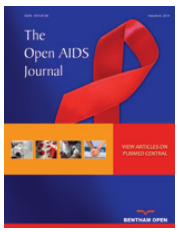

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RESEARCH ARTICLE

High Prevalence of Lower Extremity Medial Arterial Calcification in HIV-infected Patients With and Without Chronic Renal Disease: A Vascular Ultrasound Cross-sectional Study

Klaudija Višković[1,2], Annibale Marinelli[3], Katrin Nedeljko[4], Petra Povalej Bržan[5,6], Nikolina Bogdanić[2,*] and Josip Begovac[2,7]

[1]*Faculty of Health Sciences, University of Rijeka, Rijeka, Croatia*
[2]*University Hospital for Infectious Diseases "Dr. Fran Mihaljević," Zagreb, Croatia*
[3]*Nephrology and Dialysis Department, Riuniti Hospital, Anzio, Roma, Italy*
[4]*Ruđer Bošković Institute, Zagreb, Croatia*
[5]*Faculty of Medicine University of Maribor, Maribor, Slovenia*
[6]*Faculty of Electrical Engineering and Computer Science, University of Maribor, Maribor, Slovenia*
[7]*School of Medicine, University of Zagreb, Zagreb, Croatia*

Abstract:

Background:

The association between HIV infection and increased risk of atherosclerotic peripheral artery disease (PAD) has been documented. Still, the relationship between HIV infection and lower extremity medial arterial calcification (MAC) is unknown.

Objective:

We performed a cross-sectional study to compare the frequency of MAC diagnosed by vascular ultrasound in PAD-asymptomatic people living with HIV (PLWH) with and without chronic kidney disease (CKD) compared to HIV-uninfected participants as a control group.

Methods:

MAC was defined as smooth, linear, and non-stenotic hyperechogenicity in the arterial wall compared to the surrounding tissues. We studied 191 patients: 50 PLWH (25 with an estimated glomerular filtration rate (eGFR) ≥60 mL/min/1.73m^2 and 25 with an eGFR <60 mL/min/1.73m^2) and 141 HIV-uninfected patients (68 with eGFR<60 ml/min/1.73m^2).

Results:

MAC was most frequently found in PLWH with CKD (76%). The prevalence of MAC among PLWH was 54.0% (95% confidence interval [CI], 40.4-67.0%), whereas, in HIV-uninfected, it was 34.0% (95% CI, 26.7-42.2%, P=0.013). Age and CKD were consistently associated with MAC in our multivariable models, and there was also a sign that PLWH had higher odds of having MAC.

Conclusion:

We found a higher prevalence of MAC in PAD-asymptomatic PLWH compared to HIV-uninfected ones and provided evidence that HIV infection could be associated with MAC.

Keywords: HIV infection, Medial arterial calcification, Chronic kidney disease, Peripheral artery disease, Chronic renal disease, Vascular ultrasound.

Article History	Received: June 26, 2022	Revised: September 27, 2022	Accepted: November 4, 2022

1. INTRODUCTION

The introduction of antiretroviral therapy has greatly increased the life expectancy of human-immunodeficiency-virus (HIV) infected persons, also increasing the risk for age-related diseases such as cardiovascular disease (CVD), atherosclerotic peripheral artery disease (PAD) and chronic kidney disease (CKD) [1 - 3]. Besides aging, several HIV-associated risk factors for the development of CKD may be

present: viral replication, low CD4 T cell count, antiretroviral drugs, and coinfections (hepatitis B and C) [4]. The association between HIV infection and the increased risk of CVD has been widely reported [5]. A large US-based study found that after controlling for traditional factors, HIV infection carried a 19% additional risk for atherosclerotic lower extremity arterial disease [6]. Intimal arterial calcification is associated with atherosclerosis and plaque formation, consequently reducing the arterial lumen and developing atherosclerotic disease [6]. Medial arterial calcification (MAC) differs from atherosclerosis and is frequently present in patients with CKD, diabetes mellitus, and aging [7, 8]. Data on the clinical implications of asymptomatic MAC findings are still evolving; however, there is growing evidence that MAC, as a systemic, non-atherosclerotic vascular disorder that increases arterial stiffness, could be an independent risk factor for cardiovascular mortality [9 - 11] and atherosclerotic PAD [12] in non-HIV-infected persons. The relationship between MAC and HIV infection has been poorly investigated. The possible role of a class of antiretroviral drugs -protease inhibitors - as a trigger for vascular smooth muscle cell senescence and calcification, a feature of MAC, has been suggested [13]. This study aimed to examine the association between HIV infection, CKD, and MAC defined by vascular ultrasound in people living with HIV (PLWH) compared to HIV-uninfected controls.

2. MATERIALS AND METHODS

2.1. Study Design

We performed a cross-sectional study to compare the frequency of MAC diagnosed by vascular ultrasound in PLWH with and without CKD compared to HIV-uninfected participants as a control group. We also examined factors related to MAC and were interested in the relationship between HIV infection and CKD to MAC. The study was supported by the Croatian Science Foundation (project no. IP-2019-04-9702).

2.2. Study Population

We investigated 191 patients from two centres: 50 PLWH from the University Hospital for Infectious Diseases (UHID), Zagreb, Croatia, and 141 HIV-uninfected participants presenting to the Nephrology and Dialysis Department, Riuniti Hospital, Anzio (Italy). Consecutive HIV-infected participants were enrolled from the HIV/AIDS Centre at UHID from February 1st, 2018, to September 30th, 2019. The data on HIV-uninfected participants were collected retrospectively from medical records among patients who attended Nephrology and Dialysis Department, Riuniti Hospital, Anzio, Vascular laboratory, from September 4th, 2007, to March 18^{th,} 2019. Demographic and clinical data were collected from the patient's chart at enrolment.

2.3. Inclusion and Exclusion Criteria

Persons aged ≥18 years who provided informed consent were included in the study. HIV-uninfected participants were included if there were younger than 65 years of age and had no known cardiovascular disease, previous kidney transplantation, and previous episodes of acute renal failure. The study population also included persons examined at the Nephrology and Dialysis Department for a regular health check-up and with no known previous kidney disease (N=73).

Twenty-five consecutive PLWH with an estimated glomerular filtration rate (eGFR) ≥ 60 mL/min/1.73m^2 and 25 with an eGFR < 60 mL/min/1.73m^2 were included in the study. All PLWH had confirmed HIV infection by Western-Blot. Exclusion criteria were: pregnancy, breastfeeding, hormonal replacement therapy, corticosteroid and cytotoxic drug use, dementia, or involuntary movement.

2.4. Definitions

Renal function was assessed according to the NFK-KDOQI guidelines: normal renal function (stage 1) was defined as eGFR over 90 mL/min/1.73 m^2; stage 2, eGFR 89-60 mL/min/1.73 m^2; stage 3 eGFR-59-30 mL/min/1.73 m^2; stage 4 eGFR 29-15 mL/min/1.73 m^2 and stage 5-less than 15 mL/min/1.73 m^2[14]. eGFR was calculated using the equation of the Modification of Diet in Renal Disease (MDRD) formula. CKD was defined as an eGFR of less than 60 ml/min/1.73m^2 on at least 2 occasions 90 days apart. Participants' weight and height were measured and recorded, and the body mass index (BMI) was calculated (weight in kg divided by height in m^2). Diabetes mellitus was determined as confirmed fasting plasma glucose ≥ 7.0 mmol/l (126 mg/dl) or 2–h plasma glucose ≥ 11.1 mmol/l (200 mg/dl), or HbA1c ≥ 48 mmol/mol according to the World Health Organization definition (https://www.who.int/health-topics/diabetes). Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or taking antihypertensive drugs [14, 15]. Dyslipidemia was defined as random total serum cholesterol more than 240 mg/dl (6.2 mmol/l), HDL less than 35 mg/dl (0.9 mmol/L), triglyceride more than 200 mg/dl (2.2 mmol/L), or receiving lipid-lowering therapy.

2.5. Vascular Ultrasound Measures

Vascular ultrasound was performed by one radiology specialist at UHID and one nephrology specialist at Riuniti Hospital Anzio on all participants at a single visit by greyscale B-mode ultrasound for detection of MAC and intimal calcification and duplex color Doppler of the abdominal aorta and inferior extremities' arteries to detect stenosis or occlusion according to the protocol described in the published study by Marinelli and Di Napoli [16]. Vascular ultrasound was performed with the Samsung Medison UGEO H60 machine at UHID and Toshiba Corevision SSA-350A at the Riuniti Hospital Anzio, using a linear probe (7.5 MHz) for analysis of femoral, popliteal, and tibial arteries and a convex probe (3.5 MHz) for the abdominal aorta.

Transcutaneous B-mode ultrasonography was done to detect arterial intimal calcification (AIC), and MAC and duplex colour Doppler sonography was performed to detect stenosis or occlusion. The transducer frequency, focal zone, overall gain, and time-gain compensation were adjusted according to the individual subject's vessel characteristics to optimize the ultrasonography appearance of the arterial wall complex on the anechoic arterial lumen.

* Address correspondence to this author at the University Hospital for Infectious Diseases 10 000 Zagreb, Croatia; E-mail: nikolinabogdanic@gmail.com

The discrete plaque-like calcifications with luminal narrowing were regarded as AIC. MAC was defined as smooth, linear, and non-stenotic hyperechogenicity in the arterial wall compared to the surrounding tissues, disrupting the normal intima-medial wall configuration. MAC was considered as either moderate (spots or isolated segments) or diffuse (more segments) [17, 18].

According to guidelines for the assessment of the arterial circulation of the lower limbs, we considered arterial flow as normal in the presence of triphasic (no stenosis) or biphasic waveform (lumen stenosis <50%) and stenotic vessels in the presence of a flow pattern corresponding with lumen narrowing >50%. The vessel was considered occluded if no flow was observed [19].

Atherosclerotic PAD was considered present if any focal or diffuse intimal calcifications were observed in the common femoral, superficial femoral, or tibial arteries and if there were hemodynamically significant stenosis or occlusion.

2.6. Statistical Analysis

We described our data on the study population by frequency and percentages or median with the first and third quartiles (Q1–Q3). To compare different characteristics of participants with and without HIV infection and/or peripheral atherosclerosis, we used the Whitney-Mann test for continuous variables and Fisher exact test or $\chi 2$ test for categorical variables.

We assessed factors related to MAC by logistic regression with HIV infection and CKD (categorized < 60 and ≥ 60 ml/min/1.73m^2) as main predictors. The odds of having MAC was calculated in logistic regression models using the following independent variables: age, HIV status, CKD, BMI, sex, smoking, hypertension, diabetes, and dyslipidemia. We initially included all the variables in the multivariable logistic regression model analysis. However, there were only 16 patients with diabetes; hence the confidence intervals were very wide (1.01-40.96), and there were also concerns about overfitting the model, so diabetes was removed from our main model. By removing diabetes from the model, there were no substantive changes in the coefficients of the main predictors (HIV infection and CKD). The models were also examined for various interactions which were nonsignificant. Five outliers and influential observations were identified by diagnostic plots (*e.g.*, Cook's distance-type index). Since there was a significant change in the coefficient of HIV status after the removal of outliers, we present our multivariable model for the whole population and when outliers are removed. For continuous variables, the Box-Tidwell method checked the linearity assumption, tolerance statistics assessed collinearity, and the Hosmer-Lemeshow test was used to judge the overall fit of the models. Statistical analyses were performed with SAS software, version 9.4. (SAS Institute, Cary, NC, USA). The level of statistical significance was set at $P < 0.05$.

3. RESULTS

3.1. Main Characteristics

Of 191 participants, 57.6% (110/191) were male, with a median age of 51 (Q1-Q3: 42-57) years (Table **1**). All participants were Caucasians. There were more males among PLWH who were also older than HIV-uninfected persons, whereas hypertension and current smoking were more frequent among HIV-uninfected persons (Table **1**). Of 50 PLWH, 25 (50%) had CKD (eGFR <60 ml/min/1.73m^2), and of 141 HIV-uninfected, 68 (48.2%) had CKD. However, HIV-uninfected persons had more severe CKD; altogether, 35.5% (50/141) had eGFR <30 ml/min/1.73 compared to 10% (5/50) among PLWH. Dyslipidaemia was more frequently found in PLWH (Table **1**). There were no differences in BMI or prevalence of diabetes between PLWH and HIV-uninfected persons (Table **1**).

PLWH had a median of 13 (Q1-Q3: 6-17) years of HIV infection duration with a median current CD4 cell count of 540 per mm^3 (Q1-Q3: 358-720) at the time of vascular ultrasound examination. The most frequent mode of HIV infection transmission was sex between men (58%), followed by heterosexual contact and intravenous drug use (34% and 6%, respectively). At the time of vascular ultrasound examination, 5 patients were not receiving antiretrovirals, and 90% (45/50) of PLWH had plasma HIV-1 RNA < 50 copies/ml.

Table 1. Main characteristics of 191 patients by HIV status.

Variables	Total N=191	PLWH N=50	Not HIV-infected N=141	*p*-value
Gender	-	-	-	<0.001
Male	110 (57.6)	45 (90.0)	65 (46.1)	-
Female	81 (42.4)	5 (10.0)	76 (53.9)	-
Age, years	51 (42 - 57)	52.5 (45 - 63)	49 (42 - 56)	0.021
Age groups, years	-	-	-	0.005 †
18-30	13 (6.8)	3 (6.0)	10 (7.1)	-
31-40	24 (12.6)	6 (12.0)	18 (12.8)	-
41-50	58 (30.4)	11 (22.0)	47 (33.3)	-
51-60	64 (33.5)	13 (26.0)	51 (36.2)	-
≥61	32 (16.8)	17 (34.0)	15 (10.6)	-
Estimated GFR, ml/min/1.73m^2	-	-	-	0.829
≥60	98 (51.3)	25 (50.0)	73 (51.8)	-

(Table 1) contd.....

Variables	Total N=191	PLWH N=50	Not HIV-infected N=141	*p*-value
<60	93 (48.7)	25 (50.0)	68 (48.2)	-
Estimated GFR categories, ml/min/1.73m2	-	-	-	<0.001
≥90	42 (22.0)	2 (4.0)	40 (28.4)	-
60-89	56 (29.3)	23 (46.0)	33 (23.4)	-
30-59	38 (19.9)	20 (40.0)	18 (12.8)	-
15-29	23 (12.0)	2 (4.0)	21 (14.9)	-
<15	32 (16.8)	3 (6.0)	29 (20.6)	-
Hypertension	93 (48.7)	16 (32.0)	77 (54.6)	0.006
Body mass index, kg/m^2	25.6 (22.8 - 28.0)	26.5 (24.7 - 28.5)	25.3 (22.4 - 27.8)	0.083
Body mass index categories, kg/m^2	-	-	-	0.379 †
Underweight (<18.5)	4 (2.1)	1 (2.0)	3 (2.1)	-
Normal (18.5-24.9)	78 (40.8)	16 (32.0)	62 (44.0)	-
Overweight (25-29.9)	80 (41.9)	26 (52.0)	54 (38.3)	-
Obese ≥30	29 (15.2)	7 (14.0)	22 (15.6)	-
Diabetes mellitus	-	-	-	0.568 †
No	175 (91.6)	47 (94.0)	128 (90.8)	-
Yes	16 (8.4)	3 (6.0)	13 (9.2)	-
Current smoking	-	-	-	0.007
No	95 (49.7)	33 (66.0)	62 (44.0)	-
Yes	96 (50.3)	17 (34.0)	79 (56.0)	-
Dyslipidemia	-	-	-	< 0.001
No	141 (73.8)	28 (56.0)	113 (80.1)	-
Yes	50 (26.2)	22 (44.0)	28 (19.9)	-
Peripheral atherosclerosis[a]	-	-	-	< 0.001
No	165 (86.4)	36 (72.0)	129 (91.5)	-
Yes	26 (13.6)	14 (28.0)	12 (8.5)	-
Medial arterial calcification	-	-	-	0.013
No	116 (60.7)	23 (46.0)	93 (66.0)	-
Yes	75 (13.6)	27 (54.0)	48 (34.0)	-

Note: [a]Any stenosis or occlusion plus any intimal vascular calcifications.

Values are frequencies and percentages (in parenthesis) or median and first and third quartiles (in parenthesis).

†Exact test.

PLWH, people living with HIV; GFR, glomerular filtration rate. P-values are a result of comparison of PLWH and those who are not HIV infected.

3.2. Prevalence of MAC and PAD

The overall prevalence of MAC was 39.3% (75 of 191, 95% confidence intervals (CI) 32.6 to 46.3%). Among PLWH, the prevalence was 54.0% (27/50, 95% CI, 40.4 to 67.0%), and among HIV-uninfected, it was 34.0% (48/141, 95% CI, 26.7 to 42.2%, P=0.013). The prevalence was highest in PLWH with CKD (76.0%, 19/25), 32.0% (8/25) in PLWH without CKD, 41.2% (28/68) in HIV-uninfected persons with CKD, and 28.8% (21/73) in HIV-uninfected persons without CKD. Of 75 persons with MAC, 26 (34.7%) also had atherosclerotic PAD. PLWH with MAC had more frequent atherosclerotic PAD than HIV-uninfected persons (14/27, 51.9% *versus* 11/48, 22.9%, P=0.011). MAC was more frequently found in the form of spots and isolated segments compared to diffuse changes (19.9% in the superficial femoral arteries *versus* 9.9% in the right or left tibial arteries).

Atherosclerotic PAD (stenosis or occlusion plus intimal calcifications) was found in 13.6% (26/191, 95% CI, 9.5 to 19.2%) persons and was more frequent in PLWH (28.0%, 14/50) compared to HIV-uninfected persons (8.5%, 12/141, *P*< 0.001) (Tables **2** and **3**). Only one person with atherosclerotic PAD did not have MAC. All PLWH were asymptomatic, and none of the patients had clinically established atherosclerotic PAD.

Table 2. Main findings of ultrasound examinations of peripheral arteries in HIV infected persons according to the presence or absence of chronic kidney disease.

-	eGFR ≥60 ml/min/1.73m^2 N=25 (%)	eGFR < 60 ml/min/1.73m^2 N=25 (%)
Calcifications in the right common femoral artery, yes	6 (24.0)	8 (32.0)
Calcifications in the left common femoral artery, yes	6 (24.0)	8 (32.0)
Stenosis or occlusion of the left or right common femoral artery, yes	0 (0.0)	0 (0.0)

(Table 2) contd.....

-	eGFR ≥60 ml/min/1.73m^2 N=25 (%)	eGFR < 60 ml/min/1.73m^2 N=25 (%)
Calcifications in the right superficial femoral artery, yes	5 (20.0)	9 (36.0)
Calcifications in the left superficial femoral artery, yes	5 (20.0)	9 (36.0)
Stenosis or occlusion of the left or right superficial femoral artery, yes	1 (4.0)	0 (0.0)
MAC in the right superficial femoral artery, yes	6 (24.0)	17 (68.0)
MAC in the left superficial femoral artery, yes	6 (24.0)	17 (68.0)
A mild degree of MAC in the right or left superficial femoral artery, yes	1 (4.0)	6 (24.0)
Diffuse MAC in the right or left superficial femoral artery, yes	4 (16.0)	11 (44.0)
MAC in right tibial arteries, yes	7 (28.0)	16 (64.0)
MAC in left tibial arteries, yes	7 (28.0)	16 (64.0)
A mild degree of MACin the right or left tibial arteries, yes	0 (0.0)	5 (20.0)
Diffuse MAC in the right or left tibial arteries, yes	5 (20.0)	12 (48.0)
Stenosis or occlusion of the left or right tibial arteries, yes	1 (4.0)	0 (0.0)
Peripheral artery disease[a], yes	5 (20.0)	9 (36.0)
Any MAC, yes	8 (32.0)	19 (76.0)

Note: [a] Any stenosis or occlusion plus any intimal vascular calcifications.
eGFR, estimated glomerular filtration rate.
MAC-medial arterial calcification.

Table 3. Main findings of ultrasound examinations of peripheral arteries in HIV uninfected persons according to the presence or absence of chronic kidney disease.

-	eGFR ≥60 ml/min/1.73m^2 N=73 (%)	eGFR < 60 ml/min/1.73m^2 N=68 (%)
Calcifications in the right common femoral artery, yes	10 (13.7)	16 (23.5)
Calcifications in the left common femoral artery, yes	15 (20.5)	15 (22.1)
Stenosis or occlusion of the left or right common femoral artery, yes	3 (4.1)	0 (0.0)
Calcifications in the right superficial femoral artery, yes	5 (6.8)	5 (7.4)
Calcifications in the left superficial femoral artery, yes	5 (6.8)	5 (7.4)
Stenosis or occlusion of the left or right superficial femoral artery, yes	2 (2.7)	0 (0.0)
MAC in the right superficial femoral artery, yes	4 (5.5)	10 (14.7)
MAC in the left superficial femoral artery, yes	4 (5.5)	10 (14.7)
A mild degree of MAC in the right or left superficial femoral artery, yes	4 (5.5)	9 (13.2)
Diffuse MAC in the right or left superficial femoral artery. Yes	0 (0.0)	1 (1.5)
MAC in right tibial arteries, yes	2 (2.7)	6 (8.8)
MAC in left tibial arteries, yes	2 (2.7)	6 (8.8)
A mild degree of MACin the right or left tibial arteries, yes	2 (2.7)	5 (7.4)
Diffuse MAC in the right or left tibial arteries, yes	0 (0.0)	2 (2.9)
Stenosis or occlusion of the left or right tibial arteries, yes	1 (1.4)	0 (0.0)
Peripheral artery disease[a], yes	7 (9.6)	5 (7.4)
Any MAC, yes	21 (28.8)	27 (39.7)

Note: [a] Any stenosis or occlusion plus any intimal vascular calcifications.
eGFR, estimated glomerular filtration rate.
MAC-medial arterial calcification.

3.3. Analysis of Factors Related to MAC

In both univariable and multivariable logistic regression analysis, age and CKD were consistently associated with MAC (Table **4**). PLWH had higher odds of having MAC on univariable and multivariable analyses when outliers were removed (Table **4**). However, when outliers were retained in the multivariable logistic regression model, the association of HIV infection with MAC was not statistically significant (P=0.113) (Table **4**).

Among PLWH, age, hypertension, and eGFR < 60 ml/min/1.73m^2 were strongly associated with MAC. For HIV-related factors, there was a hint that previous stavudine use and lower CD4 cell counts could be associated with MAC (Table **5**).

Table 4. Factors associated with medial arterial calcifications based on vascular ultrasound examination.

-	Univariable Analysis		Multivariable Analysis[a]		Multivariable Analysis[b]	
	OR (95% CI)	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
Age, per 5 years	2.00 (1.60-2.49)	< 0.001	2.21 (1.69-2.90)	<0.001	2.89 (2.02-4.13)	<0.001
Female *vs* male	0.40 (0.22-0.74)	0.004	0.59 (0.25-1.38)	0.222	0.93 (0.36-2.40)	0.880
HIV–positive *vs* HIV-negative	2.27 (1.18-4.38)	0.014	2.44 (0.81-7.37)	0.113	4.64 (1.28-16.83)	0.020
CKD yes *vs* no	2.33 (1.28-4.22)	0.005	3.45 (1.33-8.94)	0.011	4.60 (1.57-13.49)	0.005
BMI per 5 kg/m^2	1.26 (0.91-1.73)	0.159	0.72 (0.46-1.11)	0.138	0.73 (0.45-1.19)	0.204
Current smoker *vs* non-smoker	1.46 (0.81-2.62)	0.203	2.08 (0.95-4.58)	0.068	3.30 (1.35-8.07)	0.009
Hypertension yes *vs* no	3.08 (1.68-5.65)	< 0.001	2.00 (0.74-5.41)	0.171	1.87 (0.63-5.59)	0.262
Dyslipidemia yes *vs* no	1.52 (0.84-2.75)	0.168	0.57 (0.24-1.35)	0.203	0.57 (0.22-1.45)	0.237
Diabetes yes *vs* no	7.90 (2.17-28.78)	0.002	—[c]		—[d]	

Note: Diabetes was not included in the presented multivariable models because of few cases (n=16) and concerns about overfitting the model.□[a] Full model.

[b] Model excluding five outliers.

[c] When diabetes was included its OR and 95% CI were 6.43 (1.01-40.96) (P=0.049); the OR (95% CI) for CKD was 3.87 (1.44-10.40) and for HIV status 2.78 (0.89-8.65).

[d] When diabetes was included its OR and 95% CI were 18.80 (1.97-179.28) (P=0.011); the OR (95% CI) for CKD was 5.68 (1.78-18.15) and for HIV status 5.65 (1.46-21.94).

BMI, body mass index; CKD, chronic kidney disease; OR, odds ratio; CI, confidence interval.

4. DISCUSSION

We assessed the presence of lower extremity MAC by comprehensive vascular ultrasound examination in PLWH and HIV-uninfected controls with and without CKD. MAC was most frequently found in PLWH with CKD (76%); it was particularly common in superficial femoral arteries and tibial arteries (Table **2**). MAC was also more frequently found in PLWH (54%) than in HIV-uninfected persons (34%). On multivariable analysis, older age and CKD were consistently related to MAC, whereas the relationship of HIV infection to MAC was less pronounced and was not present in all models (Table **3**). Atherosclerotic PAD was also more frequent in PLWH with MAC compared with HIV-uninfected persons with MAC.

Table 5. Main demographic, anthropometric and HIV-related variables related to medial arterial calcifications in persons living with HIV.

Variables	Medial Arterial Calcifications			
	Total N=50	**Not present N=23**	**Present N=27**	***P*-value**
Male gender	45 (90.0)	20 (87.0)	25 (92.6)	0.651[a]
Age, years	52.5 (45 - 63)	49 (36 - 53)	62 (52 - 71)	<.001
Body mass index, kg/m^2	26.5 (24.7 - 28.5)	26.8 (23.8 - 29.4)	26.1 (24.8 - 28.4)	0.815
Estimated GFR, <60 ml/min/1.73m^2	25 (50.0)	6 (26.1)	19 (70.4)	0.002
Hypertension, yes	16 (32.0)	2 (8.7)	14 (51.9)	0.001
Known duration of HIV infection, years	12.6 (6.0 - 16.9)	8.4 (6.0 - 14.6)	13.1 (7.2 - 21.5)	0.186
Current CD4 cell count per mm^3	540.0 (358.0 - 720.0)	689.0 (390.0 - 874.0)	419.0 (315.0 - 599.0)	0.028
Nadir CD4 cell count per mm^3	117 (37 - 300)	155 (78 - 346)	74 (31 - 205)	0.066
Nadir CD4 cell count categories per mm^3				0.217
< 50	17 (34.0)	5 (21.7)	12 (44.4)	
50 to ≤ 200	14 (28.0)	7 (30.4)	7 (25.9)	
>200	19 (38.0)	11 (47.8)	8 (29.6)	
Had clinical AIDS, yes	16 (32.0)	6 (26.1)	10 (37.0)	0.408
Duration of ART, years	10.5 (5.0 - 14.5)	8.1 (3.1 - 12.7)	12.7 (5.6 - 16.4)	0.056
Current ART regimen				0.996[a]
2NRTI+II	20 (40.0)	9 (39.1)	11 (40.7)	
2NRTI+NNRTI	14 (28.0)	7 (30.4)	7 (25.9)	

(Table 5) contd.....

Variables	Medial Arterial Calcifications			
	Total N=50	**Not present N=23**	**Present N=27**	***P*-value**
1NRTI+II[b]	9 (18.0)	4 (17.4)	5 (18.5)	
2NRTI or 1NRTI +PI	5 (10.0)	3 (13.0)	2 (7.4)	
Other	2 (4.0)	0 (0.0)	2 (7.4)	
Ever use of zidovudine, yes	24 (48.0)	9 (39.1)	15 (55.6)	0.247
Ever use of stavudine, yes	11 (22.0)	2 (8.7)	9 (33.3)	0.036
Ever use of protease inhibitors, yes	31 (62.0)	11 (47.8)	20 (74.1)	0.057

Note: [a]Exact test. [b]Dolutegravir + lamivudine.
Values are frequencies and percentages (in parenthesis) or median and first and third quartiles (in parenthesis).
ART, antiretroviral therapy. NRTI, nucleoside analogues reverse transcriptase inhibitors; II, integrase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitors.

It is problematic to directly compare the prevalence of MAC from published studies because the population under study differs, and the methods used to determine MAC also vary. There are also only a few studies examining MAC in PAD-asymptomatic persons. In a recent study by Konijn *et al*. [20] using computerized tomography (CT) of the lower extremities, the prevalence of lower extremity calcifications was 55.9% (66/118) in non-atherosclerotic PAD patients. The authors found mainly less severe crural dot-like calcifications in non-PAD patients that seem to fit atherosclerotic intimal calcifications [20]. One small study looking at upper extremity medial calcifications in PAD-asymptomatic CKD patients examined by vascular ultrasound found a prevalence of MAC 26% (11/41) [21]. Older studies in patients with type 2 diabetes found a prevalence of MAC based on radiological findings in 17% [9] and 41.5% [22] patients, respectively. In a more recent study in patients with diabetes, Liu *et al*. [17] reported that vascular ultrasound is more sensitive in detecting MAC, and MAC was found in 65.8% (173/263) of subjects by ultrasound examination compared to 12.2% when plain radiography was used. CKD is a well-known risk factor for MAC [8]. The prevalence of MAC measured by the ankle-brachial index (ABI ≥1.3) was reported to be 23.7%, 41.7%, 23.1%, and 3.4% in subjects with predialysis, dialysis, and kidney transplant recipients, respectively [23].

The prevalence of MAC has not yet been reported in PLWH. In a large study conducted by Beckman *et al*. on 28,714 HIV-infected veterans, the authors reported that HIV infection and atherogenic risk factors (older age, hypertension, diabetes, current smoking, and CKD) increased the risk of incident PAD [6]. PAD was defined through administrative claims to individuals who received health coverage from different insurers [24], and the risk of incident PAD was nearly 2-fold higher in persons with sustained CD4 cell counts < 200 cells per mm^3 [6]. In a study of 97 subjects in the Swiss cohort, the prevalence of atherosclerotic PAD based on the ABI index was 20.7% [25]. A recent study from Nigeria reported a higher prevalence rate of low ABI (14.6%) in PLWH compared to the 2% prevalence rate in HIV-uninfected controls [26].

Various diagnostic approaches have been reported in studies on MAC and PAD. The most commonly used noninvasive methods were the ABI and toe-brachial index (TBI) measured by the Doppler technique. An ABI of ≤0.9 and a TBI of ≤0.6 are indicative of atherosclerotic PAD [23, 27], and an ABI ≥ of 1.3 indicates MAC [23]. However, a Cochrane review found little evidence about the accuracy of the ABI for diagnosing atherosclerotic PAD in people with exertional leg pain [28]. A recent study suggested that an elevated ABI should not be used to diagnose MAC [29]. Plain foot radiograms have been used [9, 10, 22]; however, ultrasound examination is more sensitive in detecting IAC and MAC [17]. Thin-sliced computer tomography (CT) has also been used in differentiating MAC and IAC; however, CT scans are usually done for other purposes [30] and calcifications in arteries are incidental findings. Based on our study's findings, we believe that ultrasound may detect MAC and atherosclerotic PAD in asymptomatic persons and hence could be more frequently used in epidemiological studies.

MAC is considered a vascular disorder distinct from atherosclerosis [8] and is characterized by the accumulation of calcium phosphate with the formation of hydroxyapatite crystals [31]. The pathophysiology is complex and poorly understood, including cellular and molecular mechanisms of inflammation, apoptosis, calcium phosphate metabolism, osteogenic processes, extracellular matrix degradation, matrix vesicle accumulation, and genetic factors [8]. MAC increases vascular stiffening and decreases vessel compliance, affecting localized blood flow [8]. HIV infection is associated with chronic inflammation and monocyte activation [32], which might contribute to MAC. Moreover, perhaps some antiretrovirals might contribute to media calcification [13]. Despite the differences between MAC and atherosclerotic PAD, there are some risk factors common for both (e.g., older age, diabetes, and CKD), which allows clinicians to develop preventive strategies. The correlation of MAC with other viral infections has rarely been studied. Fayed *et al*. studied the prevalence, severity, and distribution of arterial calcification in hemodialysis patients (stage 5 CKD) with hepatitis B (48 patients) and hepatitis C infection (66 patients). Arterial calcification detected by histopathology of arterial tissue was present in all patients with HCV, of which 31 had medial calcification, and 19 had both intimal and medial calcifications [33].

Our study has limitations primarily because of the relatively small size of the PLWH cohort, which might have reduced the statistical power. In particular, the number of patients in some subgroups was small, resulting in wide confidence intervals (*e.g*., the presence of diabetes). Because of the small sample size, we did not compare MAC in persons with stage 3 versus stage 4/5 CKD, as reported by Hassan *et al*.

by performing mammography and examining intramammary arteries [34]. Nevertheless, as others did, we found an association between age, CKD, and diabetes with MAC. The association of HIV infection with MAC was present in bivariable analysis and univariable logistic regression, and multivariable logistic regression models when outliers were removed. Because of the small sample size, we could not assess the relationship between the type of antiretroviral drugs used and MAC and whether there might be differences in those on treatment compared to those not treated. PLWH and control group participants were enrolled at different times. However, the vascular ultrasound methodology was the same, so we believe our results are valid. Although widely accepted, we may also argue that the current system for the definition and classification of CKD is not without controversy [35].

CONCLUSION

In conclusion, we found a high prevalence of MAC and atherosclerotic PAD in HIV-infected PAD-asymptomatic persons. PLWH had MAC more frequently than non-HIV-infected patients, and CKD worsened the findings. Age and CKD were consistently associated with lower extremity MAC defined by ultrasound examination. We also provided evidence for an association of HIV infection with lower extremity MAC. Further studies are needed to examine the prevalence of MAC in PLWH and assess asymptomatic MAC's long-term outcome.

LIST OF ABBREVIATIONS

PAD	=	Peripheral Artery Disease
MAC	=	Medial Arterial Calcification
CKD	=	Chronic Kidney Disease
eGFR	=	Estimated Glomerular Filtration Rate
HIV	=	Human-Immunodeficiencyvirus

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Ethics Committee of the University Hospital for Infectious Diseases "Dr. Fran Mihaljević," Zagreb, Croatia and Riuniti Hospital, Anzio, Italy (approval number: 01-157-4-2018).

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All human research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

Written informed consent for participation was obtained from each study participant.

STANDARDS FOR REPORTING

STROBE guidelines and methodology were followed.

AVAILABILITY OF DATA AND MATERIALS

The data supporting this study's findings are available on request from the corresponding author [N.R].

FUNDING

This work was financially supported by the Croatian Science Foundation (Project No. IP-2019-04-9702).

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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