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ORIGINAL ARTICLE

Atrial fibrillation and low vitamin D levels are associated with severe vascular calcifications in hemodialysis patients

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Abstract
Background/Aims Vascular calcifications (VCs) and fractures are major complications of chronic kidney disease. Hemodialysis patients have a high prevalence of atrial fibrillation (AF) and an increased risk of thromboembolism, which should be prevented with warfarin, a drug potentially causing increased risk of VCs and fractures. Aim of this study is evaluating, in hemodialysis patients with and without AF, the prevalence of VCs and fractures, as well as identifying the associated risk factors.

Methods A total of 314 hemodialysis patients were recruited, 101 with documented AF and 213 without AF. 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 VCs and vertebral fractures.

Results In our population VCs were very common (>85 %). Severe VCs (>10 cm) were more common in patients with AF (76 %) than in patients without (33 %). Vertebral fractures were present in 54 % of patients. Multivariable analysis showed that AF (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 VCs. 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 severe VCs, especially when affected by AF. Low vitamin D levels were strongly associated with severe VCs. Prevalence of vertebral fractures was also remarkably high and associated with older age and male gender.

Keywords Atrial fibrillation · Fractures · Hemodialysis · Vascular calcifications · Vitamin D · Warfarin

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Introduction

In hemodialysis (HD) patients, chronic kidney disease mineral and bone disorder (CKD-MBD) is a significant clinical problem [1], characterized by an elevated

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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]. Atrial fibrillation 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 multicenter studies performed 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. Control patients were recruited from a cohort of HD patients without AF 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 patients with AF 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^{TM}, 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 χ^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 vs. absent or ≤10 cm—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 and by the results of univariate correlations ($p < 0.15$). 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 dataset, 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 patients with AF. Patients with AF had a significantly higher prevalence of comorbidities (heart failure, peripheral arterial disease, ischemic cardiomyopathy, and previous ischemic strokes episodes), compared to patients without. In addition, CKD-MBD characteristics of patients with AF 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 Fig. 1. Median 25(OH) vitamin D levels (1st–3rd quartiles) were markedly lower in patients with AF 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 population with AF (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, patients with AF showed a lower prevalence of vertebral fractures (40 vs. 60 %, Table 2). Considering the subgroup of population with AF, no differences were found on the prevalence of severe 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 population with AF, 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, patients with AF 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 severe VCs. The OR of patients undergoing OAT was 0.59 (0.21; 1.70), $P = 0.3$. The association between AF and VCs was not modified by the vitamin D level (P of interaction 0.8). Accordingly, an higher odd of VCs for patients with 25(OH) vitamin D levels <20 ng/ml was also observed in patients with and without AF evaluated separately, but it resulted statistically significant only in the larger group of patients without AF [OR 2.37 (1.18; 4.74), $P = 0.02$]. The lack of association between OAT and VCs was also observed in patients with AF separately.

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), $P = 0.8$ (Table 4). Coherent results were also found in patients with and without AF evaluated separately, though significance was hampered by smaller sample size (data not shown). Results were confirmed in the model using raw data.

Table 1 Demographics and clinical characteristics of patients, according to presence of 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 (years), median (1st–3rd quartiles)	72 (66–77)	71 (66–76)	74 (65–78)	0.2
Dialytic vintage (years), median (1st–3rd quartiles)	4 (2.2–7.8)	3.8 (2.3–7.1)	4.9 (2.1–10.3)	0.4
Smoking status (missing data, 22)				0.9
Never smoked	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)	<0.001
Peripheral artery disease	162 (52)	84 (39)	78 (77)	<0.001
Ischemic heart disease	96 (31)	49 (23)	47 (47)	<0.001
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/dLdl	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/dLdl	69 (22)	40 (19)	29 (29)	
3.5–5.5 mg/dLdl	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/mLml	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 data, 17)				0.001
≤190 UI/L/l	272 (92)	202 (95)	70 (83)	
>190 UI/L/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 data, 36)				<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 based 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

Table 1 continued

Characteristic	Total (n = 314) n (%)	No AF (n = 213) n (%)	AF (n = 101) n (%)	p
Cinacalcet	61 (19)	37 (17)	24 (24)	0.2
Oral anticoagulant therapy	48 (15)	–	48 (48)	

Values for categorical variables are given as number (percentage); values for continuous variables are given as median (interquartile range)

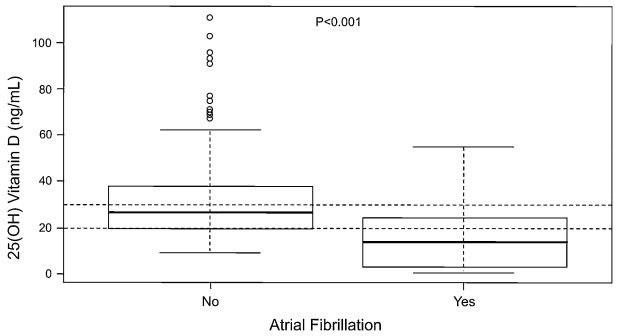

Fig. 1 Box-plot of 25(OH) vitamin D (ng/mL) levels. Vitamin D levels were markedly lower in patients with atrial fibrillation. *Black dots* represent mean values. *Dashed horizontal lines* report the reference values of 20 and 30 ng/mL. The *empty dots* represent outliers defined as patients with Vitamin 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)

Discussion

The main finding of this study performed in HD patients is the independent association of severe 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, patients with AF had markedly lower 25(OH) vitamin D levels than patients without AF. Independent predictors of vertebral fractures in our population were male gender and older age.

Data from the literature show that in patients with AF and 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 population with AF, warfarin was prescribed to 48 % of patients, but the number of patients with AF and 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. Patients with AF 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].

The number of patients treated with warfarin was lower than expected considering the current cardiologic guidelines. We confirmed that less than 50 % of dialysis patients with AF are undergoing OAT, as previously reported [10]. Even lower numbers have recently been reported in a large US Study [20], where less than 15 % of dialysis patients with newly diagnosed AF were treated with warfarin. There are several possible explanations for this finding, including a higher risk of bleeding compared to patients with normal renal function, uncertain benefits of OAT in the prevention of stroke in dialysis patients and the possibility of inducing worsening of VCs by inhibiting vitamin K dependent proteins.

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

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

	Total (n = 314) n (%)	No AF (N = 213) n (%)	AF (N = 101) n (%)	p
Vascular calcifications (missing data, 16)				<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 analysis with missing data imputed on factors associated with vascular calcifications (severe vs. absent/small or moderate)

	OR (95 % CI)	p
Atrial fibrillation: yes vs. no	5.41 (2.30–12.73)	<0.001
Gender: men vs. women	1.52 (0.87–2.66)	0.1
Age (years)	1.01 (0.98–1.05)	0.5
Dialytic vintage (years)	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. ≥20	2.05 (1.10–3.83)	0.03
Vertebral fractures: yes vs. no	1.50 (0.87–2.56)	0.1

OR odds ratio, *CI* confidence interval

Table 4 Multivariable analysis with missing data imputed on factors associated with vertebral fractures

	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 (years)	1.04 (1.01–1.07)	0.02
Dialytic vintage (years)	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

OR odds ratio, *CI* confidence interval

with severe VCs [21]. 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 [22]. FGF-23 is a peptide hormone inhibiting vitamin D [23] and Klotho expression [24]. In addition, HD patients with AF have lower circulating Klotho levels [25]. Circulating Klotho may have a role in cardiovascular protection [26] and lower levels in AF patients may favor progression of vascular damage. However, Scialla et al. [27] 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 [28].

Our data show a clear, significant, independent association between low 25(OH) vitamin D levels and severe 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 [29]. 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 [30]. 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 [29].

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 [31]. In our study, warfarin treatment is not associated with vertebral fractures, and no association was found between fractures and severe VCs. These results are contradictory to the largely accepted notion that bone demineralization go parallel to VCs. However, the number of patients treated with warfarin was limited and a role of OAT in determining vertebral fractures cannot 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. Moreover, it appears that AF status is strictly associated with a high frequency of severe VCs, which in this patient population might be at least partially independent from the mineral and bone disorder of chronic kidney disease (CKD-MBD), resulting from a complex interaction of multiple factors. Bone mineralization is only one aspect of CKD-MBD and other causative factors may be confounded in our population, where cardiovascular comorbidities may play a major role.

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 and were recruited by two multicenter parental studies. 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. Patients with AF 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. The study is also limited by the absence of assessment of bone mineral density as well as a more quantitative method of vascular calcification assessment. Because $1,25(OH)_2D_3$, FGF23, and Klotho levels are not routinely determined in clinical practice, they could not be included in our observational study. 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. Finally, the observational nature of our study cannot establish a causal association between vitamin D deficiency and AF. The same applies to the association between VCs and AF, where it might well be that patient who calcify become more prone to AF and not that AF and its associated treatments favor VCs.

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 patients with AF undergoing HD, possibly with lower basal calcification scores, such as in incident HD patients. The practical meaning of our findings lies in the concept that dialysis patients with AF should be considered at high risk of VCs, independently from active treatment with OAT. This implies determination of VCs and it has consequences on the choice of treatments for CKD-MBD abnormalities.

Low 25(OH) vitamin D levels were clearly associated with severe 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.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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