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Pathology of Peripheral Artery Disease in Critical Limb Ischemia

Navneet Narula, MD,[a,b] Andrew J. Dannenberg, MD,[a] Jeffrey W. Olin, DO,[c] Deepak L. Bhatt, MD, MPH,[d] Kipp W. Johnson, BS,[c] Girish Nadkarni, MD,[c] James Min, MD,[a] Sho Torii, MD,[e] Priti Poojary, MD, MPH,[c] Sonia S. Anand, MD,[f] Jeroen J. Bax, MD, PhD,[g] Salim Yusuf, MD,[f] Renu Virmani, MD,[e] Jagat Narula, MD, PhD[c]

ABSTRACT

BACKGROUND Critical limb ischemia (CLI) is the most serious complication of peripheral artery disease (PAD).

OBJECTIVES The purpose of this study was to characterize pathology of PAD in below- and above-knee amputation specimens in patients presenting with CLI.

METHODS Peripheral arteries from 95 patients (121 amputation specimens) were examined; 75 patients had presented with CLI, and the remaining 20 had amputations performed for other reasons. The pathological characteristics were separately recorded for femoral and popliteal arteries (FEM-POP), and infrapopliteal arteries (INFRA-POP).

RESULTS A total of 299 arteries were examined. In the 239 arteries from CLI patients, atherosclerotic plaques were more frequent in FEM-POP (23 of 34, 67.6%) compared with INFRA-POP (79 of 205, 38.5%) arteries. Of these 239 arteries, 165 (69%) showed ≥70% stenosis, which was due to significant pathological intimal thickening, fibroatheroma, fibrocalcific lesions, or restenosis in 45 of 165 (27.3%), or was due to luminal thrombi with (39 of 165, 23.6%) or without (81 of 165, 49.1%) significant atherosclerotic lesions. Presence of chronic luminal thrombi was more frequently observed in arteries with insignificant atherosclerosis (OR: 16.7; $p = 0.0002$), more so in INFRA-POP compared with FEM-POP (OR: 2.14; $p = 0.0041$) arteries. Acute thrombotic occlusion was less frequently encountered in INFRA-POP than FEM-POP arteries (OR: 0.27; $p = 0.0067$). Medial calcification was present in 170 of 239 (71.1%) large arteries.

CONCLUSIONS Thrombotic luminal occlusion associated with insignificant atherosclerosis is commonly observed in CLI and suggests the possibility of thromboembolic disease. The pathological characteristics of arteries in CLI suggest possible mechanisms of progression of PAD to CLI, especially in INFRA-POP arteries, and may support the preventive role of antithrombotic agents. (J Am Coll Cardiol 2018;■:■–■) © 2018 Published by Elsevier on behalf of the American College of Cardiology Foundation.

From the [a]New York Presbyterian Hospital and Weill Cornell Medicine, New York, New York; [b]NYU Langone Medical Center, New York, New York; [c]Mount Sinai Heart and Icahn School of Medicine at Mount Sinai, New York, New York; [d]Brigham & Women's Hospital and Harvard Medical School, Boston, Massachusettes; [e]Cardiovascular Pathology Inc., Gaithersburg, Maryland; [f]Population Health Research Institute and McMaster University, Hamilton, Ontario, Canada; and the [g]Leiden University Medical Center, Leiden, the Netherlands. Dr. Olin has served on the International Steering Committee for the Euclid Trial, sponsored by AstraZeneca; has served on the Medical Advisory Board of Janssen Pharmaceuticals; and has served on the Steering Committee of the TRAP 2 Prevention Trial sponsored by Merck. Dr. Deepak L. Bhatt has served on the advisory board of Cardax, Elsevier Practice Update Cardiology, Medscape Cardiology, and Regado Biosciences; has served on the board of directors of Boston VA Research Institute, Society of Cardiovascular Patient Care, and TobeSoft; has served as chair of American Heart Association Quality Oversight Committee; has served on the data monitoring committees for Baim Institute for Clinical Research (formerly Harvard Clinical Research Institute, for the PORTICO trial, funded by St. Jude Medical, now Abbott), Cleveland Clinic, Duke Clinical Research Institute, Mayo Clinic, Mount Sinai School of Medicine, and Population Health Research Institute; has received honoraria from the American College of Cardiology (Senior Associate Editor, Clinical Trials and News, ACC.org; Vice-Chair, ACC Accreditation Committee), Baim Institute for Clinical Research (formerly Harvard Clinical Research Institute; RE-DUAL PCI clinical trial steering committee funded by Boehringer Ingelheim), Belvoir Publications (Editor in Chief, Harvard Heart Letter), Duke Clinical Research Institute (clinical trial steering committees), HMP Global (Editor in Chief, Journal of Invasive Cardiology), Journal of the American College of Cardiology (Guest Editor; Associate Editor), Population Health Research Institute (for the COMPASS operations committee, publications committee, steering committee, and USA national co-leader, funded by Bayer), Slack Publications (Chief Medical Editor, Cardiology Today's Intervention), Society of Cardiovascular Patient Care (Secretary/Treasurer),

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ABBREVIATIONS AND ACRONYMS

AIT = adaptive intimal thickening

AKA = above-knee amputation

ALI = acute limb ischemia

BKA = below-knee amputation

CAD = coronary artery disease

CLI = critical limb ischemia

FA = fibroatheroma

FC = fibrocalcific plaque

FEM-POP = femoral and popliteal arteries

INFRA-POP = infrapopliteal arteries

PAD = peripheral artery disease

PIT = pathological intimal thickening

Peripheral artery disease (PAD) accounts for a large proportion of cardiovascular disease (CVD) prevalence in most world regions (1). Global estimates suggest that >200 million people worldwide were afflicted with PAD by 2010. From 2000 to 2010, this represented a 28.7% increase in prevalence in low- and middle-income countries and a 13.1% increase in high-income countries (2,3). The prevalence is even higher compared with coronary artery disease (CAD), as 110.55 million prevalent cases were reported for the same period (1). The reasons for the underappreciation of PAD include low awareness and screening rates by primary care physicians, and classic claudication occurs infrequently and, when there is claudication present, it is often assumed to be due to musculoskeletal problems in older individuals. Only a small proportion of patients with chronic stable PAD progress to acute events. Whereas there were an estimated 7 million acute myocardial infarctions and 8.92 million deaths due to CAD, only 52,500 deaths were reported from PAD in 2015. The morbidity, procedural interventions, hospitalization, and readmission rates are high, and quality-of-life indexes are poor in PAD patients. About one-fourth of the PAD population experience intermittent claudication, but only about 1% to 3% develop critical limb ischemia (CLI). Clinical follow-up of CLI patients over the ensuing 1 year is associated with high mortality, frequent amputation, or continuing CLI, but resolution of symptoms occurs in only 25% of patients (3,4).

There are multiple arteries in the lower extremity, and there is significant potential for collateralization. Therefore, it is important to understand the mechanisms that simultaneously affect several arterial territories resulting in CLI, and such understanding is necessary to develop strategies that help prevent progression of asymptomatic or symptomatic PAD to CLI (5). Little is known about the pathology and plaque composition of arteries of lower extremities in PAD patients, and it is generally assumed to be similar to the pathology of atherosclerosis in the coronary and carotid arteries (6-10). The present study was performed in above-knee amputation (AKA) and below-knee amputation (BKA) specimens to describe the pathology of lower-extremity arteries in patients with CLI to better understand the mechanisms leading to amputation in patients with CLI.

METHODS

PATIENTS AND AMPUTATION SPECIMENS. In this retrospective study, the lower-extremity arteries from patients who underwent amputation at Weill Cornell Medicine from 2014 to 2017 were examined. Based on the clinical history obtained from the electronic medical record, the amputations from patients with CLI (Rutherford category 4 to 6), acute limb ischemia (ALI) (Rutherford category 3), gangrene due to alternative causes that were not PAD-related (gangrene, non-PAD), and amputations for causes other than gangrene (nongangrene, non-PAD) were included in this study. The charts were also reviewed for age, sex, and the presence of risk factors for atherosclerosis (diabetes, hyperlipidemia, hypertension, smoking history) as documented in the history. The documented history of CAD, cerebrovascular disease, and chronic kidney disease was also recorded. Smoking was considered to be present in patients who were either former or current

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smokers. Institutional Review Board approval was obtained from Weill Cornell Medicine.

Arteries from 121 amputations from 95 patients were available for analysis. Of these, there were 98 amputation specimens from 75 patients with CLI. The amputations were performed in the remaining 20 of 95 patients for ALI (3 patients, 3 amputation specimens), gangrene of non-PAD origin (9 patients, 12 amputations specimens), and nongangrene non-PAD causes (8 patients, 8 amputations). In the CLI group, there were 27 AKA and 71 BKA specimens; 19 patients in the CLI group had more than 1 amputation procedure performed either on the same day (bilateral amputations), or subsequently as revised ipsilateral amputation and contralateral amputation. The ALI group included 2 AKA and 1 BKA. The gangrene non-PAD group comprised of 11 BKA and 1 AKA specimens, and all 8 patients in the nongangrene, non-PAD group underwent BKA.

The pathological characteristics were recorded separately for the femoral and popliteal arteries (FEM-POP) from AKA specimens and the anterior tibial, posterior tibial, peroneal, and dorsalis pedis (INFRA-POP) arteries from both AKA and BKA specimens. Arteries that were present on the sections submitted from the specimen but not specifically designated in the gross description were designated as unspecified arteries; 11 unspecified arteries in the CLI group from below-knee amputations were included in the INFRA-POP group and the unspecified artery from 1 patient with revised AKA was included in the FEM-POP group. The arteries were fixed in formalin and decalcified when necessary using the standard protocol. The arteries were serially sectioned along the length of the artery, and the segment from each artery with the maximum stenosis was embedded in paraffin. From the paraffin block, 5-μm tissue sections were prepared and stained with hematoxylin and eosin; Movat pentachrome and/or Verhoeff's elastic stains were performed in a selected few.

HISTOPATHOLOGICAL EVALUATION OF LARGE ARTERIES. A detailed histopathological characterization of 299 arteries, 1 section per artery with the most severe stenosis, was undertaken. Of these, 239 arteries were from the CLI group and included 205 INFRA-POP and 34 FEM-POP arteries (Table 1). The remaining 60 arteries were obtained from amputation specimens from ALI (n = 10), gangrene non-PAD (n = 29), and nongangrene non-PAD (n = 21) patients. When there was more than 1 lesion in the same artery with an equally severe luminal stenosis, then the lesion with luminal thrombosis (if any) was analyzed as it was more likely to be the culprit lesion. The percent luminal stenosis was estimated visually at low power magnification (×20).

Atherosclerotic plaques when present, were classified according to the modified AHA classification (11) as adaptive intimal thickening (AIT), pathological intimal thickening (PIT), fibroatheroma (FA), or fibrocalcific plaque (FC) (Figure 1A). Briefly, AIT was defined as the presence of smooth muscle cells in a proteoglycan matrix, whereas PIT was defined as the presence of smooth muscle cells with extracellular lipid. FA were identified by a well-defined fibrous cap with an underlying necrotic core. Plaques rich in collagen and calcification were classified as FC plaques. Arteries with AIT and normal intima were classified as insignificant atherosclerosis, and those with PIT, FA, and FC plaques were classified as significant atherosclerosis.

The presence or absence of luminal thrombi was noted (Figure 1B). The thrombi were classified as acute or organizing when predominant fibrin or at least residual fibrin was seen. Acute thrombi demonstrated fibrin with platelets and entrapped red blood cells. The organizing thrombi showed ingrowth of endothelial cells and smooth muscle cells. On the other hand, the luminal thrombi were described as chronic (also called organized) when they consisted of capillary channels, inflammatory cells, and smooth muscle cells and collagen in varying proportions. The presence or absence of an atherosclerotic plaque in a thrombosed artery (both acute and chronic thrombi) and the type of plaque were noted. An acute thrombus secondary to plaque rupture was defined as that associated with a disrupted fibrous cap showing communication with the underlying necrotic core or when an acute luminal thrombus was associated with a fibroatheromatous plaque even if a rupture site was not identified in the section. Arteries with acute or organizing thrombi were considered as 100% stenosis even if the thrombus was not completely occlusive. Restenotic lesions were defined as proliferation of myofibroblasts in a myxoid matrix in patients with history of prior intervention.

The type and extent of calcification in the neointimal plaques and also in the medial layer of the large arteries was semiquantitatively assessed. Calcification in the neointima was classified as microcalcification (calcium particles <15 μm), punctate calcification (≥15 μm and <1 mm), and fragment-like calcium (≥1 mm); sheet calcium was described when the calcific fragment involved >1 quadrant of the vessel. The degree of circumferential involvement of both the intimal and medial calcification was noted and was graded as <25%, ≥25 to <50%, ≥50 to <75%, and ≥75% of the vascular circumference.

TABLE 1 Vessel Pathology in Patients Presenting With CLI

	Stenosis ≥70% (n = 165)				All Stenosis (n = 239)			
	FEM-POP (n = 31)	INFRA-POP (n = 134)	Odds Ratio*	p Value*	FEM-POP (n = 34)	INFRA-POP (n = 205)	Odds Ratio*	p Value*
Insignificant atherosclerosis	9 (29.0)	72 (53.7)			10 (29.4)	121 (59.02)		
Atherosclerotic lesions	21 (67.7)	60 (44.8)	3.3	0.0262	23 (67.6)	79 (38.5)	5.47	0.00284
PIT	0 (0.0)	1 (1.7)			1 (4.3)	8 (10.1)		
Fibroatheroma	9 (42.9)	20 (33.3)			9 (39.1)	24 (30.4)		
Fibrocalcific lesions	12 (57.1)	39 (65.0)			13 (56.5)	47 (59.5)		
Restenotic lesions	1 (3.2)	2 (1.5)			1 (2.9)	5 (2.4)		
	FEM-POP (n = 31)	**INFRA-POP (n = 134)**	**Odds Ratio***	**p Value***	**FEM-POP (n = 34)**	**INFRA-POP (n = 205)**	**Odds Ratio***	**p Value***
No medial calcification	13 (41.9)	37 (27.6)			14 (41.2)	55 (26.8)		
Medial calcification	18 (58.1)	97 (72.4)	2.38	0.16457	20 (58.8)	150 (73.2)	2.89	0.0799
	FEM-POP (n = 31)	**INFRA-POP (n = 134)**	**Odds Ratio†**	**p Value†**	**FEM-POP (n = 34)**	**INFRA-POP (n = 205)**	**Odds Ratio†**	**p Value†**
Medial calcification, extent								
No calcification	13 (41.9)	37 (27.6)	3.94	0.00589	14 (41.2)	55 (26.8)	3.35	0.00632
<25% of circumference	9 (29.0)	22 (16.4)			10 (29.4)	30 (14.6)		
≥25 to <50% of circumference	4 (12.9)	14 (10.4)			4 (11.8)	19 (9.3)		
≥51 to <75% of circumference	2 (6.5)	18 (13.4)			2 (5.9)	26 (12.7)		
≥75% of circumference	3 (9.7)	43 (32.1)			4 (11.8)	75 (36.6)		

Values are n (%). *Computed using a hierarchical generalized linear model with a logistic link function, controlling for fixed effects of age, sex, hypertension, diabetes, hyperlipidemia, congestive heart failure, cerebrovascular disease, ischemic heart disease, and smoking history. Nested random effects were included per patient and per surgical procedure to control for within-patient and within-surgical-procedure correlations. The odds ratio may be interpreted as the increased odds for plaque type of insignificant atherosclerosis (Table 1) or increased odds for medial calcification in infrapopliteal artery samples vs. femoral and popliteal vessel samples. †Computed with hierarchical generalized linear regression model with a cumulative logit link function and random effects fit via the Laplace method, controlling for fixed effects of age, sex, diabetes, hypertension, hyperlipidemia, congestive heart failure, cerebrovascular disease, ischemic heart disease, and smoking history. We included a random effect per patient and per surgical procedure to control for within-patient and within-surgical-procedure correlations. The odds ratio may be interpreted as the increased odds for extent of medial calcification in the infrapopliteal artery samples compared to femoral and popliteal artery samples.

FEM-POP = femoral and popliteal arteries; INFRA-POP = infrapopliteal arteries; PIT = pathological intimal thickening.

The arterial sections were reviewed by 2 pathologists (N.N. and R.V.).

HISTOPATHOLOGICAL EVALUATION OF SUBCUTANEOUS SMALL VESSELS. Small subcutaneous arteries, arterioles, and venules were evaluated for medial calcification and intimal fibrosis, luminal occlusion resulting from thrombi, and cholesterol emboli (Figures 1A and 1B). Small artery calcification was considered to be present when observed in any section from a given patient. Calciphylaxis was defined as the presence of calcification of small arterioles, venules, and small subcutaneous arteries in addition to calcification of soft tissue.

STATISTICAL METHODS. All statistical analysis was performed using the R Statistics Programming software version 3.4.0 (R Foundation for Statistical Computing, Vienna, Austria) (12). Because much of our analysis was considered at the artery level, it was vital to control for within-patient and within-procedure correlations. Thus, we used generalized linear mixed effect regression models with random effects nested: 1) per limb procedure; and 2) per patient to account for these correlations. Procedures were considered to be different if they occurred on different limbs, or if an initial surgical procedure was later revised on the same limb (for example, from BKA to AKA). Specifically, we used the R package *lme4* to fit generalized mixed binomial regression models with a logistic link function and the R package *ordinal* to fit mixed ordinal regression models with a cumulative logistic link function (13). When variables could be analyzed as either binary variables or as ordinal variables considering the extent of presence, we generally preferred to analyze as ordinal variables to obtain more accurate, powerful, and clinically relevant effect estimates. In our mixed-effect regression models, we controlled for fixed effects of age, sex, hypertension, hyperlipidemia, diabetes, renal disease, smoking history, ischemic heart disease history, cerebrovascular disease history, and heart failure disease history. In models for acute and chronic thrombi, for computational reasons we could not obtain model convergence when considering diabetes as a fixed effect, and so this variable was omitted. The p values for regression model fixed

effects were obtained using the lmerTest package, which implements the Satterthwaite approximation to the effective degrees of freedom (14). Following standard procedure, we exponentiated regression coefficients to obtain the corresponding odds ratios for binary and ordinal regression models (15). We interpreted all p values in the context of their corresponding effect size (odds ratio estimates). All p values were derived from 2-tailed tests.

RESULTS

CLINICAL CHARACTERISTICS OF CLI PATIENTS. The median age of the 75 patients was 75 years (mean 70.3 ± 12.7 years; range: 37 to 92 years), of which 65.3% were male. Clinical history included high rates of diabetes (n = 59; 79.7%), hypertension (n = 58; 77.3.7%), hyperlipidemia (n = 53; 70.7%), and smoking (n = 40; 53.3%). Of these 75 patients, 41 (54.7%) had a clinical history of CAD, 20 (26.7%) cerebrovascular disease, and 5 (6.7%) had heart failure of unspecified etiology; 48 patients had documented kidney disease (64.0%).

SIGNIFICANT ATHEROSCLEROTIC LESIONS ESPECIALLY IN INFRA-POP ARTERIES WERE NOT COMMON IN CLI. Characteristics of the 239 arteries from CLI patients is shown in Table 1; 205 were INFRA-POP and 34 were FEM-POP arteries. Significant atherosclerotic lesions (including PIT, FA, and FC) were present in 23 of 34 (67.6%) FEM-POP arteries and 79 of 205 (38.5%) INFRA-POP arteries. The multivariable-adjusted odds ratio (OR) for increased odds of insignificant atherosclerosis in INFRA-POP arteries versus FEM-POP arteries was 5.47 (p = 0.00284). Pure restenotic lesions were present in 6 arteries.

Calcification within the atherosclerotic plaques was observed in 80% of the FEM-POP and 70.6% of the INFRA-POP lesions. We did not observe a significant difference between calcification type (microcalcification, punctate calcification, fragmented calcification, and plate calcification) or extent of plaque calcification between INFRA- and FEM-POP arteries.

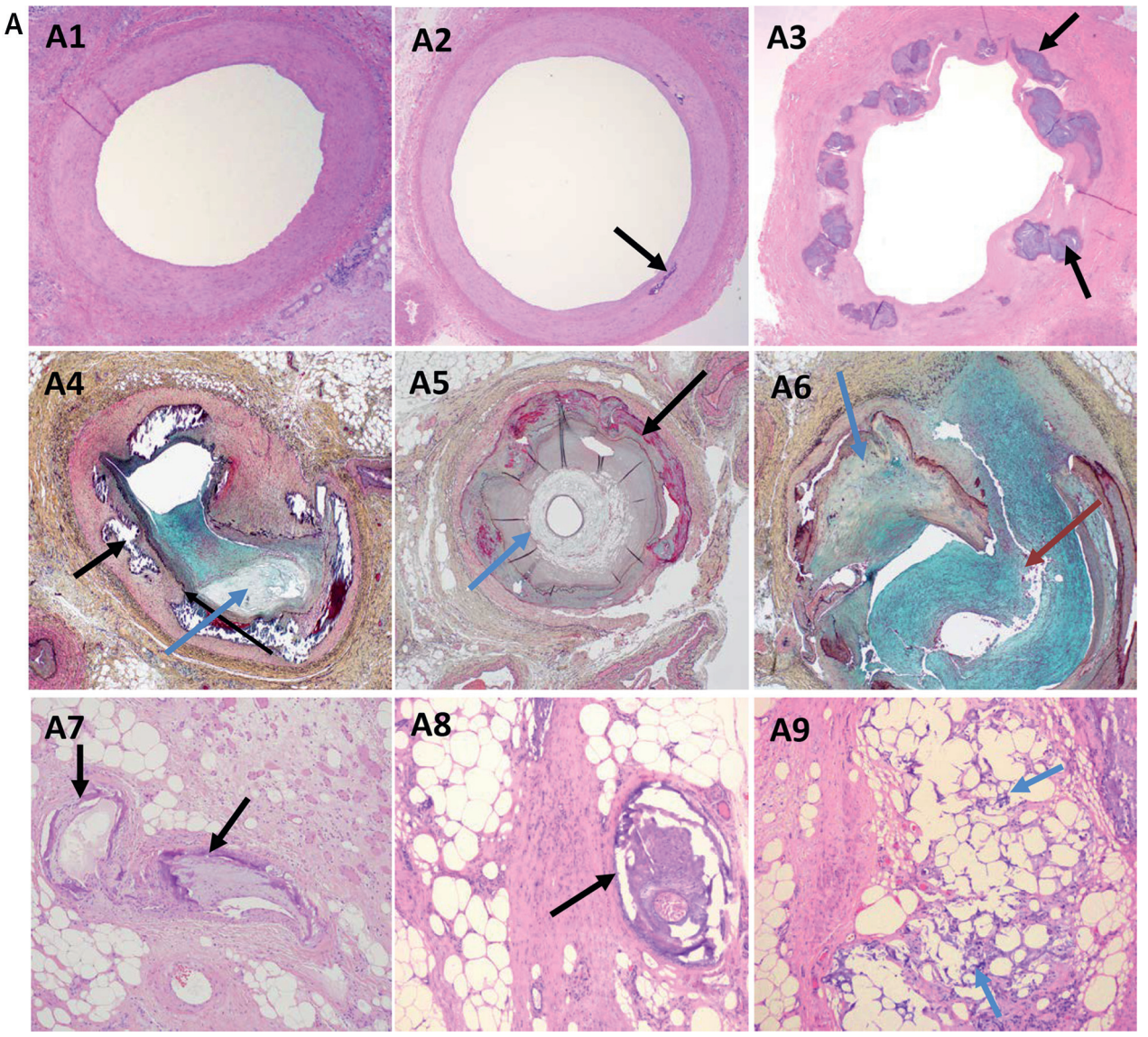
A total of 165 of 239 (69%) arteries showed ≥70% luminal stenosis. Of these 165 arteries, 134 were INFRA-POP and 31 were FEM-POP arteries. Even in the presence of ≥70% luminal stenosis, insignificant atherosclerosis was seen in 72 of 134 (53.7%) INFRA-POP arteries and 9 of 31 (29.0%) of the FEM-POP arteries; in these 72 + 9 arteries, luminal stenosis of ≥70% resulted from presence of thrombi associated with insignificant vessel wall pathology. In arteries with ≥70% luminal stenosis, the multivariable-adjusted OR for increased odds of insignificant compared with significant atherosclerosis in INFRA-POP arteries versus FEM-POP arteries was 3.3 (p = 0.0262).

THROMBOTIC OCCLUSION REGARDLESS OF VESSEL WALL PATHOLOGY IS COMMON IN PAD, ESPECIALLY IN INFRA-POP ARTERIES. Of the 165 arteries with ≥70% luminal stenosis, acute or chronic thrombi were observed in 120 arteries (72.7%) (Table 2, Figure 1B, Figure 2). In the remaining 45 arteries with ≥70% luminal compromise, the stenosis was caused by significant atherosclerosis without acute or chronic thrombi in 42 (25.5%), and due to restenosis in patients with prior intervention in 3 arteries (1.8%). Of the 120 arteries with thrombi, 81 arteries (67.5%) demonstrated insignificant atherosclerotic lesions. The presence of luminal thrombi with insignificant atherosclerosis could likely suggest a thromboembolic phenomenon. In the remaining 39 of 120 arteries with ≥70% luminal stenosis due to chronic or acute thrombi, the thrombotic process was associated with fibrocalcific plaques in 23 arteries, fibroatheromatous plaques in 15 arteries, and PIT in 1 artery. Of the 27 arteries from revised amputations, 4 arteries showed acute thrombi, and chronic thrombi were present in 13 arteries.

The presence of chronic thrombi was observed significantly more frequently in arteries with insignificant than with significant atherosclerotic lesions (multivariable-adjusted OR= 16.7; p= 0.0002). However, the presence of acute thrombi was not similarly associated with insignificant atherosclerosis (multivariable adjusted OR: 0.71; p = 0.41). INFRA-POP arteries had higher odds for chronic thrombi compared with FEM-POP vessels (multivariable adjusted OR: 2.14; p = 0.0041) but lower odds for acute thrombi (multivariable adjusted OR: 0.27; p = 0.0067).

MEDIAL CALCIFICATION IS COMMON IN CLI, MORE SEVERE IN BKA ARTERIES. Medial calcification of the large arteries was present in 170 of 239 (71.1%) arteries. The multivariable-adjusted odds ratio for presence of medial calcification, considered as a binary, dichotomized variable (e.g., presence vs. complete absence) in INFRA-POP compared with FEM-POP arteries was 2.89 (p = 0.0799). However, when the extent of calcification was considered as an ordinal variable (none, <25%, ≥25% to <50%, ≥50% to <75%, and ≥75% to 100%), the multivariable-adjusted odds ratio for increased extent of medial calcification in INFRA-POP versus FEM-POP arteries was 3.35 (p = 0.0063) as analyzed with multivariable-adjusted hierarchical regression. In addition to INFRA-POP versus FEM-POP, renal disease was also significantly associated with the extent of medial

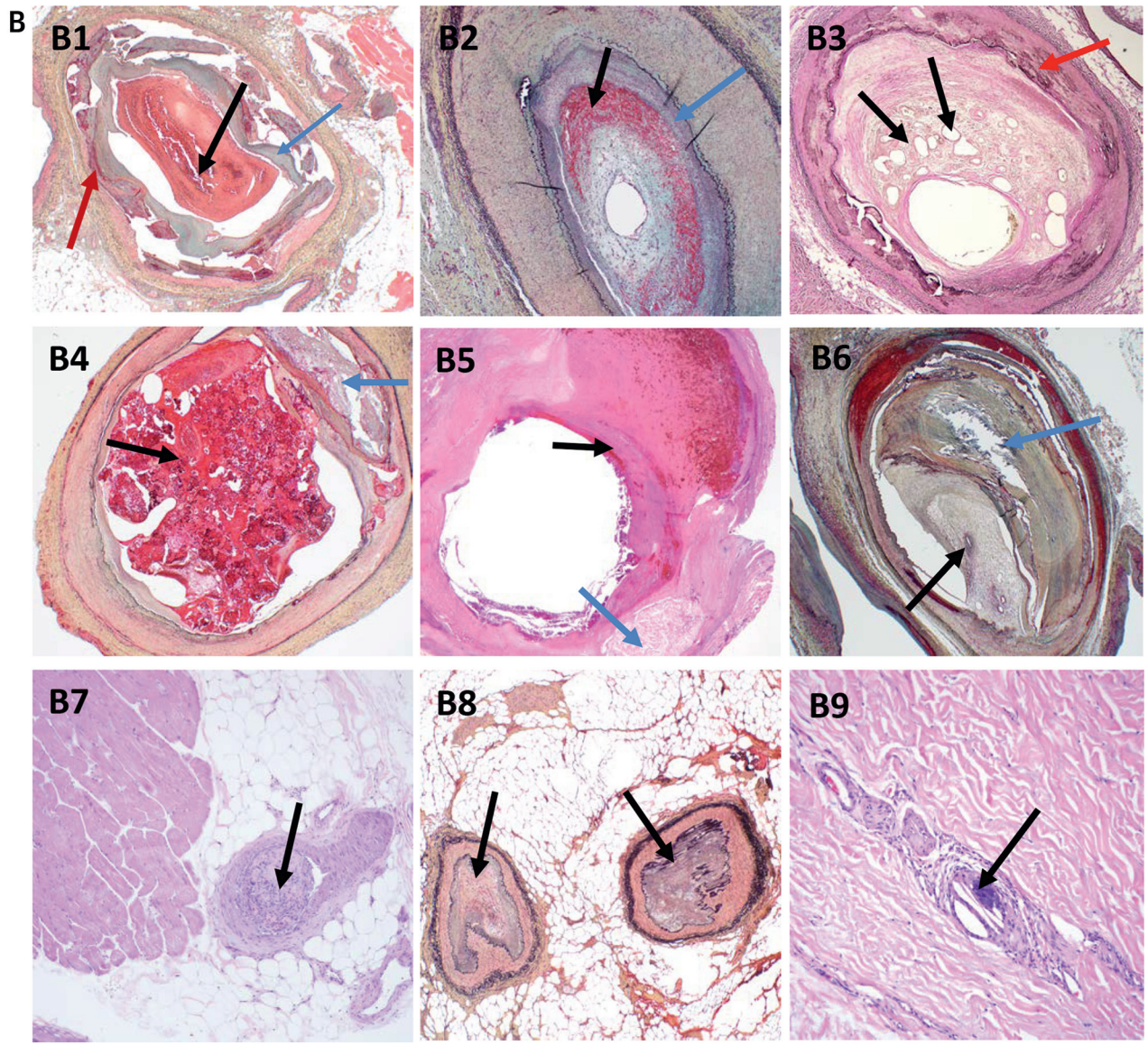
FIGURE 1 **Vessel Wall Pathology and Luminal Thrombi in CLI**



(A) Vessel wall pathology of critical limb ischemia (CLI). Normal-appearing posterior tibial artery (A1, magnification 40×). There is no evidence of intimal thickening or medial calcification. The anterior tibial artery (A2, 40×) shows adaptive intimal thickening with focal calcification of the internal elastic lamina **(arrow)**. The dorsalis pedis artery (A3, 40×) shows adaptive intimal thickening and medial calcification **(arrows)** involving more than three-fourths of the circumference of the artery. Posterior tibial artery (A4, 40×) shows a fibroatheromatous plaque (**blue arrow** points to necrotic core) with medial calcification **(black arrow)** involving more than three-fourths of the circumference of the artery. Peroneal artery (A5, 40×) with a fibrocalcific plaque **(blue arrow)** and medial calcification **(black arrow)** involving more than three-fourths of the circumference. The anterior tibial artery (A6, 40×) shows a fibrocalcific plaque **(blue arrow)** with a restenotic lesion consisting of myofibroblastic proliferation in a proteoglycan-rich matrix **(red arrow)** in a patient with prior intervention. Calcification (A7, 100×) highlighted by **black arrows** and intimal fibrosis of small subcutaneous arteries. Images A8 and A9 (magnification 100×) are from a patient with calciphylaxis in which calcification of small subcutaneous arteries **(black arrow)**, arterioles/venules **(blue arrows)**, and soft tissue calcification is seen. The arterial sections with normal intima and with adaptive intimal thickening were referred to as insignificant atherosclerotic disease. Images A1 to A3 and A7 to A9 are stained with hematoxylin and eosin and A4 to A6 are stained with Movat pentachrome stain.

Continued on the next page

FIGURE 1 **Continued**



(B) Luminal thrombosis vis-à-vis vessel wall pathology in lower extremity vessels. Anterior tibial artery (B1, 20×) reveals adaptive intimal thickening **(blue arrow)** and an acute luminal thrombus **(black arrow)**. Circumferential medial calcification **(red arrow)** is present. Dorsalis pedis artery (B2, 40×) shows an organizing thrombus with residual fibrin **(black arrow)**; adaptive intimal thickening is present **(blue arrow)**. The anterior tibial artery (image B3, 40×) shows a recanalized thrombus (**black arrows** point to vascular channels) with 90% luminal stenosis and medial calcification **(red arrow)** involving the entire circumference of the artery; underlying adaptive intimal thickening is also present. Popliteal artery (B4, 20×) showing a fibrocalcific plaque **(blue arrow)** with 30% luminal stenosis, wherein the lumen of the artery is occluded by a thrombus consisting of calcified nodules **(black arrow)** and fibrin. Popliteal artery (B5, 20×) demonstrates an ulcerated/disrupted fibroatheromatous plaque with a necrotic core **(blue arrow)** with luminal thrombus **(black arrow)**. Posterior tibial artery (B6, 20×) with a fibrocalcific plaque consisting of sheet-like calcium **(blue arrow)** and chronic luminal thrombus **(black arrow)**. The sheet-like calcification involves more than 25% circumference of the fibrocalcific plaque. Chronic recanalized thrombi (B7 and B8, 100×) in the subcutaneous arteries **(black arrows)**. Cholesterol embolus in a distal small arteriole **(arrow)** (image B9, magnification 200×). Images B5, B7, B9 are stained with hematoxylin and eosin and images B1, B2, B4 and B6 are stained with Movat pentachrome, and images B3 and B8 are stained with Verhoeff's elastic stain.

TABLE 2 Vessel Wall Pathology of CLI in ≥70% Luminal Stenosis, FEM-POP, and INFRA-POP Arteries, Acute or Chronic Thrombi

	FEM-POP (n = 31)	INFRA-POP (n = 134)	OR*	p Value
Chronic thrombi	12 (38.7)	77 (57.5)		
Chronic thrombi with insignificant atherosclerosis	5 (41.7)	62 (80.5)	16.71	<0.0001
Chronic thrombi with significant atherosclerosis	7 (58.3)	15 (19.5)		
Chronic thrombi with PIT	0 (0)	1 (1.3)		
Chronic thrombi with FA	3 (25.0)	3 (3.9)		
Chronic thrombi with FC	4 (33.3)	11 (14.3)		
Acute thrombi	12 (38.7)	19 (14.2)		
Acute thrombi with insignificant atherosclerosis	4 (33.3)	10 (52.6)	0.71	0.41
Acute thrombi with significant atherosclerosis	8 (66.6)	9 (47.4)		
Acute thrombi with PIT	0 (0)	0 (0.0)		
Acute thrombi with FA	4 (33.3)	5 (26.3)		
Acute thrombi with FC	4 (33.3)	4 (21.1)		

Values are n (%). *Computed with generalized linear mixed model hierarchical regression with a logistic link function, controlling for fixed effects age, sex, diabetes, hypertension, hyperlipidemia, congestive heart failure, cerebrovascular disease, ischemic heart disease, and smoking status.

Odds for Thrombi	Groups	Adjusted OR†	95% CI	p Value
Chronic thrombi	Insignificant vs. significant atherosclerosis	16.7	(5.3–50.4)	0.0002
Acute thrombi	Insignificant vs. significant Atherosclerosis	0.71	(0.3–1.6)	0.41
Chronic thrombi	INFRA-POP vs. FEM-POP	2.14	(1.4–3.3)	0.0041
Acute thrombi	INFRA-POP vs. FEM-POP	0.27	(0.1–0.7)	0.0067

†Computed using a hierarchical generalized linear model with a logistic link function, controlling for fixed effects of age, sex, hypertension, hyperlipidemia, congestive heart failure, cerebrovascular disease, ischemic heart disease, and smoking history. Nested random effects were included per patient and per surgical procedure to control for within-patient and within-surgical-procedure correlations. The odds ratio may be interpreted as the odds for chronic thrombi or acute thrombi in: 1) insignificant atherosclerosis compared with significant atherosclerosis; or 2) in the infrapopliteal vessels compared with the femoral and popliteal vessels. Due to numerical instability, we could not specify an interaction term in the hierarchical regression model to assess if there was an interaction between atherosclerosis type and vessel type associated with either acute or chronic thrombi.

CI = confidence interval; FA = fibroatheroma; FC = fibrocalcific lesions; FEM-POP = femoral and popliteal arteries; INFRA-POP = infrapopliteal arteries; OR = odds ratio; PIT = pathological intimal thickening.

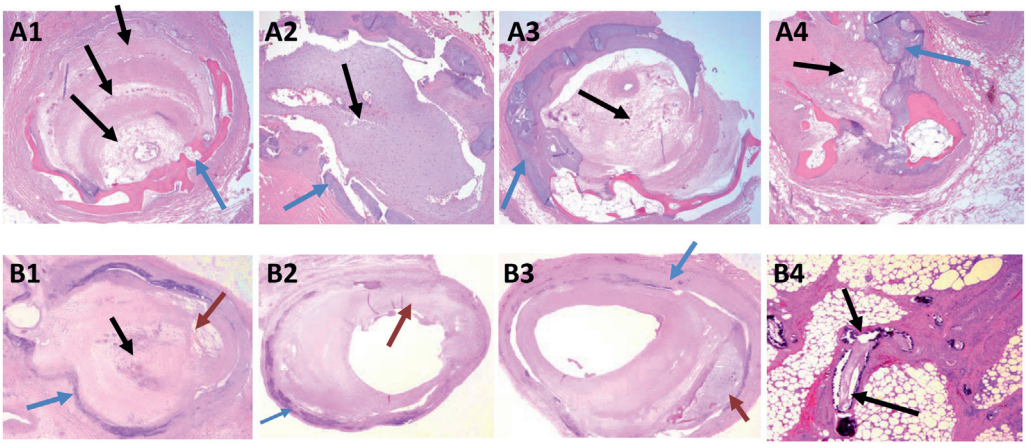
calcification (OR: 5.29; p = 0.01). Other control variables were not significant at the p = 0.05 level. Examination of models including variables for luminal stenosis or an interaction between vessel type and stenosis did not significantly improve the fit of the model versus vessel type alone (Bayesian Information Criterion 708.2 vs. 710.7 and 711.4, respectively; lowest best). Considering only the arteries with ≥70% luminal stenosis, the multivariable-adjusted odds ratio for increased extent of medial calcification in INFRA-POP versus FEM-POP arteries was 3.94 (p = 0.00589) when analyzed with multivariable-adjusted hierarchical ordinal regression.

DISTAL SMALL-VESSEL PATHOLOGY IS COMMON IN CLI. Distal small-artery medial calcification was present in 43 of 75 patients (57.3%) and was accompanied by varying degrees of intimal fibrosis, resulting in mild to severe luminal stenosis. Thrombi and cholesterol emboli were present in the small arteries and arterioles. Two patients with CLI had calciphylaxis, both of whom had renal disease.

VASCULAR PATHOLOGY IN AMPUTATIONS PERFORMED FOR CLINICAL ALI. A total of 10 arteries from 3 amputations (2 AKA and 1 BKA) with clinical ALI were available for analysis. The previous bypass graft was occluded in 1 AKA, and in the other, acute thrombus was found associated with fibroatheroma in the POP artery. In 2 INFRA-POP arteries, chronic thrombus was associated with fibroatheroma in one and with insignificant atherosclerosis in another. In the BKA specimen, the INFRA-POP arteries demonstrated chronic thrombi, one of which was associated with fibrocalcific plaque and another with insignificant atherosclerosis.

VASCULAR PATHOLOGY IN AMPUTATIONS PERFORMED FOR NON-PAD ETIOLOGY. A total of 50 arteries were reviewed in specimens wherein amputations were performed for non-PAD etiology. Of these, 29 arteries were available from amputations performed for gangrene but no clinical evidence of ALI or CLI. The remaining 21 of 50 arteries were obtained from specimens where amputations were performed in patients

FIGURE 2 **Clinical Examples of CLI With Minimal Atherosclerotic Disease in BKA Arteries, and Plaque Rupture in AKA Specimen**



The **top row** (A1 to A4) presents histomorphological characteristics of the left lower leg arteries from a 73-year-old woman who underwent a below knee amputation for gangrene. Her past medical history was significant for diabetes, hypertension, hyperlipidemia, ischemic heart disease, stroke, as well as renal disease. All of the lower leg arteries, including posterior tibial (A1, 40×), anterior tibial (A2, 100×), peroneal (A3, 40×), and dorsalis pedis (A4, 40×) show chronic thrombotic occlusions **(black arrows)** and medial calcification/ossification **(blue arrows)** involving at least 75% of the vessel wall circumference. The posterior tibial artery (A1) shows evidence of repeated recanalized thrombi **(arrows)** depicted by multilayered appearance. Atherosclerosis is not present in any artery. All sections are stained with hematoxylin and eosin. The second patient, an 82-year old woman, presented in the **bottom row** (B1 to B4), underwent a left above-knee amputation. The patient had prior left toe amputation and a failed femoral and popliteal artery bypass graft. She continued to have rest pain. The medical history was significant for diabetes and hypertension. The anterior tibial artery (B1, 40×) shows a fibroatheromatous plaque **(red arrow)** with an organizing luminal thrombus **(black arrow)**. There is medial calcification **(blue arrow)** involving three-fourths of the circumference of the vessel. The posterior tibial artery (B2, 20×) has a fibroatheromatous plaque with ulceration/rupture **(red arrow)**. The medial calcification **(blue arrow)** involves 60% of the circumference. The popliteal artery (B3, 20×) shows a 70% occlusive fibrocalcific plaque with sheet-like calcium **(red arrow)** and medial calcification involving greater than one-half of the circumference of the artery **(blue arrow)**. The small subcutaneous arteries (**arrow**; B4, 40×) show calcification with intimal fibrosis. All sections have been hematoxylin and eosin-stained.

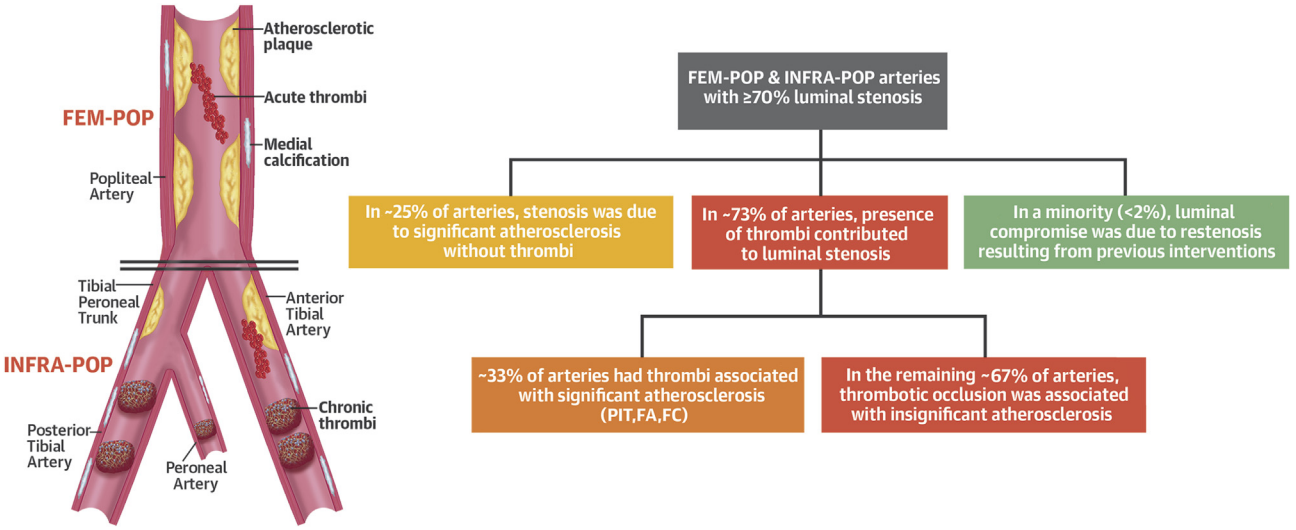
who neither had gangrene nor PAD. Acute thrombi were present in 10 of 50 arteries from 6 patients of the non-CLI group. The acute thrombi were due to frostbite, systemic vasculitis, heparin-induced thrombocytopenia in a patient with burns, atrial fibrillation that led to stroke and gangrene, ankle fracture, and Charcot foot deformity with an open wound. Chronic thrombi were present in 5 of 50 arteries from 4 patients with clinical history of infected gangrene, systemic vasculitis, ankle fracture, and complications of surgery for aortic dissection.

Even in this group of patients, where the amputations were performed for non-PAD reasons, significant atherosclerosis was present in 12 of 50 (24%) arteries. The multivariable-adjusted OR for the significant atherosclerosis in CLI-related arteries compared with non-CLI arteries was 2.38 ($p = 0.026$). However, during multivariable hierarchical regression analysis, when presence of medial calcification was treated as a binary variable or extent of medial calcification was treated as an ordinal variable, neither demonstrated a significant difference between arteries from and not from CLI patients (OR: 3.07; $p = 0.17$; and OR: 3.4; $p = 0.17$, respectively). We, thus, do not have enough evidence to conclude that medial calcification is more prevalent or occurs to a greater extent in CLI cases. This finding may be attributable to insufficient non-CLI sample size. However, there was a significantly lower odds of small artery calcification in non-CLI cases compared with CLI patients. Small artery calcification was seen in 5 of 17 (29.4%) non-CLI patients, and the multivariable-adjusted odds ratio for increased frequency of small artery calcification in patients with CLI versus non-CLI patients was 4.2 ($p = 0.042$).

DISCUSSION

PATHOLOGY OF CLI. This study presents histopathological characteristics of PAD in patients presenting with CLI (Central Illustration). Evaluation of arteries with ≥70% luminal stenosis from amputation specimens demonstrated significantly more atherosclerosis in FEM- than INFRA-POP arteries (Figure 2). The

CENTRAL ILLUSTRATION **Pathological Characterization of Large Arteries in Amputations for Critical Limb Ischemia**



Narula, N. et al. J Am Coll Cardiol. 2018;■(■):■-■.

In critical limb ischemia, chronic thrombotic occlusions are frequently observed, more often without significant atherosclerosis, especially in infrapopliteal arteries. Luminal thrombosis with insignificant atherosclerosis suggests the possibility of thromboembolic disease and supports the role of antithrombotic agents. The medial calcification is very common. FA = fibroatheroma; FC = fibrocalcific plaque; FEM-POP = femoral and popliteal; INFRA-POP = infrapopliteal; PIT = pathological intimal thickening.

luminal compromise in the INFRA-POP arteries was more commonly secondary to the thrombotic occlusion, and was more likely to occur in absence of significant atherosclerosis. While chronic thrombi were more frequently observed in arteries with insignificant atherosclerosis, acute thrombi were seen in arteries both with significant or insignificant atherosclerosis. INFRA-POP arteries had higher odds for chronic thrombotic occlusion compared with FEM-POP arteries, but had lower odds for acute thrombi. Also, the distal small vessels showed medial calcification, intimal fibrosis, luminal thrombi, and cholesterol emboli. Unlike what is uniformly observed in coronary arteries, luminal thrombosis was less frequently associated with fibroatheromatous or fibrocalcific lesions. In addition to a high degree of luminal stenosis regardless of the intimal pathology, a variable degree of medial calcification was frequently seen. Therefore, unlike the atherosclerotic process in the coronary arterial bed, CLI most frequently resulted from thrombotic occlusion of peripheral vessels even though the vessel wall may not necessarily have harbored significant atherosclerotic disease.

MECHANISTIC IMPLICATIONS OF PATHOLOGY FINDINGS IN CLI. The pathological characterization of the lower-extremity arteries suggests that luminal stenosis due to atherosclerotic disease occurred less frequently, particularly in the INFRA-POP arteries. Luminal thrombi, especially in INFRA-POP arteries, with luminal occlusion of 2 or more major arteries with insignificant atherosclerosis and distal small vessel obliteration, suggests a possibility of thromboembolic phenomenon (Figure 1B, Figure 2). The presence of layered occlusive thrombi supports repeated embolic burden (Figure 2). If this is true, such an embolic shower could explain the relatively lower incidence of CLI even though the prevalent PAD population is large (1). It is especially convincing because 3 major vessels are available in the lower leg to take over from one another if only 1 vessel was to develop thrombus locally. We also observed continuous thrombotic occlusion extending from the major vessel into small arteries. It seems likely that these emboli would have originated from aortic, femoral, and popliteal atherosclerotic plaques or even upstream atherosclerotic disease in the same vessel. Although thromboembolic phenomenon seems to be

a logical explanation of progression of PAD to CLI, it is quite possible that the noncompliant lower leg vessels (both large and small), due to medial calcification, infection, and loss of mobility (16), may also somehow favor development of luminal thrombi from the stasis of blood. Although not tested in this study, it is also possible that local or systemic coagulation abnormalities contribute to the thrombotic milieu. Blood rheological factors have been correlated to ankle-brachial systolic pressure index and arterial narrowing in PAD in the Edinburgh Artery Study (5).

PREVENTIVE AND THERAPEUTIC IMPLICATIONS OF PATHOLOGICAL FINDINGS. A subset of PAD patients are at risk of acute limb events. The mainstay of treatment for these patients has included the use of an antiplatelet agent to prevent major adverse events (17). An analysis of 7,470 people with PAD from the recent COMPASS (Cardiovascular Outcomes for People Using Anticoagulation Strategies) trial (18) showed that the combination of low-dose rivaroxaban (2.5 mg twice daily) with low-dose aspirin substantially reduced major adverse limb events (composed of acute and chronic limb ischemia), including major vascular amputation. The combination of rivaroxaban and aspirin was superior to either drug used alone and supports the hypothesis that both the proximal sites of origin of thromboembolism and the lower legs at the receiving end might need to be protected. Furthermore, the patients who are most at risk of major adverse limb events were those with severe limb ischemia at baseline (Fontaine Class 3 or 4), those who were taking aspirin only, and those with a prior history of lower limb revascularization/amputation (4). Similar findings were also shown in a pre-specified subgroup of 3,787 patients with qualifying PAD in the TRA2°P (Thrombin Receptor Antagonist Secondary Prevention Trial) randomized to vorapaxor or placebo in addition to background therapy with aspirin and/or clopidogrel. There were lower rates of hospitalization for acute limb ischemia and of peripheral artery revascularization in patients randomized to vorapaxor compared with placebo (19,20).

The therapeutic options in CLI management include open or endovascular revascularization with or without adjunctive mechanical thrombectomy and/or thrombolysis. If the thromboembolic hypothesis of CLI in lower legs is correct, then catheter-based therapy (mechanical thrombectomy and low-dose thrombolytic therapy) or surgical thrombectomy may provide rapid restoration of blood flow (21-24).

STUDY LIMITATIONS. There are several important limitations that arise from the retrospective nature of the current study. It would be more informative if we could histopathologically examine systematically performed serial sections at 5-mm intervals across the entire length of the lower-limb arteries. Therefore, a prospective study would need to validate the results of this study with a complete computed tomography or magnetic resonance imaging-based roadmap of the entire abdominal aorta, pelvic arteries, and lower-limb arteries. A history of atrial fibrillation would be important. Although we have observed repeated and layered embolic occlusion of lower leg vessels and distal subcutaneous vasculature, a global vascular picture will need to be mapped out for each individual. There is no information available in the collateral blood vessels, such as the profunda femoral artery and the geniculate arteries around the knee. The contribution of coagulation profile will also need to be investigated to understand the phenomenon of thrombotic occlusion associated with insignificant intimal pathology in the lower legs. Although we have provided the prevalence of renal disease based on the history obtained from Epic, estimated glomerular filtration rate would have been more accurate. Sections with no atherosclerosis might have had minimal adaptive intimal thickening, which could not be identified in the absence of the availability of elastic stains in all sections. The process of decalcification of the arteries may have affected the assessment of calcification, particularly atherosclerotic microcalcification when the decalcification was undertaken for extensive medial layer calcification.

CONCLUSIONS

The histopathological characterization of the lower leg vessels in CLI reveals thrombotic luminal occlusion commonly with insignificant atherosclerosis and distal small vessel occlusion, and suggests the possibility of thromboembolic phenomenon. Medial calcification in these vessels is frequently observed. The in situ thrombosis secondary to plaque rupture as classically reported in coronary disease is less common. The pathological findings in lower-extremity arteries suggest possible mechanisms of progression of PAD to CLI, and may support the preventive role of antithrombotic agents.

ADDRESS FOR CORRESPONDENCE: Dr. Navneet Narula, NYU Langone Medical Center, 550 First Avenue, Suite TH4 14A, New York, New York 10016. E-mail: navneet.narula@nyumc.org. Twitter: @nyulangone.

PERSPECTIVES

COMPTENCY IN MEDICAL KNOWLEDGE: The histopathological characterization of the lower leg vessels in CLI reveals significant atherosclerotic lesions in FEM-POP compared with INFRA-POP arteries. The INFRA-POP arteries frequently showed thrombotic luminal occlusion associated with insignificant atherosclerosis. This suggests a possibility of thromboembolic phenomenon in addition to less frequent an in situ thrombosis secondary to plaque rupture, which is classically reported in patients with CAD.

TRANSLATIONAL OUTLOOK: The pathological findings in the lower extremity arteries suggest possible mechanisms of progression of PAD to CLI, and may support the preventive role of direct antithrombotic agents.

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