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Abstract:	Background/Aims: Vascular calcifications and fractures are major complications of chronic kidney disease. Hemodialysis patients have a high prevalence of atrial fibrillation and an increased risk of thromboembolism, which should be prevented with warfarin, a drug potentially causing increased risk of vascular calcifications and fractures. Methods: A total of 314 hemodialysis patients were recruited, 101 with documented atrial fibrillation and 213 non atrial fibrillation patients. Comorbidities, chronic kidney disease mineral and bone disorder blood tests and therapies were collected. Vertebral quantitative morphometry was carried out centrally for the detection of fractures, defined as vertebral body reduction by ≥20 %. In the same radiograph, the length of aortic calcification was also measured. Logistic regression models were applied for evaluating the independent predictors of presence of vascular calcifications and vertebral fractures. Results: In our population vascular calcifications were very common (>85%). Severe vascular calcifications (>10 cm) were more common in atrial fibrillation (76%) than in non atrial fibrillation patients (33%). Vertebral fractures were present in 54% of patients. Multivariable analysis showed that atrial fibrillation (OR=5.41, 95%CI 2.30-12.73) and 25(OH)vitamin D <20 ng/mL (OR=2.05, 95%CI 1.10-3.83) were independent predictors of vascular calcifications. Age (OR=1.04/year, 95%CI 1.01-1.07) and male gender (OR=1.76, 95%CI 1.07-2.90) predicted vertebral fractures. Conclusions: Hemodialysis patients had an elevated prevalence of vascular calcifications, especially when affected by atrial fibrillation. Low vitamin D levels were strongly associated with vascular calcifications. Prevalence of vertebral fractures was also remarkably high and associated with older age and male gender.
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Atrial fibrillation and low vitamin D levels are associated with vascular calcifications in a population of hemodialysis patients

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ABSTRACT

Background/Aims: Vascular calcifications and fractures are major complications of chronic kidney disease. Hemodialysis patients have a high prevalence of atrial fibrillation and an increased risk of thromboembolism, which should be prevented with warfarin, a drug potentially causing increased risk of vascular calcifications and fractures.

Methods: A total of 314 hemodialysis patients were recruited, 101 with documented atrial fibrillation and 213 non atrial fibrillation patients. Comorbidities, chronic kidney disease mineral and bone disorder blood tests and therapies were collected. Vertebral quantitative morphometry was carried out centrally for the detection of fractures, defined as vertebral body reduction by ≥20 %. In the same radiograph, the length of aortic calcification was also measured. Logistic regression models were applied for evaluating the independent predictors of presence of vascular calcifications and vertebral fractures.

Results: In our population vascular calcifications were very common (>85%). Severe vascular calcifications (>10 cm) were more common in atrial fibrillation (76%) than in non atrial fibrillation patients (33%). Vertebral fractures were present in 54% of patients. Multivariable analysis showed that atrial fibrillation (OR=5.41, 95%CI 2.30-12.73) and 25(OH)vitamin D <20 ng/mL (OR=2.05, 95%CI 1.10-3.83) were independent predictors of vascular calcifications. Age (OR=1.04/year, 95%CI 1.01-1.07) and male gender (OR=1.76, 95%CI 1.07-2.90) predicted vertebral fractures.

Conclusions: Hemodialysis patients had an elevated prevalence of vascular calcifications, especially when affected by atrial fibrillation. Low vitamin D levels were strongly associated with vascular calcifications. Prevalence of vertebral fractures was also remarkably high and associated with older age and male gender.

Key words: atrial fibrillation, fractures, hemodialysis, vascular calcifications, vitamin D, warfarin.

INTRODUCTION

In hemodialysis (HD) patients, chronic kidney disease mineral and bone disorder (CKD-MBD) is a significant clinical problem (1), characterized by an elevated prevalence of several important adverse outcomes, including an increased bone turnover associated with secondary hyperparathyroidism, low bone turnover (adynamic bone), cardiovascular calcifications, bone fractures and increased mortality (2-3).

Atrial fibrillation (AF) is the most common arrhythmia in the general population (4) and it is even more frequent in HD patients (5). AF is associated with an increased incidence of ischemic stroke, prompting cardiologic guidelines to recommend treatment with oral anticoagulants (warfarin or novel oral anti-coagulants) in patients with AF (6). However, use of warfarin has been associated to an increased risk of osteoporotic fractures in the elderly (7). In addition, in HD patients the possible causal relationship between warfarin treatment and vascular calcifications (VCs) has been proposed, as already demonstrated in experimental studies (8,9).

Aim of this study is evaluating, in HD patients with and without AF, the prevalence of VCs and vertebral fractures, as well as identifying the risk factors with a significant role in their determination.

METHODS

This study has been approved by Local Review Boards. All clinical investigation has been conducted according to the principles expressed in the Declaration of Helsinki. Written informed consent has been obtained from the participants

Study design

Patients with and without AF were recruited from two cohorts previously studied by our group (2, 10). From a cohort of 290 hemodialysis (HD) patients with documented AF (10), 101 patients accepted to participate in further analysis and were included in the study. Non-AF control patients were recruited from a cohort of HD patients previously studied for the assessment of fractures and VCs (2). From this cohort, all patients within the same range of age and dialytic

age with respect to AF patients were recruited (N=213). Inclusion criteria were broad, i.e. adult patients of both genders on HD, willing to give informed consent to the use of their medical records for the study.

At recruitment we collected demographical data, dialytic age and the presence of comorbidities (hypertension, diabetes mellitus, peripheral artery disease, ischemic heart disease, heart failure, previous ischemic strokes) in all patients. Moreover, CKD-MBD characteristics (plasma calcium, phosphate, parathyroid hormone, alkaline phosphatase, 25(OH)vitamin D levels) and therapies (aluminium binders, calcium based binders, calcium free binders, calcitriol, cinacalcet, paricalcitol) were collected at recruitment. Among the 101 AF patients, 48 were taking oral anticoagulation treatment (OAT).

In all patients, a radiograph of the thoracic and lumbar regions of the spinal column in the latero-lateral view with the patient in the lateral recumbent position was obtained. Each participating dialysis center was given a detailed information sheet explaining how to carry out spinal column X-rays correctly. The radiograph was to be carried out always by the same technician using the same film distance (100 cm) and the same focus for the central ray, namely D7 for the dorsal region and L3 for the lumbar region; D12 had to be visible in both the dorsal and the lumbar tract radiographs. Assessment of the radiographs was centralized at CNR in Padua, Italy and performed separately by two blinded physicians according to the quantitative method (Quantitative Vertebral Morphometry, QVM) using a dedicated software (SpineAnalyzer™, Version 3.2, Optasia Medical Ltd., Cheadle, UK). Vertebral fracture was defined as a deformity of the vertebral body due to reduction in one of its dimensions (anterior, middle and posterior heights) by more than 20%. Vertebral fracture assessment was carried out based on the indications of Genant et al (11), but using QVM. Quantification was carried out assigning 6 points to the level of the upper and lower margin of each vertebra from D5 to L4, defining three measures: anterior [ha], middle [hm], and posterior [hp] height. Abnormal modifications of these measures allow diagnosing three types of deformity, namely wedge deformity (reduction in anterior [ha] vs. posterior [hp] height by 20%: ha/hp<80%), biconcave deformity (reduction in middle height [hm] as compared to the posterior height

[hm/hp<80%] and crush deformities (all three dimensions reduced by more than 20% as compared to the average height of the two adjacent vertebrae, upper and lower). The severity of the vertebral deformities was estimated as mild, moderate or severe (reduction in height: 20–25%, 25–40% or >40%, respectively) (12,13).

The same radiograph was used to calculate the VC score according to Witteman et al. (14), quantifying the length of the calcium deposits along the aortic wall (mild 0.1-5 cm, moderate 5.1-10 cm, and severe > 10 cm).

Statistical Methods

We evaluated the distribution of demographic, CKD-MBD characteristics and therapies according to AF. We presented continuous variables as quartiles and categorical variables as proportions. Differences across AF were assessed using $\chi 2$ tests for categorical variables and Wilcoxon rank sum test for continuous variables.

In order to evaluate the associations of AF with VCs and vertebral fractures, the logistic regression model was applied. Vascular calcifications were classified in two levels (>10 cm versus absent or ≤10cm – small numbers precluded consideration of more classes below 10 cm).

The covariates included in the multivariable models have been selected based on their biologically plausible potential to confound the association between AF and outcomes. Multivariable models were all adjusted for gender, age, dialytic age, OAT, heart failure, peripheral artery disease, ischemic heart disease. The model on VCs was further adjusted for previous stroke, 25(OH)vitamin D and vertebral fractures, while the model on vertebral fractures was adjusted for VCs. Odds ratios (ORs) and 95% confidence intervals (CIs) were reported. We also evaluated the interactions between AF and the variables included in the model. Parameter estimates in these models were combined according to Rubin (15), after a multiple imputation procedure, by a Markov chain Monte Carlo method, performed to take care of missing data on VCs (16 patients-5% due to technical difficulties in reading radiographs) and on 25(OH)vitamin D (36 patients-11%). Imputation assumed multivariate normality and considered demographic, clinical and CKD-MBD characteristics of patients besides the outcomes (20 imputed data-set, MI SAS procedure, SAS

version 9.4). We assumed missing at random, given that lack of data was due to technical issues not related to the outcome itself. Sensitivity analyses were performed adopting different imputation methods and similar results were found.

Descriptive data were reported as raw percentages on the complete case series. Multivariable models were carried out both on the complete case series and on the imputed data. Test were two-sided. Analyses were performed with SAS 9.4.

RESULTS

Demographic and clinical characteristics of patients are described in Table 1, according to AF status. Age and time spent on dialysis were similar. Oral anticoagulation treatment was administered to 48% of AF patients. Atrial fibrillation patients had a significantly higher prevalence of comorbidities (heart failure, peripheral arterial disease, ischemic cardiomyopathy, and previous ischemic strokes episodes), compared to non-AF patients. In addition, CKD-MBD characteristics of AF patients were different: higher levels of alkaline phosphatase, lower 25(OH)vitamin D levels (<20 ng/ml), lower use of calcium free binders and higher use of calcitriol (Table 1). The distribution of 25(OH)vitamin D levels was strikingly different, as illustrated in Figure 1. Median 25(OH)vitamin D levels [1^{st} -3^{rd} quartiles] were markedly lower in AF patients than in controls, 12[3-24] vs 27[20-38] ng/mL, respectively.

In the overall population, VCs of any degree were highly prevalent (89% of patients), and significantly more prevalent in the AF population (96% of patients) (Table 2). When considering patients with severe calcifications (>10 cm) they were markedly more common among AF patients (76%), compared to non-arrhythmic patients (33%). On the contrary, AF patients showed a lower prevalence of vertebral fractures (40% vs. 60%, Table 2). Considering the subgroup of AF population, no differences were found on the prevalence of vascular VCs (P=0.2) or vertebral fractures (P=0.9) among patients under OAT or not. Similar results were obtained taking into account the time of being exposed to OAT before study recruitment (data not shown).

Univariate analysis showed an association between severe VCs and peripheral arterial disease (p=0.01), ischemic heart disease (P=0.001), 25(OH)vitamin D levels <20 ng/ml (P<0.001), and C-reactive protein (CRP) > 0.5 mg/dL (P=0.02). Prevalence of vertebral fractures was negatively associated with peripheral artery disease (P=0.008) and diabetes mellitus (P=0.002). Considering the subgroup of AF population, no association between OAT and vascular VCs or vertebral fractures was found (P=0.3 and P=0.9, respectively).

At multivariable analysis with imputation of missing data, AF patients and patients with 25(OH)vitamin D levels < 20 ng/ml had higher odds [OR 5.41 (CI 2.30;12.73) and 2.05 (CI 1.10; 3.83), respectively] of having severe VCs (Table 3). No other factor in the model was an independent predictor of VCs. The OR of patients undergoing OAT was 0.58 (0.2; 1.69), P=0.8. The association between AF and VCs was not modified by the vitamin D level (P of interaction 0.8).

Results were coherent with a model based on the raw data [AF, OR 21.09 (CI 5.65 ; 78.65); vitamin D <20 ng/ml, OR 2.55 (CI 1.33;4.9)], where however estimates were of much lower precision due to missing data. For vertebral fractures, independent predictors were male gender [OR 1.76 (1.07;2.90), P=0.03] and older age [OR 1.04 per year (1.01;1.07), P=0.02]. The OR of patients undergoing OAT was 0.92 (0.40; 2.10), P=0.8. Results were confirmed in the model using raw data (Table 4).

DISCUSSION

The main finding of this study performed in HD patients is the independent association of VCs with AF and 25(OH)vitamin D levels <20 ng/ml. We also reported a very high prevalence of VCs in this population, especially in those affected by AF. In addition, AF patients had markedly lower 25(OH)vitamin D levels than non-AF patients. Independent predictors of vertebral fractures in our population were male gender and older age.

Data from the literature show that in AF patients with normal renal function, warfarin treatment is associated with coronary artery, aortic and mitral valve calcifications (16-17). An increased risk of

VCs due to OAT has been demonstrated in experimental CKD (9) and suggested for dialysis patients (18-19).

In our AF population, warfarin was prescribed to 48% of patients, but the number of AF patients with VCs was impressively higher: 96% showed some degree of calcification, while 76% of them had aortic calcifications >10 cm, compared to 33% of patients with sinus rhythm. Given this elevated prevalence of VCs, the role of warfarin might be underestimated. Atrial fibrillation patients showed a high degree of cardiovascular impairment, as demonstrated by the high number of cardiovascular comorbidities, which could facilitate the calcification process of arteries. This process can mask the pro-calcifying action of warfarin, a vitamin K antagonist (8-9).

In a previous study, factors associated with VCs in HD patients treated with long dialysis sessions and good adherence to guidelines indications for CKD-MBD were assessed. In this study, only age, serum levels of Fibroblast Growth Factor 23 (FGF-23) and diabetes were associated with severe VCs (20). Recent data can shed some light on the relationship between AF and CKD-MBD. Incidence of AF was associated with higher circulating FGF-23 levels, which may explain in part the link between chronic kidney disease and AF (21). FGF-23 is a peptide hormone inhibiting vitamin D (22) and Klotho expression (23). In addition, HD patients with AF have lower circulating Klotho levels (24). Circulating Klotho may have a role in cardiovascular protection (25) and lower levels in AF patients may favor progression of vascular damage. However, Scialla et al (26) recently showed that FGF-23 is not associated with arterial calcification and does not promote calcification experimentally, although higher FGF-23 levels are independently associated with greater risk of cardiovascular events, particularly heart failure, in patients with CKD stages 2-4 (27).

Our data show a clear, significant, independent association between low 25(OH)vitamin D levels and VCs: patients with vitamin D levels <20 ng/ml had a double risk of severe (>10 cm) aortic calcifications. Previous reports on the effects of vitamin D on VCs are contradictory. Experimental data in cells and animals suggest that it may facilitate calcification through up-regulation of Runx2/Cbfa1 (Runt-related transcription factor 2 / Core binding Factor A1), osterix (a transcription

factor for osteoblast differentiation) and osteocalcin (Bone Gla Protein), thus increasing intracellular calcium transport in vascular smooth muscle cells (28). However, the hypothesis on the pro-calcifying effect of vitamin D through osteocalcin is not supported by the finding of the association between higher osteocalcin levels and reduced progression of aortic calcification in a population of 51-85 year-old males prospectively followed for 10 years (29). Additional data indicating a protective role of vitamin D on VCs derive from studies showing that vitamin D increases the expression of proteins inhibiting calcification, such as MGP (matrix Gla protein) and osteopontin, while reducing pro-inflammatory cytokines, such as IL6, IL1b and TFG-beta (28).

Bone fractures are an important and poorly studied outcome in dialysis patients. In this study, being older and of male gender are factors significantly associated with a higher probability of vertebral fractures. A higher prevalence of fractures in male patients has been previously reported, in particular in warfarin treated subjects (30). In our study, warfarin treatment is not associated with vertebral fractures. However the number of patients treated with warfarin was limited and a role of OAT in determining vertebral fractures can not be excluded. In addition, the high number of cardiovascular comorbidities in our AF population might be associated with reduced mobility and therefore with a reduced risk of falls.

A limitation of the study is that patients with and without AF were recruited from two different patient populations. However, both cohorts of HD patients shared the same inclusion criteria, except for AF. Differences between the two cohorts in terms of comorbidities and therapies are likely due essentially to AF and are accounted for in the multivariable analyses. Atrial fibrillation patients are heavily calcified and the number of patients treated with warfarin is limited, therefore the calcifying effect of OAT shown in other populations may have been blunted. Another limitation is the presence of missing data in VCs and vitamin D. We accounted for this lack of data by multiple imputation. Moreover, sensitivity analyses were applied and the observed association between AF and vascular calcification remained highly significant.

In conclusion, prevalent HD patients had a very high prevalence (> 85%) of VCs. Overall, severe calcifications were present in 45% of patients, but those presenting AF were affected in 76% of

cases. Warfarin apparently was not implicated in this phenomenon, but this unexpected result should prompt further studies in larger population of AF patients undergoing HD, possibly with lower basal calcification scores, such as in incident HD patients.

Low 25(OH)vitamin D levels were clearly associated with VCs in our population. This observation is important because it justifies a prospective study evaluating the effects of cholecalciferol supplementation on cardiovascular calcification in HD patients, with target levels of 25(OH)vitamin D higher than 20 ng/mL. Interestingly, low vitamin D levels were also associated with AF in our patients.

Previous reports of a relevant prevalence (54% of affected patients) of vertebral fractures in the HD population were also confirmed by this study, underscoring the need of further investigations for identifying preventable risk factors.

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Figure 1: Box-plot of 25 (OH) Vitamin D (ng/mL) levels. Vitamin D levels were markedly lower in patients with atrial fibrillation.

Black dots represent mean values. Dashed horizontal lines report the reference values of 20 and 30 ng/mL. The empty dots represent outliers defined as patients with Vitamin D that is more than 1.5 times the interquartile range above the third quartile (or more than 1.5 times the interquartile range below the first quartile).

Table
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Table 1: Demographics and clinical characteristics of patients, according to atrial fibrillation (AF) presence.

Characteristic	Total (N=314) n(%)	NO AF (N=213) n(%)	AF (N=101) n(%)	P
Males	199(63)	133(62)	66(65)	0.6
Age (yr), median [1st -3rd quartiles]	72[66-77]	71[66-76]	74[65-78]	0.2
Dialytic Age (yrs), median [1st -3rd quartiles]	4[2.2-7.8]	3.8[2.3-7.1]	4.9[2.1-10.3]	0.4
Smoke (22 missing)				0.9
Never smoke	173(59)	119(59)	54(61)	
Smoker	35(12)	24(12)	11(12)	
Ex-smoker	84(29)	60(29)	24(27)	
Hypertension	255(81)	173(81)	82(81)	0.9
Heart Failure	53(17)	23(11)	30(30)	<.001
Peripheral Artery Disease	162(52)	84(39)	78(77)	<.001
Ischemic Heart Disease	96(31)	49(23)	47(47)	<.001
Ischemic Stroke	27(9)	12(6)	15(15)	0.007
Diabetes Mellitus	75(24)	52(24)	23(23)	0.8
Parathyroidectomy	17(5)	8(4)	9(9)	0.06
Calcium				0.5
≤8.4 mg/dL	35(11)	23(11)	12(12)	
8.4-|9.5 mg/dL	208(66)	138(65)	70(69)	
>9.5 mg/dL	71(23)	52(24)	19(19)	
median [1st -3rd quartiles]	9.1[8.7-9.5]	9.1[8.8-9.5]	8.9[8.7-9.4]	
Phosphate				0.1
≤3.5 mg/dL	69(22)	40(19)	29(29)	
3.5-|5.5 mg/dL	193(61)	138(65)	55(54)	
>5.5 mg/dL	52(17)	35(16)	17(17)	

median [1st -3rd quartiles]	4.3[3.6-5.3]	4.3[3.7-5.3]	4.3[3.5-5.2]	
Parathyroid Hormone				0.8
0-100 pg/mL	41(13)	26(12)	15(15)	
101-500 pg/mL	236(75)	161(76)	75(74)	
≥500 pg/mL	37(12)	26(12)	11(11)	
median [1st -3rd quartiles]	230[143-342]	230[140-341]	225[154-342]	
Alkaline Phosphatase (17 missing)				0.001
≤190 UI/L	272(92)	202(95)	70(83)	
>190 UI/L	25(8)	11(5)	14(17)	
median [1st -3rd quartiles]	87[70-116]	82[65-105]	107[77-148]	
25(OH)Vitamin D (36 missing)				<0.001
< 20 ng/mL	101(36)	54(26)	47(68)	
20|-30 ng/mL	77(28)	68(33)	9(13)	
≥ 30 ng/mL	100(36)	87(42)	13(19)	
median [1st -3rd quartiles]	24[16-35]	27[20-38]	12[3-24]	
C-Reactive Protein				0.3
≤ 0.5 mg/dL	99(32)	71(33)	28(28)	
> 0.5 mg/dL	215(68)	142(67)	73(72)	
median [1st -3rd quartiles]	1.6[0.5-4.8]	1.6[0.5-5.0]	1.5[0.5-4.0]	
Aluminium Binders	55(18)	39(18)	16(16)	0.6
Calcium Based Binders	127(40)	85(40)	42(42)	0.8
Calcium Free Binders	142(45)	106(50)	36(36)	0.02
Calcitriol	155(49)	93(44)	62(61)	0.003
Paricalcitol	56(18)	42(20)	14(14)	0.2
Cinacalcet	61(19)	37(17)	24(24)	0.2
Oral Anticoagulant Therapy	48(15)	-	48(48)	

Note: Values for categorical variables are given as number (percentage); values for continuous variables are given as median [interquartile range].

Table 2: Vascular calcifications and vertebral fractures, according to atrial fibrillation (AF) presence

	Total (N=314) N (%)	NO AF (N=213) N (%)	AF (N=101) N (%)	P
Vascular Calcifications (16 missing)				<0.001
Absent	32(11)	29(14)	3(4)	
<1 cm	3(1)	3(1)	0	
1.1-5 cm	47(16)	40(19)	7(8)	
5.1-10 cm	81(27)	71(33)	10(12)	
>10 cm	135(45)	70(33)	65(76)	
Vertebral Fractures	168(54)	128(60)	40(40)	0.003

Table 3: Multivariable analyses on data with missing imputed. Vascular calcifications and associated factors (Sever vs Absent/Small and Moderate).

	OR(95%CI)	P
Atrial Fibrillation: Yes vs No	5.41(2.30-12.73)	<.001
Gender: Men vs Women	1.52(0.87-2.66)	0.1
Age (yrs)	1.01(0.98-1.05)	0.5
Dialytic Age (yrs)	1.04(0.99-1.09)	0.1
Oral Anticoagulant Therapy: Yes vs No	0.59(0.21-1.70)	0.3
Heart Failure: Yes vs No	0.99(0.46-2.13)	0.9
Peripheral Artery Disease: Yes vs No	0.94(0.54-1.65)	0.8
Ischemic Heart Disease: Yes vs No	1.45(0.78-2.70)	0.2
Previous stroke: Yes vs No	1.83(0.72-4.67)	0.2
25(OH)Vitamin D (ng/mL): <20 vs $\geq$ 20	2.05(1.10-3.83)	0.03
Vertebral Fractures: Yes vs No	1.50(0.87-2.56)	0.1

Table 4: Multivariable analyses on data with missing imputed. Vertebral fractures and associated factors.

	OR(95%CI)	P
Atrial Fibrillation: Yes vs No	0.50(0.25-1.01)	0.05
Gender: Men vs Women	1.76(1.07-2.90)	0.03
Age(yrs)	1.04(1.01-1.07)	0.02
Dialytic Age (yrs)	1.01(0.98-1.05)	0.5
Oral Anticoagulant Therapy: Yes vs No	0.92(0.4-2.10)	0.8
Heart Failure: Yes vs No	0.69(0.35-1.36)	0.3
Peripheral Artery Disease: Yes vs No	0.63(0.38-1.04)	0.07
Ischemic Heart Disease: Yes vs No	0.88(0.51-1.55)	0.7
Vascular Calcifications Severe or Moderate vs Absent or Small	1.11(0.91-1.35)	0.3

Figure
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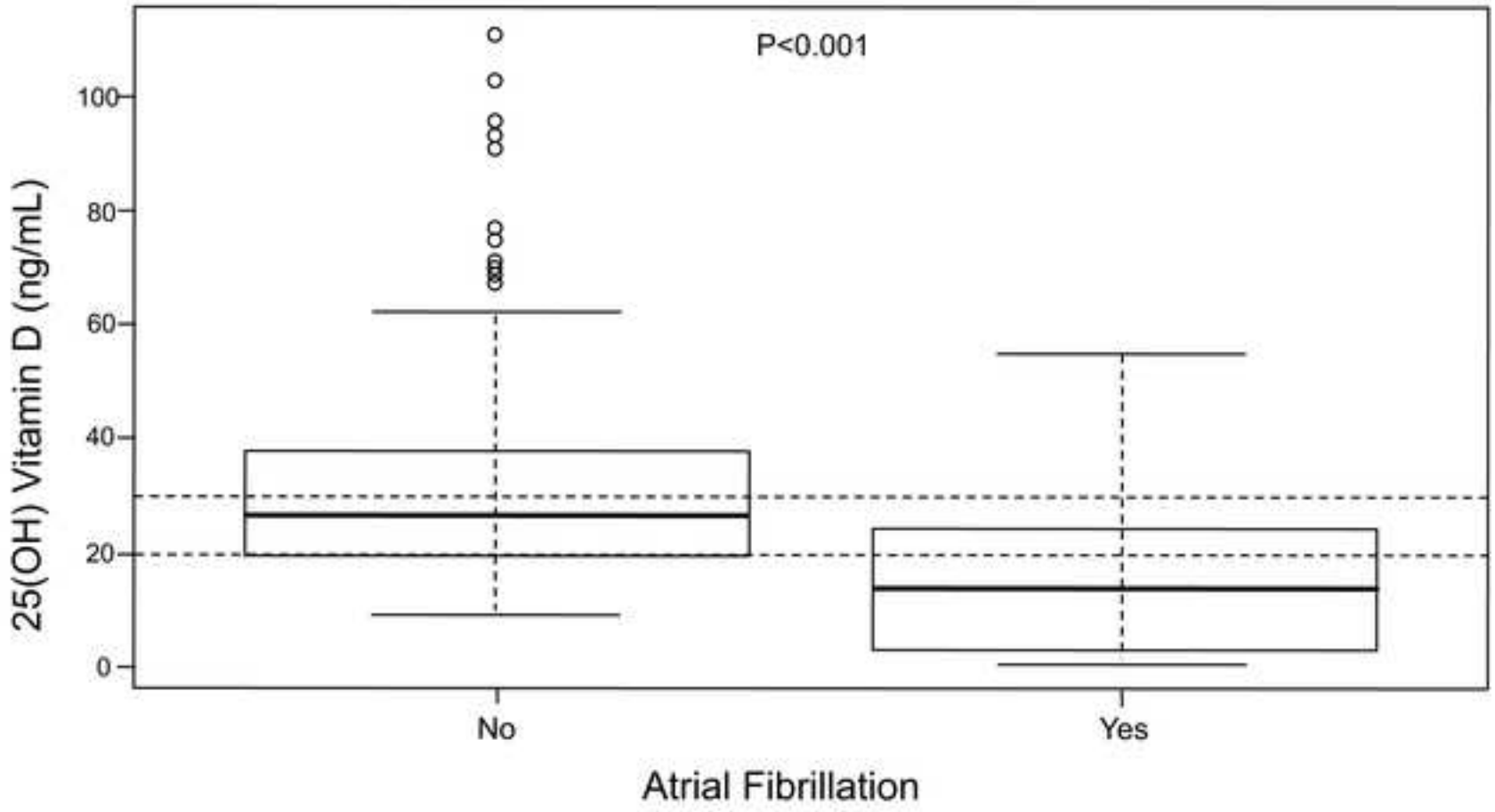

Figure 1