this analysis who were alive as of April 1, 2003, with the baseline as a participant's first clinic visit on or after this date. Participants were followed from baseline to the minimum of the date of lower-extremity amputation, death, or December 31, 2014. Our analyses were truncated at the end of 2014 to coincide with the end of our Medicare data files. We excluded participants with previous amputation based on administrative *International Classification of Diseases, Ninth Revision* (*ICD-9*), or Current Procedural Terminology codes before their baseline date or within 180 days postbaseline to account for delayed reporting. Because VACS was initially designed to look at the impact of HIV but was not a focus of this manuscript, we additionally excluded participants who seroconverted to HIV+ during follow-up. After these exclusions (n=7931), our final sample included 125 674 veterans.

Independent Variable

The presence of MVD was determined on the basis of at least 1 inpatient or 2 or more outpatient VA, VA fee for service, or Medicare *ICD-9* or Current Procedural Terminology codes for peripheral neuropathy or retinopathy related to MVD (Table I

in the online-only Data Supplement) before baseline or within 180 days postbaseline or a urine protein test result of +1 or greater, indicative of proteinuria within 180 days on either side of baseline. PAD was defined using the description from Bali et al,[9] which we have previously used in VACS,[10] less codes for lower-extremity amputation based on presence of 1 or more inpatient or at least 2 outpatient VA, VA fee for service, or Medicare *ICD-9* or Current Procedural Terminology codes before baseline or within 180 days postbaseline (Table II in the online-only Data Supplement). We then defined a 4-level categorical variable to serve as our exposure of interest, with the 4 levels being neither MVD nor PAD (referent), MVD alone, PAD alone, and both MVD and PAD.

Dependent Variable

Our primary outcome, incident lower-extremity amputation, was based on the presence of 1 or more inpatient or at least 2 outpatient VA, VA fee for service, or Medicare *ICD-9* or Current Procedural Terminology codes in the absence of any exclusion codes (Table II in the online-only Data Supplement). The lower-extremity amputation and exclusion codes described by Bali et al[9] were used in this analysis.

Covariates

In the present analysis, age, sex, race/ethnicity, HIV infection, hypertension, diabetes mellitus, lipid levels, smoking status, hepatitis C infection, renal disease, body mass index, anemia, total bilirubin, chronic obstructive pulmonary disease, alcohol abuse or dependence, cocaine abuse or dependence, and prevalent cardiovascular disease served as our covariates. Ascertainment sources and definitions of these variables in VACS have been previously described.[10]

Statistical Analysis

We analyzed descriptive statistics for all variables by baseline MVD and PAD status using χ^2 tests for categorical variables and Kruskal-Wallis tests for continuous variables. Next, we plotted the cumulative incidence of amputation over the follow-up period by baseline vascular disease status and performed log-rank tests to compare the various groups. We determined that there were no serious violations of the proportional hazards assumption through inspection of log([minus]log) plots where we observed approximately parallel lines and then utilized unadjusted and multivariable-adjusted Cox proportional hazards regression models to compare the risk of incident amputation in veterans with at least 1 subtype of vascular disease compared with veterans free of MVD and PAD. Because participants could change vascular disease status during follow-up, the primary analyses included vascular disease status as a time-varying exposure. Our multivariable-adjusted Cox proportional hazards regression model included age, sex, race/ethnicity, established atherosclerotic risk factors, and other comorbidities described above. In supplemental analyses, baseline vascular disease status was assumed constant throughout the follow-up period.

Additional analyses were further limited to participants free of diabetes mellitus, participants free of renal disease, never smokers, and those free of HIV infection, because these factors are associated with MVD, PAD, or both.[10] In further supplemental analyses, we investigated the association between vascular disease groups and incident major adverse cardiac events (MACE) to support our MVD definition in comparison with prior findings regarding the impact of MVD and PAD on MACE. Here, as described by Hawn et al,[11] MACE was defined as acute myocardial infarction or coronary revascularization with and without all-cause mortality. For these analyses, individuals with prevalent cardiovascular disease were excluded (n=19 954) for an analytic sample of 105 720 individuals.

In the present investigation, we used multiple imputation by chained equation techniques that generated 5 complete data sets to handle missing covariate data through predictive mean-matching. A 2-sided *P* value of <0.05 was used to determine statistical significance. Multiple imputation was performed in R version 3.2.5, and all other analyses were performed using SAS version 9.4 (Cary, NC).

RESULTS

There were 125 674 veterans without evidence of amputation before baseline. We analyzed veterans according to the presence or absence of prevalent PAD, MVD, or the combination of PAD and MVD (Table 1). Compared with participants with neither manifestation of vascular disease, participants with PAD, MVD, or the combination were more likely to have hypertension, diabetes mellitus, anemia, and chronic obstructive pulmonary disease.

Amputation

The rate of incident amputation over a median of 9.3 years of follow-up was 1.16 per 1000 person-years. At the time of amputation, retinopathy was present in 69%, nephropathy in 67%, and neuropathy in 78% of participants. In unadjusted analyses that were time-updated for changes in PAD and MVD status to reflect this, the presence of MVD alone was associated with a 6.8-fold (95% CI, 5.6–8.4) increased risk of amputation; PAD alone conferred a 19.7-fold (95% CI, 16.3–23.8) elevated risk of amputation; and the combination of PAD and MVD was associated with a 56.9-fold (95% CI, 47.8–67.8) increased risk of amputation (Table 2). Even after multivariable adjustment for demographic characteristics, cardiovascular disease risk factors, and other potential confounders, our results were highly significant. Compared with those without either vascular disease, the presence of MVD alone was associated with a 3.7-fold (95% CI, 3.0–4.6) increased risk of amputation; PAD alone conferred a 13.9-fold (95% CI, 11.3–17.1) elevated risk of amputation; and, notably, the combination of PAD and MVD was associated with a 22.7-fold (95% CI, 18.3–28.1) increased risk of amputation (Table 2).

The location of amputation also varied by vascular bed involvement (Table 3). Using the type of vascular

Table 1. Baseline Characteristics of Veterans Stratified by PAD and Microvascular Disease

Baseline Characteristic*	No Microvascular Disease or PAD (n = 109 447)	Microvascular Disease Only (n = 9125)	PAD Only (n = 5313)	Microvascular Disease and PAD (n = 1789)
Age, years				
Mean (SD)	49.1 (10.1)	52.7 (9.6)	57.4 (10.4)	58.6 (10.2)
Median	49.0	53.0	56.0	58.0
Male sex, %	97.1	97.8	98.5	99.2
Race/ethnicity, %				
Black	47.2	56.0	46.0	53.9
White	39.5	34.0	44.6	36.2
Hispanic	7.7	6.6	6.1	6.9
Other	5.5	3.4	3.3	3.0
Prevalent CVD, %	12.3	26.4	54.2	65.5
HIV infection, %	29.1	44.7	27.2	32.4
Framingham risk factors, %				
Hypertension†				
None	36.8	18.5	11.8	6.8
Controlled	34.3	43.7	52.7	50.9
Uncontrolled	29.0	37.9	35.5	42.3
Diabetes mellitus	12.9	40.7	32.3	61.3
Lipids, mg/dL†				
LDL cholesterol <100	37.2	47.3	47.5	57.5
LDL cholesterol 100–129	32.3	28.4	29.0	23.8
LDL cholesterol 130–159	20.2	15.6	16.0	12.0
LDL cholesterol ≥160	10.4	8.7	7.5	6.8
HDL cholesterol ≥60	13.3	12.7	11.1	11.6
HDL cholesterol 40–59	44.6	39.0	41.8	36.8
HDL cholesterol <40	42.1	48.4	47.1	51.7
Triglycerides ≥150	40.5	46.1	43.3	44.9
Smoking†				
Current	51.8	49.2	56.8	48.9
Former	17.3	20.4	21.2	25.1
Never	30.9	30.4	22.0	26.0
Other risk factors, %				
HCV infection	17.0	24.9	20.8	24.5
Renal disease, mL/min per 1.73 m^2				
eGFR ≥60	95.1	80.6	83.6	60.6
eGFR 30–59	4.4	13.9	11.5	23.1
eGFR <30	0.5	5.5	5.0	16.3
Body mass index, kg/m^2†				
Mean (SD)	28.3 (5.8)	28.3 (6.6)	28.4 (6.9)	28.6 (6.8)
Median	27.6	27.3	27.6	27.6
Anemia, g/dL†				
Hemoglobin ≥14	68.4	48.5	51.5	35.4
Hemoglobin 12–13.9	26.0	34.4	34.2	37.6
Hemoglobin 10–11.9	4.7	13.3	11.0	20.5
Hemoglobin <10	1.0	3.8	3.3	6.6

(*Continued*)

Table 1. Continued

Baseline Characteristic*	No Microvascular Disease or PAD (n = 109 447)	Microvascular Disease Only (n = 9125)	PAD Only (n = 5313)	Microvascular Disease and PAD (n = 1789)
Bilirubin, mg/dL†				
Mean (SD)	0.7 (0.6)	0.7 (0.6)	0.7 (0.6)	0.6 (0.7)
Median	0.6	0.6	0.6	0.5
History of alcohol abuse, %	26.1	28.9	30.9	28.1
History of cocaine abuse, %	16.2	18.2	16.8	17.2
COPD, %	9.9	15.7	26.7	27.1

Système international conversion factors: To convert HDL and LDL to millimoles per liter, multiply by 0.0259; to convert hemoglobin to grams per liter, multiply by 10; and to convert triglycerides to millimoles per liter, multiply by 0.0113. COPD indicates chronic obstructive pulmonary disease; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and PAD, peripheral artery disease.

*All characteristics were statistically different among veterans in various groups ($P<0.05$) using χ^2 test or Kruskal-Wallis test.

†All variables had complete data except the following. Hypertension data were available on 105 436 (no microvascular disease or PAD), 5240 (PAD only), 9041 (microvascular disease only), and 1771 (micro- and macrovascular disease); LDL cholesterol data were available on 85 544 (no micro- or macrovascular disease), 4529 (PAD only), 8022 (microvascular disease only), and 1589 (micro- and macrovascular disease); HDL cholesterol data were available on 86 627 (no microvascular disease or PAD), 4571 (PAD), 8089 (microvascular disease only), and 1600 (micro- and macrovascular disease); triglyceride data were available on 86 977 (no microvascular disease or PAD), 4603 (PAD only), 8170 (microvascular disease only), and 1623 (micro- and macrovascular disease); smoking data were available on 74 253 (no microvascular disease or PAD), 4000 (PAD only), 6787 (microvascular disease only), and 1373 (micro- and macrovascular disease); eGFR data were available on 98 789 (no micro- or macro vascular disease), 5044 (PAD only), 8883 (microvascular disease only), and 1735 (micro- and macrovascular disease); body mass index data were available on 103 830 (no micro- or macrovascular disease), 5186 (PAD only), 9004 (microvascular disease only), and 1753 (microvascular disease and PAD); anemia data were available on 98 288 (no microvascular disease or PAD), 5056 (PAD only), 8838 (microvascular disease only), and 1719 (micro- and macrovascular disease); and bilirubin data were available on 95 243 (no microvascular disease or PAD), 4925 (PAD only), 8720 (microvascular disease only), and 1709 (micro- and macrovascular disease).

disease at the time of amputation, participants with MVD alone had 18% of all amputations, 21% of below-ankle amputations, 15% of below-knee amputations, and 6% of all above-knee amputations. Participants with PAD alone at baseline had 22% of all amputations, 17% of below-ankle amputations, 25% of below-knee amputations, and 39% of above-knee amputations. The combination of MVD and PAD accounted for 45% of all amputations and caused the most amputations at all limb levels. There was a statistically significant variation in vascular involvement and level of amputation, with MVD more likely to cause a below-ankle amputation and PAD more likely to cause below- and above-knee amputations ($P<0.001$). Further, we assessed the location of MVD at the time of amputation. Amputation at all levels was associated with all manifestations of MVD, alone and in combination (Table III in the online-only Data Supplement). The graded risk of amputation from neither vascular disease to MVD alone, PAD alone, and the combination was maintained after adjustment, using time-updated exposures, in participants without diabetes mellitus, free of renal disease, never smokers, and those free of hypertension and HIV infection (Figure 1).

Analyses assuming baseline vascular disease status remained constant throughout follow-up also demonstrated increased risk of amputation among those with at least 1 vascular disease subtype compared with those free of vascular disease, but the effects were greatly attenuated (Figure 2, Table IV in the online-only Data Supplement) because at study baseline, only 31% of the participants who later required amputation had been diagnosed with MVD.

Diabetes Mellitus

Because of the strong association of diabetes mellitus with MVD, we assessed the graded risk of amputation in subjects with diabetes mellitus as well. Participants with diabetes mellitus comprise 41% of those with MVD alone, 32% of those with PAD alone, and 61% of those with MVD and PAD compared with 13% of those with neither manifestation. Time-updating vascular disease status reinforced the role of both MVD and PAD in lower-extremity amputation incidence in participants with diabetes mellitus. After multivariable adjustment, compared with those diabetic participants without either vascular disease, the presence of MVD alone was associated with a 3.1-fold (95% CI, 2.4–4.0) increased risk of amputation; PAD alone conferred a 7.9-fold (95% CI, 5.9–10.5) elevated risk of amputation; and, even more impressively, the combination of PAD and MVD was associated with a 15.9-fold (95% CI, 12.4–20.6) increased risk of amputation (Table V in the online-only Data Supplement).

MACEs

The rate of incident MACE over a median 9.2 years of follow-up was 24 events per 1000 person-years. In unadjusted analyses, compared with those without vascular disease, the presence of MVD alone was

Table 2. Time-Updated Risk of Amputation by Combination of Microvascular Disease and PAD

Group	Person-Years	Events	Incidence Rate per 1000 Person-Years	Unadjusted Model		Adjusted Model	
				Hazard Ratio (95% CI)	*P* Value	Hazard Ratio (95% CI)	*P* Value
No microvascular disease or PAD	795 802	182	0.23	1.00	—	1.00	—
Microvascular disease	130 584	207	1.59	6.84 (5.60–8.35)	<0.0001	3.74 (3.03–4.62)	<0.0001
PAD	58 395	265	4.54	19.68 (16.25–23.83)	<0.0001	13.86 (11.25–17.07)	<0.0001
Microvascular disease and PAD	40 182	531	13.22	56.92 (47.80–67.78)	<0.0001	22.71 (18.34–28.12)	<0.0001

The adjusted model includes age, sex, race, HIV status, prevalent cardiovascular disease, hypertension, diabetes mellitus, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, smoking status, hepatitis C virus infection, renal failure, body mass index, anemia, total bilirubin, alcohol abuse or dependence, cocaine abuse or dependence, and chronic obstructive pulmonary disease. PAD indicates peripheral artery disease.

associated with a 2.0-fold (95% CI, 1.9–2.1) increased risk of MACE; PAD alone conferred a 2.7-fold (95% CI, 2.5–2.9) elevated risk of MACE; and the combination of PAD and MVD was associated with a 3.9-fold (95% CI, 3.4–4.3) increased risk of MACE (Table VI in the online-only Data Supplement). After adjustment for demographic characteristics, cardiovascular disease risk factors, and other potential confounders, the association was attenuated for all vascular disease subsets but remained statistically significant. These results were similar when death was removed from the MACE end point (Table VI in the online-only Data Supplement).

DISCUSSION

In this cohort of veterans, the presence of MVD was associated with a significantly increased risk of lower-extremity amputation. The increase in amputation risk among those with MVD was independent of the presence of PAD, augmented the risk when PAD was present, and remained robust after adjusting for demographics, cardiovascular risk factors, and other factors associated with vascular disease. In contrast, adjusting for similar covariates attenuated the association between MVD and MACE, and MVD did not augment the MACE risk when added to PAD.

In this cohort, we show that MVD is common and strongly associated with adverse limb events in a manner that is potentiated in participants with concomitant PAD. This suggests that MVD plays an important and independent role in creating conditions that place patients at risk for amputation. In a large series of patients with critical limb ischemia undergoing revascularization, 21% did not have evidence of reduced perfusion pressure,[12] numbers similar to our population. MVD may play an important role in determining the PAD population at particularly high risk for critical limb ischemia and amputation in addition to suggesting the need for active foot surveillance in patients with MVD alone. In our cohort, more than 40% of all amputations occurred in the group with both PAD and MVD, despite representing about 4% of the population. The novelty of these findings becomes clear when put into the current framework of critical limb ischemia. In a recent state-of-the-art review of critical limb ischemia, MVD as a whole or its components did not receive a single mention.[13] Our work shows that MVD helps identify a population not previously considered at particularly high risk for amputation and, when added to PAD, identifies a group of patients at very high risk for amputation. One take-home message from this work is that MVD alone is associated with 18% of all amputations and 15% of all below-knee amputations, implicating MVD as an important risk for amputation. The results of this work provide additional avenues of inquiry to reduce rates of amputation.

In contrast to the strong association between MVD and amputation, we describe a more modest relationship for myocardial infarction, coronary revascularization, and death. Our findings are similar to those previously reported and provide a positive control for our definition, link our definition for MVD to those

Table 3. Level of Amputation by Type of Vascular Disease Manifestation at Time of Amputation

Vascular Disease Manifestation	Level of Amputation			
	Below Ankle	Below Knee	Above Knee	Unknown Location
No microvascular disease or PAD	110 (15%)	34 (14%)	31 (16%)	7 (26%)
Microvascular disease only	152 (21%)	37 (15%)	11 (6%)	7 (26%)
PAD only	124 (17%)	62 (25%)	73 (39%)	6 (22%)
Microvascular disease and PAD	333 (46%)	117 (47%)	74 (39%)	7 (26%)
Number of amputations	719	250	189	27

Cells contain N (column percent). There is an association between level of amputation and vascular disease manifestation (χ^2=60.5, *df*=9, *P*<0.0001). PAD indicates peripheral artery disease.

Risk of Amputation by combination of Microvascular Disease and PAD in subsets of VACS Participants

Subset	Group	Person-Years	Events	Hazard Ratio [95% CI]	p-value
	No Microvascular Disease or PAD	747,310	124	1.00	
Free of	Microvascular Disease Only	51,532	26	2.27 [1.48, 3.51]	<0.0001
Diabetes	PAD Only	50,782	181	15.72 [12.00, 20.58]	<0.0001
	Microvascular Disease and PAD	15,254	99	24.29 [18.04, 32.71]	<0.0001
	No Microvascular Disease or PAD	602,720	214	1.00	
Free of	Microvascular Disease Only	62,159	106	2.44 [1.92, 3.12]	<0.0001
Renal Disease	PAD Only	49,084	246	9.21 [7.47, 11.36]	<0.0001
	Microvascular Disease and PAD	20,210	261	14.53 [11.76, 17.95]	<0.0001
	No Microvascular Disease or PAD	174,635	50	1.00	
Never	Microvascular Disease Only	18,396	39	2.66 [1.69, 4.16]	<0.0001
Smoker	PAD Only	10,793	46	8.37 [5.31, 13.19]	<0.0001
	Microvascular Disease and PAD	5817	97	16.00 [10.44, 24.52]	<0.0001
	No Microvascular Disease or PAD	306,787	54	1.00	
Free of	Microvascular Disease Only	16,137	8	1.79 [0.85, 3.80]	0.13
Hypertension	PAD Only	10,957	37	12.38 [7.34, 20.89]	<0.0001
	Microvascular Disease and PAD	2948	26	22.49 [12.73, 39.76]	<0.0001
	No Microvascular Disease or PAD	615,787	228	1.00	
HIV	Microvascular Disease Only	46,446	102	2.49 [1.94, 3.20]	<0.0001
Uninfected	PAD Only	51,669	249	8.18 [6.66, 10.05]	<0.0001
	Microvascular Disease and PAD	21,212	344	13.72 [11.13, 16.90]	<0.0001

Figure 1. Risk of amputation by time-updated combination of microvascular disease and PAD in subsets of VACS participants.
Specific subsets include those who were free of diabetes mellitus, free of renal disease (estimated glomerular filtration rate > 60), never smokers, free of hypertension, and free of HIV infection. PAD indicates peripheral artery disease; and VACS, Veterans Aging Cohort Study.

previously reported, and help validate our definition for MVD.[7,14]

MVD may modify symptomatic status importantly in patients with atherosclerosis more generally.[15] For example, coronary microvascular dysfunction is a stronger predictor of symptom severity than measured stenosis in patients with CAD[16–19] and associates well with functional capacity even in patients without CAD.[20] When measured in more than 900 patients, coronary flow reserve predicted cardiovascular death and heart failure,

Figure 2. Cumulative incidence of amputation by baseline microvascular disease and PAD status.
Kaplan-Meier survival curves illustrating the time to first amputation incident over 9.3 median years of follow-up for veterans with neither microvascular disease nor PAD, microvascular disease alone, PAD alone, and microvascular disease and PAD at baseline. PAD indicates peripheral artery disease.

but not myocardial infarction.[21] MVD may represent an important mediator of symptoms in both CAD and PAD and a target for new therapies.

We examined the impact of MVD in subpopulations free of conditions known to cause MVD. Notably, the risk of amputation was similar to the cohort as a whole in participants who never smoked, were free of diabetes mellitus, were free of renal disease, and were free of hypertension. These results suggest the risk of amputation associates with MVD, per se. Indeed, the organizing principle may be that symptoms are dependent on MVD rather than the specific disease that may cause it.[22]

One important question in these findings is the role of diabetes mellitus. Our data demonstrate a similar increase in associated risk of amputation in the presence or absence of diabetes mellitus. Diabetes mellitus is an important contributor to the MVD and PAD population burden and provides multiple mechanisms by which amputation may develop. Although we cannot exclude the possibility of patients with undiagnosed diabetes mellitus, the likelihood that undiagnosed diabetes mellitus contributes significantly to the findings in our nondiabetic population is modest. The validated definition of diabetes mellitus we use[23] considers glucose measurements, antidiabetic agent use, or at least 1 inpatient or at least 2 outpatient *ICD-9* codes for this diagnosis.

Interestingly, participants with diabetes mellitus represent the minority of patients who develop MVD in our cohort. This finding mirrors other work in MVD prevalence. In the Diabetes Prevention Program, approximately 8% of all participants with prediabetes had retinopathy, similar to the number of prediabetic participants with retinopathy in the Gutenberg Health Study in Germany.[24,25] In the PREVEND study (Prevention of Renal and Vascular Endstage Disease), 7% of the participants had evidence of microalbuminuria.[7] Of 3200 participants in PREVEND with urinary albumin excretion of >20 mg/L, 7% had diabetes mellitus, 21% had hypertension, and 82% had neither. Diabetes mellitus remains an important contributor to MVD development, but focusing on it alone may miss opportunities for amputation prevention present in a larger population at risk.

This work advances the notion that microvascular dysfunction may be a systemic phenomenon that leads to adverse clinical events akin to those described for conduit arteries.[26–28] Dysfunction of the microvasculature in beds remote from clinical presentation has been similarly demonstrated. Both retinal arteriolar and skin arteriolar dysfunction directly correlate with albuminuria, in the presence or absence of diabetes mellitus.[29] The presence of retinopathy[30] and nephropathy[31,32] has been shown to associate inversely with coronary flow reserve. With particular relevance to amputation, both retinopathy and nephropathy are associated with impaired skin microvessel function and lower-extremity amputation.[33–35] We surmise that clinical evidence of MVD diagnosed in any vascular bed increases the risk for dermal microvascular dysfunction, poor wound healing, and amputation. We believe that MVD is a systemic phenomenon. Viewed as such, our work extends the multiple previous observations of individual bed MVD and amputation.[36] We aggregated the 3 major MVD manifestations into a single phenotype, demonstrating the independent association of this combined MVD phenotype and amputation, and showed its additive effect to PAD in fostering adverse limb outcomes. Indeed, similar to atherosclerosis, the associated risk of amputation increases with the presence of symptoms in more than 1 microvascular bed, raising the possibility of a similar polyvascular disease phenomenon.

There are some limitations of this work to be noted. First, the diagnostic codes used to identify both PAD and MVD do not find wide agreement in the literature. For PAD, we have used the criteria defined by Bali et al[9] and Beckman et al.[10] The criteria we selected for MVD are listed in the online-only Data Supplement. We would submit that missing MVD cases would likely be distributed evenly through the groups, would bias the results toward the null, and should not significantly affect the findings of this study. A single, unifying MVD phenotype has not been studied before; however, our MVD definition associated with MACE events similar to other cohorts of MVD components, supporting our contention that it is a valid marker. Because our study population is a cohort of veterans, our results are most generalizable to a cohort of men with a relatively high burden of disease. Finally, because this is an observational cohort, we cannot exclude the possibility of residual or unmeasured confounding.

We conclude that in the VACS cohort, the diagnosis of MVD alone increased the risk of amputation, further increased the risk in patients with PAD, and identified a greater population of patients than previously recognized at risk for amputation. MVD had a modest effect on MACE and did not increase the risk beyond the diagnosis of PAD. MVD likely participates importantly in the development of adverse limb events in PAD and suggests additional patient populations who may benefit from greater foot surveillance to minimize amputation.

ARTICLE INFORMATION

Received March 6, 2019; accepted May 20, 2019.

Guest Editor for this article was Filippo Crea, MD.

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The online-only Data Supplement is available with this article at https://www.ahajournals.org/doi/suppl/10.1161/circulationaha.119.040672.

Correspondence

Joshua A. Beckman, MD, 1215 21st Avenue South, MCE 5472, Nashville, TN 37215. Email Joshua.a.beckman@vanderbilt.edu

Affiliations

Division of Cardiovascular Medicine (J.A.B., M.S.D., Q.S.W., A.W.A., M.S.F.), Division of General Internal Medicine and Public Health (H.A.T.), Department of Pharmacology (J.V.B.), Vanderbilt University, Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine (D.H.W.), Nashville, TN. Department of Surgery, Perelman School of Medicine, University of Pennsylvania (S.M.D.) and Corporal Michael Crescenz VA Medical Center (S.M.D.), Philadelphia. Infectious Disease Section, VA North Texas Health Center and University of Texas Southwestern Medical Center, Dallas (R.J.B.). Department of Medicine, Weill Cornell Medical College, New York, NY (A.A.B). Atlanta Veterans Affairs Medical Center and Emory University School of Medicine, GA (V.C.M.). VA Connecticut Healthcare System, West Haven (J.J.S.). Yale School of Medicine, Departments of Neurology and Internal Medicine, New Haven, CT. Geriatric Research Education and Clinical Centers, Veterans Affairs Tennessee Valley Healthcare System, Nashville (H.A.T.). University of Colorado School of Medicine and Colorado Prevention Center Clinical Research, Aurora (M.P.B.).

Sources of Funding

This work was supported by American Heart Association Strategically Focused Research Network in Vascular Disease grants 18SFRN33960373 (to Drs Beckman, Freiberg, Wasserman, Wells, and Barnett), HL131977 (to Dr Beckman), and HL125032 and U01 AA020790 (to Dr Freiberg). This work was also supported by US Department of Veterans Affairs grant IK2-CX001780 (to Dr Damrauer). This publication does not represent the views of the Department of Veterans Affairs or the United States government.

Disclosures

Dr Beckman reports consulting with AstraZeneca, Bristol Myers Squibb, Amgen, Merck, Sanofi, Antidote Pharmaceutical, and Boehringer Ingelheim. He serves on the Data Safety Monitoring Committee for Bayer and Novartis. The other authors report no conflicts.

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