VASCULAR CALCIFICATIONS, VERTEBRAL FRACTURES AND

MORTALITY IN HAEMODIALYSIS PATIENTS

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ABSTRACT

Background: Vascular calcifications and the bone fractures caused by abnormal bone fragility, also called osteoporotic fractures, are frequent complications associated to chronic kidney diseases (CKD). The aim of this study was to investigate the association between vascular calcifications, osteoporotic bone fractures and survival in haemodialysis (HD) patients.

Methods: A total of 193 HD patients were followed up to 2 years. Vascular calcifications and osteoporotic vertebral fractures (quoted just as vertebral fractures in the text) were assessed by thoracic, lumbar spine, pelvic and hand X-rays and were graded according to their severity. Clinical, biochemical and therapeutical data gathered during the total time spent on HD were collected.

Results: The prevalence of aortic calcifications was higher in HD patients than in a random-based general population (79% versus 37.5%, $p<0.001$). Total time on any renal replacement therapy (RRT) and diabetes were positively associated to a higher prevalence of vascular calcifications. In addition to these factors, time on HD was also positively associated to the severity of vascular calcifications, and higher haemoglobin levels were associated with a lower prevalence of severe vascular calcifications in large and medium caliber arteries. The prevalence of vertebral fractures in HD patients was similar to that of the general population (26.5% versus 24.1%). Age and time on HD showed a positive and statistically significant association with the prevalence of vertebral fractures. Vascular calcifications in the medium caliber arteries were associated to a higher rate of prevalent vertebral fractures. In women, severe vascular calcifications and vertebral fractures were positively associated to mortality [RR=3.2 (1.0-10.0) and RR=4.8 (1.7-13.4) respectively].

Conclusions: Positive associations between vascular calcifications, vertebral fractures and mortality have been found in patients on HD.

Keywords: Haemodialysis, mortality, osteoporotic fractures, fragility fractures, vascular calcifications, vertebral fractures.

Short summary:

In HD patients, vascular calcifications were more frequent than in the general population, in addition, vascular calcifications in medium caliber arteries were positive and significantly associated with a higher rate of prevalent vertebral fractures. In women, the presence of severe vascular calcifications and vertebral fractures were positively associated with mortality [RR=3.2 (1.0-10.0) and RR=4.8 (1.7-13.4) respectively].

INTRODUCTION

Vascular calcifications have been associated with adverse clinical outcomes in CKD patients, including ischaemic cardiac events, claudication and mortality [1-6]. The pathogenesis of vascular calcifications is complex and not fully understood. It does not only consist of a simple precipitation of calcium (Ca) and phosphorus (P), it is also an active and modifiable process. Vascular calcification in CKD may be interpreted as the result of the dysregulation of the equilibrium between calcification promoters and inhibitors in which several uremic factors, including abnormalities in the mineral metabolism, are implicated [7-11]. Other risk factors for vascular calcification, such as age, diabetes mellitus, dyslipidemia, hypertension and smoking may play a role not only in vascular calcification but also in bone health.

Bone mineral density and osteoporotic fractures are well known markers of bone health in both general and CKD populations [12, 13]. However, until recently, they had not been linked or associated with changes in the vascular system. Experimental data have clearly shown that vascular smooth muscle cells can modify their phenotype becoming osteoblast-like cells able to induce vascular calcifications[14-19]. This fact has opened a fascinating area of cross-link research between vascular and mineral metabolisms. In addition, recent data from general population and CKD patients have shown that not only vascular calcifications but also osteoporotic fractures are associated with an increased risk of mortality [20,21].

The aim of this multicenter study carried out in HD patients was to investigate the association between vascular calcifications, osteoporotic fractures and survival in HD patients.

MATERIAL AND METHODS

The study was carried out in 193 HD patients (121 men and 72 women) from a total of 258 patients from 7 HD units in Asturias (Spain) followed up for 2 years. The clinical practice protocols and the type of dialysis in all dialysis units were similar and the quality control of aluminium (Al) in dialysis water in all units was performed in the reference center (Hospital Universitario Central de Asturias). Once the informed consent was obtained, only the HD patients who completed the radiological studies were subsequently included in the study. Patients who did not give the informed consent and patients with senile dementia were not included in the study. The mean age was 64±14 years (men) and 68±13 years (women); the mean time on HD was 3.1±3.6 years. Most women (91.7%) were post-menopausal but they never received estrogen, selective estrogen receptor modulators or bisphosphonates. Vascular calcifications and vertebral fractures were diagnosed following the methods described below. Peripheral osteoporotic fractures reported by patients were confirmed by medical records. Eighty four different parameters, including clinical, biochemical and therapeutical information gathered during the total time spent on HD were collected. The arithmetic mean of the monthly biochemical values since the beginning of HD until the date of beginning of the study was obtained. Moreover, the cumulative doses of Ca, calcitriol and Al-containing phosphate binder received since the beginning of dialysis were also investigated.

In order to diagnose vascular calcifications all patients underwent X-Ray studies (thoracic, lumbar spine, pelvic and hand). The analyses were done grouping the arteries into three types according to their size and the predominant constituent of the tunica media: large or elastic arteries (aorta and iliac) in which elastic fibers are the main component of the media; medium (muscular or distributive) arteries (femoral,

uterine-spermatic and radial) in which smooth muscle is the predominant constituent of the media; and small arteries (palm arch and digital) in which also a minor quantity of smooth muscle is present. Abdominal aortic calcifications were graded according to their severity into mild (isolated punctiform calcifications), moderate (linear calcifications with a maximum length of two vertebral bodies or one dense plaque) and severe (linear calcifications with a length of two or more vertebral bodies and/or two or more dense plaques). Iliac, femoral, uterine/spermatic, radial, palm arch and digital vascular calcifications were also graded and grouped as mild-moderate when non-confluent calcifications or the partial section of the vessel was calcified, and severe if contiguous calcifications involving multiple segments or the whole section of the vessel were calcified.

Thoracic and lumbar spine X-ray were used to diagnose vertebral fractures by a standard procedure detailed elsewhere [22]. Vertebral fractures were classified according to Genant´s semi-quantitative method [23], which considers osteoporotic vertebral fractures as a reduction of the vertebral height (anterior, posterior or middle) higher than 20% after excluding other causes of vertebral deformities.

Non-vertebral (peripheral) osteoporotic fractures were included in the analysis only if they had been previously confirmed in the medical records. Fractures were defined as osteoporotic when they fulfilled the next two criteria: a) they were caused by a minor trauma or by a fall from a maximum height equal to or below the upright position of the patient, and b) localised in typical areas of osteoporotic fractures excluding skull, hands and feet. Vascular calcifications and vertebral fractures were blindly evaluated by two independent experts with an inter-observer concordance of 90% and a kappa index of 0.73 [20].

The HD patients were prospectively followed up for two years; patients contributed as person-time until they underwent kidney transplantation, reached the end of the follow-up period, died or were lost to follow-up.

The prevalence of aortic calcifications and vertebral fractures were compared to a random-based general population cohort from the same geographic area (308 men and 316 women; mean age 65±9 years) who participated in the European vertebral osteoporosis study [EVOS], in which X-Ray evaluations of thoracic and lumbar spine were carried out following the aforementioned standard procedure [22, 24].

The clinical, biochemical and therapeutical data gathered during the total time spent on HD were:

- General and clinical data: gender, date of birth, profession, center of dialysis, height, weight, dialysis treatment, dairy products intake after starting dialysis, physical activity, age of menarche, age of menopause, number of births, breast-feeding, hysterectomy and peripheral and vertebral fractures.

- Renal disease and general HD data: Etiology of renal disease (nephrosclerosis and/or ischemic renal disease, diabetes, polycystic kidney disease, glomerular disease, tubulointersticial disease, other etiology, unknown etiology), total time on dialysis, age of starting dialysis, number of renal transplants, time on transplant, time on replacement therapy (the sum of time on HD, time on peritoneal dialysis and time on transplant), localization of functioning arterious-venous fistula, time of each HD session, bath solution use in HD session (bicarbonate, Ca acetate), Kt/V, PCR.

- Risk factors and background: diabetes, time on diabetes, alcohol habits (actual hard-drinking, non actual hard-drinking, no hard-drinking), tobacco habits (current

smoker, non-smoker, former-smoker person), high blood pressure, years of high blood pressure, bone diseases, endocrine diseases, neurological diseases, gastroenterology diseases, respiratory diseases, cardiovascular diseases, urologic diseases, parathyroidectomy, age of parathyroidectomy.

- Biochemical data: serum Ca, P, CaxP product, parathyroid hormone (PTH), alkaline phosphatase, Al, total cholesterol, HDL-cholesterol, triglycerides, total proteins, albumin, haemoglobin, haematocrit and bicarbonate.

- Treatment: cumulative dose of Ca, calcitriol and Al-containing compounds, corticosteroids, anticoagulants, thyroid hormones, lipid-lowering drugs and insulin.

The study protocol was conducted according to the Helsinki Declaration. Informed consent was obtained from all the recruited patients and approved by the Clinical Research Ethics Committee of Asturias. Statistical analyses were performed with the SPSS v.8.0 software for Windows. The prevalence of vascular calcifications and vertebral fractures were expressed as percentages with 95% confidence intervals (CI). Differences in clinical and biochemical data and the association between clinical and biochemical variables and vascular calcifications were compared using t-test analyses, multivariable logistic regression analyses and also non-parametric tests (U Mann Whitney) if required. The strength of the association between the prevalence of vascular calcifications and the prevalence of vertebral fractures was compared calculating the odds ratio (OR) and 95% CI using multivariable logistic regression analyses. All these analyses were adjusted by age, time on dialysis, time on RRT, haemoglobin, diabetes, sex and all treatments administered.

Kaplan-Meier survival curves were used to examine crude survival. Cox multiple regression analyses for censored survival data were performed to compare survival

between subjects with and without prevalent vertebral fractures and vascular calcifications. The analyses were performed separately for men and women after adjusting by age and time on dialysis.

RESULTS

The main clinical and biochemical data collected are summarized in Table I; there were no statistically significant differences between both sexes.

Vascular calcifications, osteoporotic fractures and clinical and biochemical parameters

Based on the caliber of the arteries, the prevalence of vascular calcifications was: 86.4% in large caliber, 70.5% in medium caliber and 20.2% in small caliber arteries.

The prevalence of aortic calcifications was higher in HD patients than in the general population (79% vs. 37.5%, p<0.001). This difference was observed in both sexes (77.8% vs. 29.7% in women, p<0.001 and 80.3% vs. 45.4% in men, p<0.001). In HD patients, the Odds Ratio (OR) for having prevalent aortic calcifications (all grades) compared with the general population was 8.7 (5.0-15.0). In women, the OR was slightly higher than in men [OR=9 (3.8-21) and OR=7.7 (3.7-16.1), respectively].

Mean serum Ca, P, CaxP, median iPTH and the median cumulative intake of calcitriol, Ca or Al-containing phosphate binders, corticosteroids and other therapies were not significantly associated with the prevalence of any type of vascular calcifications (Table 2). From the rest of factors analyzed, only age, time on HD, total time on any renal replacement therapy (RRT) (HD, peritoneal dialysis or renal transplantation) and diabetes were positively and significantly associated to a higher prevalence of vascular calcifications (Table 3). After age and sex adjustments, the multivariate logistic regression analysis showed that only time on RRT and diabetes maintained the positive association.

Age was associated to vascular calcifications in large and medium caliber arteries while time on HD and total time on RRT were associated with an increased risk of vascular calcification, only in medium caliber arteries, such as femoral arteries [OR=1.26 (1.07-1.48) and OR=1.17 (1.04-1.32)], radial arteries [OR=1.17 (1.02-1.33) and OR=1.13 (1.02-1.24)] and uterine/testicular arteries [OR=1.20 (1.04-1.39) and OR=1.15 (1.03-1.29)].

Diabetes was the most relevant variable associated with an increase risk of vascular calcifications, mainly in small and medium caliber arteries, such as digital [OR=18.2 (4.7-70.2), palmar-arch OR=17.6 (4.8-65), radial OR=13.4 (3.8-47.7), uterine/spermatic arteries OR=3.1 (1.1-9.1) and femoral arteries [OR=5.5 (1.6-18.5)] but no association was found in large caliber arteries, iliac and aorta arteries [OR=0.4(0.1-1.5) and OR=1.1(0.3-3.9), respectively]. The other multiple clinical conditions detailed before showed no association with vascular calcifications at any site in the univariate and multivariate analyses.

The prevalence of severe vascular calcifications was 63.6%, 70.7% in large caliber arteries, 55.6% in medium caliber arteries and 14.1% in small caliber arteries. The severity of vascular calcifications in small caliber arteries was significantly higher in men than in women (20.3% vs. 5%, p<0.05). There was no association between the severity of vascular calcifications and biochemical parameters or the cumulative dose of drugs received.

In large and medium caliber arteries, higher haemoglobin levels were associated with a lower prevalence of severe vascular calcifications [OR=0.74 (0.55-0.99) and OR=0.65 (0.44-0.96), respectively]. Among the other registered and analyzed factors, time on RRT was positively and significantly associated to the prevalence of severe vascular calcifications in large and medium caliber arteries [OR=1.23 (1.03-1.47) and

OR=1.18 (1.02-1.36), respectively], the time on HD was also positively and significantly associated with the prevalence of severe vascular calcifications in medium caliber arteries [OR=1.47 (1.15-1.87)].

Similarly, diabetes was also positively associated with the severity of vascular calcifications [OR=6.43 (1.65-25.11)]. This effect was more remarkable in small and medium caliber arteries compared with large caliber arteries [small caliber: OR= 83.17 (11.81-585.86), medium caliber: OR= 33.38 (3.69-301.88) and large caliber: OR=2.07 (0.57-7.54)].

The prevalence of vertebral fractures in HD patients was 26.5%, slightly higher in women than in men (33.3% vs. 21.7%, p=0.162) and similar to the values found in the general population 24.1% (27.3% in women vs. 20.7% in men, p=0.056)[25]. It was more common to have two or more vertebral fractures in women than in men (25.5% vs. 6%, p<0.05). Among all the different clinical and biochemical parameters analysed, only age and time on HD showed a significant, positive association with the prevalence of vertebral fractures. Diabetes was not associated with an increased risk of vertebral fractures.

The prevalence of non-vertebral osteoporotic fractures in HD patients was 13.5% (26 fractures in total: 12 forearms, 6 hip fractures and 8 at other sites). Among all the different variables analysed (general, clinical, biochemical and therapeutical), only age was positively associated with the prevalence of peripheral osteoporotic fractures. In fact, the risk of having peripheral osteoporotic fractures increased 7% per year.

Relationship between vascular calcifications, fragility fractures and mortality

Vascular calcifications in medium caliber arteries were statistically significant and positively associated to a higher rate of prevalent vertebral fractures but there were

not relationship between vascular calcifications and peripheral osteoporotic fractures. At other sites such as, large caliber arteries there was a trend, but not statistically significant differences (table 4).

After two years of follow-up, 25 women (34.7%), and 39 men (32.2%) died. In men, no significant association was observed between vascular calcifications and mortality. By contrast, the 93.3% of women who died had severe vascular calcifications at least in one of the studied areas. In women (figure 1A), severe vascular calcifications, was positively associated with mortality after adjustments by age and time on HD (Cox multiple regression analyses) [RR=3.2 (1.0-10.0), p<0.05]. When serum iPTH, serum Ca and cumulative intake of calcitriol were included as covariates, a similar behaviour was observed [RR=3.8 (1.1-13.6, p<0.05), but only severe vascular calcifications were independently associated with mortality.

As figure 1B depicted, women with vertebral fractures showed also a higher mortality rate [RR=4.8 (1.7-13.4)] adjusted by age, time on HD and severe vascular calcifications (Cox multiple regression analysis)]. When serum iPTH, serum Ca and cumulative intake of calcitriol were included as covariates, a more marked effect was observed [RR=6.3 (2.0-20.3, p<0.005), but only the prevalence of vertebral fracture was independently associated with mortality. No differences were found in men. No relationship between non-vertebral fractures and mortality was found either in men or in women.

DISCUSSION

In recent years, the study of vascular calcifications, demographic, clinical and biochemical parameters and bone fractures in patients with CKD has gained great interest, mainly due to their great impact on morbidity and mortality [5, 26].

Calcification in the vessel walls occurs at two sites: the intima and the media. Calcification of the intima is frequently localized in the aorta and coronaries and associated to atherosclerotic burden. Calcification of the media occurs in the elastic lamina of the large and medium caliber arteries, it increases with age and it is frequent in CKD and diabetic patients. The complications of these two types of vascular calcifications are different; however, they greatly account for the increase in morbidity and mortality of CKD patients [27-29].

In this study, prevalent aortic calcifications were significantly higher in HD patients (79%) than in a random-based general population of the same age, sex and region (37.5%)[25]. Similar results have been reported in other studies with HD patients [30]. Moreover, women on HD showed an increased risk of having severe aortic calcifications compared with women from the general population probably due to a combination of atherosclerosis and arteriosclerosis with intima and media vascular calcifications.

Most of the previous studies have focused their interest on the aorta and coronary arteries. In this study, however, we also looked at other sites (digital, palm arch, radial, uterine-spermatic, femoral and iliac arteries) and we also looked at the severity of vascular calcifications using a semiquantitative method. Other original and important aspect of the present work was to investigate the relationship between the prevalence of vascular calcifications in different arterial territories -classified according

to their size and the predominant constituent of tunica media- and the different variables studied [31-33]. We found a relationship between the prevalence of vascular calcifications and the caliber of the arteries. In HD patients, the prevalence of vascular calcifications in large caliber arteries was higher than in medium and small caliber arteries, - four times in the latter -. These results could be at least partly due to the different arterial wall structure of the three categories of arterias studied [34], which may also imply relevant functional changes in the vasculature.

In addition, the severity of vascular calcifications in small caliber arteries was significantly higher in men than in women (19.4% vs 4.8%, $p<0.05$), a finding that could be partly related with the protective role of estrogens in inflammation, atherosclerosis and calcification, a common finding in young women but also verifiable even in elderly women [35-37].

Among demographic, general and clinical parameters, the age was positively associated with vascular calcifications in large and medium caliber arteries, reinforcing the role of age as risk factor for atherosclerosis and arteriosclerosis[38]. Time on HD and total time on RRT were positively associated with vascular calcifications, particularly in medium caliber arteries, in which each year on RRT increased the risk of having vascular calcifications by approximately 15%. These results are in agreement with previous studies that have identified the time spent on dialysis as an important risk factor, mainly for the medial but also for the intima arterial calcifications [3, 4, 38], [39].

Other meaningful clinical or biochemical variables, such as hypertension, hyperlipidaemia, hypercalcaemia, hyperphosphataemia, hyper- or hypoparathyroidism, corticosteroids treatment, cumulative intake of Ca, calcitriol and Al-containing phosphate binders or elevated CaxP product did not show any significant association with the prevalence and severity of vascular calcifications.

Interestingly, high serum haemoglobin levels were associated with a lower prevalence of severe vascular calcifications in large and medium caliber arteries. This finding is in agreement with the well-known fact that chronic anaemia results in an increased cardiac output which, over time, leads to ventricular dilation, hypertrophy and the arterial remodelling of central elastic arteries such as the aorta and the carotids, leading to arterial enlargement, compensatory arterial intima-media thickening and arteriosclerosis [40].

Diabetes, a highly prevalent condition in HD patients, was strongly associated with the prevalence of calcifications, particularly in small caliber arteries. In fact, the risk of having any type of vascular calcification increased from 18 to 38 times in all the comparisons carried out between diabetic and non-diabetic HD patients. This strong, positive association has been already reported in diabetic patients with no CKD [41-43].

So far, few studies in HD patients have focused on the prevalence of vertebral fractures [44], clinical and biochemical parameters and current treatments. We found the prevalence of vertebral fractures (26.5%) was higher than that documented in previous studies [44-46], but similar to that observed in a general population (25%) recruited in the same geographical area with similar age and sex patterns to those of the cohort of HD patients studied [24]. Only age and time on HD showed a positive and statistically significant association with a higher prevalence of vertebral fractures. In agreement with other recently published studies, none of the biochemical variables or previous treatments showed any significant association with vertebral fractures [46].

Non-vertebral (peripheral) osteoporotic bone fractures, particularly hip fractures, seem to be more prevalent in dialysis patients [47]. In agreement with the DOPPS II study (2.6%), we observed a 3% prevalence of hip fractures and a clearly positive association of hip fractures with age [48]. Although PTH levels might be an independent risk factor

for osteoporotic fractures [12, 47], the association of these two parameters is difficult to prove in cross-sectional or short-term follow-up studies (2 years) as it is the case of our study. Therefore, similarly to us, Stehman-Breen et al. [49] did not find any association between PTH and hip fracture in HD patients. In any case, the small number of hip fractures (n=6) in our study might be insufficient to infer a more detailed explanation.

Vascular calcifications, osteoporotic fractures and mortality

Even though the pathogenetic factors linking vascular calcifications and bone fragility are not clear, several studies have shown a greater predisposition to develop vascular calcifications when bone turnover is low [50-52]. In our study we have found a positive relationship between osteoporotic vertebral fractures and vascular calcifications in some large and medium calibre arteries, mainly in femoral and uterine-spermatic, but not in small calibre arteries. This finding could be related to the reduced amount of muscle cells present in small calibre arteries. In fact, only vascular smooth cells derived from the media from large and medium size caliber arteries are those which undergo calcification and bone and cartilage-like phenotypic changes under several in vitro experimental conditions[15]. In addition, the reduced number of patients with calcification in small calibre arteries makes difficult to draw definitive and more solid conclusions in this area.

In agreement with our results, other authors have also found a relationship between large size arteries (aorta) and osteoporotic fractures in the general population [53]. , reinforcing the idea that there is a positive association between vascular calcifications and osteoporotic fractures in both, general and HD population.

The Framminghan study carried out in general population has shown that vascular calcifications are independent predictors of vascular morbidity and mortality [54]. In our study, only women on HD showed a positive association between prevalent

severe vascular calcifications and mortality. Conversely, no association was found in men, supporting the hypothesis that there are relevant sex differences in which the arterial remodelling effect of hormones could be implicated. Cardiovascular risk factors may have different impact according to the sex, as example, in general population, cholesterol is more important for cardiovascular risk in men than in postmenopausal women, in whom hypertension, diabetes and its combination play a major role [55].

Similarly to what has been described in the general population [20, 56], HD women showed a positive association between prevalent vertebral fractures and mortality. This finding, after only two years of follow-up, stresses the role and importance of bone health as an independent parameter related not only with morbidity and quality of life but also with mortality.

We are aware of some of the limitations of our study. Firstly, the relatively small sample size but also the only 2 years of follow-up may have prevented from obtaining associations among some of the other relevant variables studied. Secondly, since we used a non-highly sensitive technique such as X-Rays to detect vascular calcifications, it is possible that the use of more specific and sensitive techniques, such as electron beam computed tomography (EBCT) or spiral computed tomography [57-60] might have added additional useful information. However, in favour of our results, recent studies have shown a good correlation between EBCT and X-Rays [61, 62, 55]. In any case, the fact we found a positive association with a medium size sample and using a less sensitive but widely available technique as the standardized X-Rays demonstrates the strength of the association between vascular calcifications, bone fractures and mortality.

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CONFLICT OF INTEREST STATEMENT

All the authors of this manuscript declare that the results presented in this paper have not been published previously in whole or part, except in abstract format.

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Table 1. Clinical variables in HD patients by sex.

	Men (n=121)	Women (n=72)
Age (years)	64±14	68±13
Time on RRT (years)	3.3±4.2	3.9±4.3
Time on HD (years)	2.7±2.9	3.8±4.5
Mean Ca (mg/dl)	9.4±0.8	9.5±0.7
Mean P (mg/dl)	5.4±1.2	5.8±1.3
Mean CaxP (mg^2/dl^2)	51±13	53±12
iPTH (pg/ml)	141(17-877)	203(8-1581)
Cumulative intake of Ca (kg)	1.1 (0.5-2.4)	0.9 (0.3-2.4)
Cumulative intake of calcitriol (mg)	46 (22-107)	59 (23-165)
Cumulative intake of Al$(OH)_3$ (kg)	0.5 (0.3-2.5)	0.3 (0.1-1.5)
Diabetes mellitus	23.1%	19.4%
Any treatment with corticoids	23.1%	23.6%
Treatment with cholesterol-lowering drugs	12.5%	8.3%
Previous renal transplant	10.2%	9.4%

No statistically significant differences were found according gender. iPTH and cumulative intake of Ca, calcitriol and Al was expressed as median and interquartile range. In this case, the Mann-Whitney test was used for comparison.

Table 2. Mean and standard deviation values of biochemical parameters (Ca, P and PTH), median values and interquartile range of PTH, cumulative dose received of calcitriol, Ca and Al in patients with (yes) or without (no) vascular calcifications in different arterial localizations. No statistically significant differences were found.

	Calcifications in Large caliber arteries		Calcifications in Medium caliber arteries		Calcifications in Small caliber arteries	
	Yes	**No**	**Yes**	**No**	**Yes**	**No**
Ca (mg/dl)	9.5±0.7	9.4±1	9.5±0.8	9.4±0.6	9.4±0.7	9.5±0.7
P (mg/dl)	5.4±1.2	5.6±1.3	5.3±0.9	5.7±1.4	5.6±1	5.5±1.3
CaxP (mg^2/dl^2)	50±10	52±14	50±10	53±12	51±9	51±11
iPTH (pg/ml)	141 (72-245)	269 (129-342)	143 (80-278)	147 (67-295)	138 (108-231)	143 (69-289)
Cumulative intake of calcitriol (mg)	50 (22-150)	77 (23-98)	50 (24-160)	77 (19-95)	67 (21-179)	50 (22-107)
Cumulative intake of Ca-containing phosphate binders (kg)	1.1 (0.5-2.9)	1.0 (0.3-2.4)	1.1 (0.5-3.1)	0.9 (0.3-2.3)	0.9 (0.4-3.2)	1.2 (0.4-2.8)
Cumulative intake of Al-containing phosphate binders (kg)	0.5 (0.2-1.0)	0.4 (0.1-1.7)	0.5 (0.2-1.1)	0.4 (0.1-0.6)	0.4 (0.2-0.8)	1.1 (0.1-1.)

Table 3. Age, time on haemodialysis (HD), time on renal replacement therapy (RRT) in patients with (yes) or without (no) vascular calcifications at different caliber arteries (* significant differences, p< 0.05)

	Calcifications in Large caliber arteries			Calcifications in Medium caliber arteries			Calcifications in Small caliber arteries		
	Yes	**No**	**p**	**Yes**	**No**	**p**	**Yes**	**No**	**p**
Age	**66±14**	**51±20**	**0.01***	**66±14**	**57±19**	**0.019***	64±13	64±16.5	NS
Time on HD (years)	2.8±3	2.2±3.3	NS	**3.2±3.5**	**1.7±1.1**	**0.001***	2.8±3.1	2.9±3.2	NS
Time on RRT (years)	3.5±4.5	2.8±4	NS	**3.9±4.9**	**2.2±2.8**	**0.039***	4.4±6.2	3.2±3.8	NS

Table 4. Relative risk (OR) of vertebral fractures in haemodialysis patients with (yes) or without (no) vascular calcifications in different caliber arteries, adjusted by age and sex (*significant differences, p< 0.05).

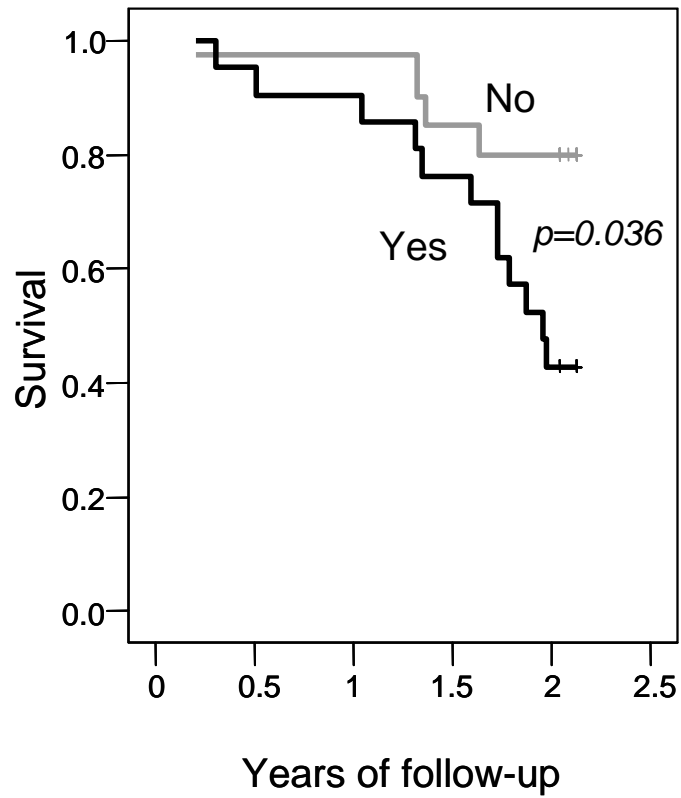|		Vertebral fractures (%)	OR (95%CI)
Calcifications in Large caliber arteries	**Yes**	26.5	3.8(0.5-31.6)
	No	6.3	
Calcifications in Medium caliber arteries	**Yes**	30.9	*6.5(1.4-29.8)**
	No	6.5	
Calcifications in Small caliber arteries	**Yes**	23.8	0.96(0.3-3.1)
	No	23.1	

Legends:

Figure 1. Kaplan-Meier analysis in women A) with prevalent severe vascular calcifications at any vascular site, B) with prevalent vertebral fractures.

A)

Severe Vascular calcifications

No

Yes

p=0.036

Survival

Years of follow-up

B)

Vertebral fractures

No

Yes

p=0.012

Survival

Years of follow-up