Original Article

Different type and dosage of heparin were not associated with the progression of coronary artery calcification in haemodialysis patients

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SUMMARY AT A GLANCE

This study shows no significant effects of different types and dosages of heparin on baseline and progression of coronary artery calcification in hemodialysis patients.

ABSTRACT:

Aim: **Several studies have verified that unfractionated heparin (UFH) and low molecular heparin (LWMH) can induce bone loss, and bone mineral density has been inversely associated with vascular calcification in some clinical researches. But few have focused on the relationship between types and dosages of heparin and the progression of vascular calcification. We observed the progression of coronary artery calcification (CAC) in maintenance haemodialysis (MHD) patients who were treated with UFH and LMWH.**

Methods: **This was a prospective prevalent cohort study of MHD patients. Computed tomography was performed at enrolment and 2 years after enrolment, and CAC score was obtained. Demographic and clinical data, baseline and time-average laboratory indices were collected. Multiple linear regression and logistic regression were used to estimate the influencing factors of progression of CAC.**

Results: **In this study, (i) we initially enrolled 69 HD patients, and then 56 patients finished the follow-up. (ii) Among the total 56 patients, 27 patients (48.2%) were treated with UFH, 14 (25.0%) with LMWH and 15 (26.8%) with both. The median baseline CAC scores of three groups (UFH, LMWH and both users) were 91.0 (1.0, 1052.0), 134.0 (0, 1292.0) and 250.5 (27.0, 1139.0), respectively, with no significant difference ($P = 0.663$); the median CAC progression scores were 42.0 (0, 364.0), 172.0 (7.0, 653.0) and 118.5 (0, 434.0), respectively, with no significant difference ($P = 0.660$). (iii) Pearson and spearman correlation analysis shown that the progression of CAC was not associated with cumulative dosage of heparin used. (iv) After adjusted for diabetes mellitus, time-averaged intact parathyroid hormone, phosphate and alkaline phosphatase, logistic regression analysis showed using different types of heparin was not an independent risk factor for CAC progression; and multiple linear regression analysis showed that the type of heparin used was not associated with CAC progression.**

Conclusion: **There were no significant differences in the effects of the types and dosages of heparin on CAC progression in patients on haemodialysis.**

The incidence of cardiovascular disease (CVD) in end-stage renal disease (ESRD) patients is 20–30 times higher than that of the general population.[1] Vascular calcification is a highly prevalent complication in ESRD patients, an important cause of CVD, and an independent predictor of all-cause death and cardiovascular death in haemodialysis (HD) patients.[2] The incidence of coronary artery calcification (CAC) in new dialysis patients is about 60%, and in maintenance HD (MHD) patients, the incidence of CAC increased

to 70–83%.[3–5] The association of long-term use of heparin with vascular calcification was studied.

It has been wildly known that long-term use of unfractionated heparin (UFH) is associated with osteoporosis and bone fracture.[6,7] In 1965, Griffith *et al.*[8] analyzed 117 patients on long-term use of UFH for the treatment of thromboembolic disease, and they found that the use of UFH caused bone loss, osteoporosis and increased risk of fracture. In a study of patients with deep venous thrombosis

who had been on UFH or low molecular heparin (LMWH), Monreal et al.[7] found that the incidence of fractures was significantly higher in patients treated with UFH than in patients treated with LMWH. Although LMWH had relatively fewer side effects than UFH,[9] long-term use of LMWH was not risk-free.[10] In vivo, Meng et al. treated rats with different doses of UFH and LMWH, they observed that UFH-induced bone loss in chronic kidney disease (CKD) rats with secondary hyperparathyroidism was mainly caused by inhibition of osteoblast activity and promotion of osteoclast activity.[11] In vitro, the mechanism of UFH caused osteoporosis was that UFH promoted osteoclastogenesis by inhibiting the activity of osteoprotegerin.[12,13]

The relationship among abnormalities of serum biochemical markers, renal bone disease and vascular calcification in ESRD patients had been established. Chen et al.[14] indicated that low bone density is an independent risk factor of vascular calcification in non-dialysis CKD patients. Tangvoraphonkchai and Davenport reported that lower BMD at the femoral neck was associated with greater aortic pulse wave velocity, reinforcing the hypothesis of a link between bone disease and vascular disease in dialysis patients.[15] Aleksova et al.[16] showed that trabecular bone score, an instrument for measurement of bone microarchitecture integrity, was inversely related to vascular calcification in dialysis patients, which means that patients with low trabecular bone scores tended to have more severe vascular calcification.

Unfractionated heparin and LMWH have remained the most widely used anticoagulant in HD.[17,18] Previous studies have observed the phenomenon that long-term, high-dose using of heparin can cause bone density decrease and osteoporosis.[7,9,19,20] And in pre-dialysis CKD and dialysis patients, some studies indicated that bone density is inversely related to vascular calcification.[15,16] Taking all these together, we hypothesized that high dosage or long-time usage of UFH and LWMH may accelerate the progression of vascular calcification in HD patients. This was confirmed by Meng et al. [11] in rats in 2013, in this study, they had four groups: normal rats, CKD control rats, low-dose UFH CKD rats and high-dose UFH CKD rats. They found that calcium and phosphorus contents in the thoracic aorta in the high-dose UFH CKD rats were higher than those in the low-dose UFH CKD group and CKD control group.

To date, the effects of long-term use of UFH or LMWH on the occurrence and progression of vascular calcification in patients on MHD were not reported. In this study, we analyzed types and cumulative dosages of heparin on progression of CAC in HD patients in a prospective cohort.

METHODS

Study design and subjects

This was a single-centre prospective cohort study with MHD patients. Enrolled patients were received multi-slice spiral computed tomography to evaluate CAC at the time of enrolment and the end of 2 years follow-up period. The influences of type and dosage of heparins on progression of CAC were analyzed using logistic model and linear regression model. This study was approved by the Ethics Committee of Peking University People's Hospital (IRB00001052-11055).

We enrolled MHD patients with ESRD in our dialysis center from February 2012 to January 2015. Inclusion criteria for participants: (i) age >18 years old, dialysis vintage ≥6 months and stable clinical condition; (ii) use UFH and/or LMWH as anticoagulant during dialysis. Exclusion criteria: (i) conditions making computed tomography (CT) technically impossible or unreliable (such as severe cardiac arrhythmias); (ii) patients with acute complications, such as heart failure, severe infection, malignant tumour and life expectancy less than 3 months.

Demographic and clinical data

Baseline demographics were collected, including age, gender, dialysis vintage, the presence or absence of diabetes and body mass index (BMI). Laboratory indices were tested at baseline and every 3 months thereafter, and then averaged as the time-average value, including serum corrected calcium (cCa), phosphate (P), intact parathyroid hormone (iPTH), alkaline phosphatase (ALP), albumin (ALB), uric acid (UA), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (T-Chol), serum creatinine (Scr), carbon dioxide combining power (CO2CP), haemoglobin (Hgb), total urea clearance (Kt/V). We also recorded the use of the drug during the follow-up period, such as calcium-based phosphate binders, non-calcium-based phosphate binders, vitamin D analogue and Cinacalcet.

The type and dose of anticoagulants used for each dialysis during follow-up were recorded and the cumulative dosage used during the period was calculated. Patients were grouped as UFH users (UFH was used in more than 90% dialysis sessions), LMWH users (LMWH was used in more than 90% dialysis sessions), and UFH/LMWH users.

Evaluation of CAC

Computed tomography scans were performed at enrolment and 2 years after enrolment in the department of radiology of our hospital, CAC scores assessed blindly by two radiologists according to the method previously described by Agaston et al.[21] CAC progression score was defined as the difference in follow-up CAC scores minus baseline CAC scores, reflecting the progression of CAC during the 2 years follow-up period. To describe the various rate of progression of calcification, subjects were also classified as the progression of CAC score <100, 100–500, and >500.

Statistical methods

Continuous variables were expressed as mean ± standard deviation or median (25th, 75th), and categorical data were expressed as number and percentages. As the number in each group is small, differences in mean and median values among UFH, LWMH or both users were tested using Kruskal–Wallis test, which is a method of nonparametric test. Categorical variables among groups were compared using χ^2 test. Pearson and Spearman correlation analysis were used to examine the relationship between the cumulative dosage of heparin substances and the progression of CAC, the relationship between the types of heparin substances and the progression of CAC. In the one-way analysis of variance, there were significant differences of the proportion of DM, time-averaged iPTH, and time-averaged P among groups of CAC progression. We put these factors and the type of heparin into the models of multivariate linear regression analysis and logistic regression analysis, which were used to analyze the independent influencing factors for CAC progression. P value <0.05 was considered to be statistically significant. Statistical analysis was performed using SPSS software, version 22.0 (IBM Corp., Armonk, NY, USA).

RESULT

Demographic data and clinical characteristics

We initially enrolled 69 HD patients, but during the follow-up period 13 patients were excluded, the baseline characteristics of these patients were shown in Table 1. Reasons of elimination: three patients only had baseline CT scans, three patients received kidney transplantation, three patients died during follow-up, two patients had CT that could not assess coronary calcification score due to severe motion artefacts or stents, one patient received bypass surgery and one patient transferred to other hospital. Finally, 56 patients were included in the present study, including 37 males (66.1%), with an average age of 52.3 ± 13.7 years and a median dialysis duration of 39.0 (13.5, 80.8) months. Primary causes of ESRD were predominantly chronic glomerulonephritis (n = 28, 50.0%), followed by diabetic nephropathy (n = 12, 21.4%), chronic tubulointerstitial nephropathy (n = 5, 8.9%), hypertensive nephropathy (n = 4, 7.1%), and others (n = 7, 12.5%). There were 13 patients (23.2%) complicated with diabetes (Table 2).

Usage of heparin

Of the 56 patients, 27 (48.2%) were UFH users, 14 (25.0%) were LMWH user and 15 (26.8%) were both users. The cumulative dosages of UFH and LMWH in the three groups were shown in Table 3. There were no significant differences in baseline demographics of age, dialysis vintage, proportion of diabetes and laboratory indices of serum cCa, P

Table 1 Baseline characteristics of patients on haemodialysis or peritoneal dialysis

	HD (n = 69)
Age	52.09 ± 13.33
Dialysis vintage (months)	38.00 (12.00,75.00)
Male (n, %)	47 (68.12)
DM (n, %)	17 (24.64)
History cardiovascular disease, n (%)	19 (27.54)
BMI	22.64 ± 3.65
Hb (g/L)	111.78 ± 12.52
Alb (g/L)	39.94 ± 2.84
cCa (mmol/L)	2.22 ± 0.19
P (mmol/L)	1.62 ± 0.65
iPTH (pg/ml)	124.35 (55.45322.20)
ALP (U/L)	69.50 (53.50, 81.50)
CO_2CP (mmol/L)	24.15 ± 4.01
Scr (mmol/L)	1027.57 ± 252.41
UA (umol/L)	440.60 ± 87.64
TG (mmol/L)	1.71 (1.16,2.56)
LDL-C (mmol/L)	2.04 (1.72,2.37)
HDL-C (mmol/L)	0.98 ± 0.25
T-Cho (mmol/L)	4.27 ± 0.89
25-OH-VD (nmol/L)	7.69 (5.26, 11.25)
Kt/V	1.48 ± 0.25
Medication use	
Calcium-based phosphate binder[†] (n, %)	66 (97.06)
Non-calcium-based phosphate binder[‡] (n, %)	3 (4.35)
Cinacalcet (n, %)	2 (2.90)
Vitamin D analogue (n, %)	36 (52.17)
CAC Score	97.00 (0.50743.50)

[†]Calcium carbonate. [‡]Lanthanum carbonate or sevelamer. Alb, albumin; ALP, alkaline phosphatase; BMI, body mass index; CAC: coronary artery calcification; cCa, corrected calcium; DM, diabetes mellitus; Hb, haemoglobin; HDL-C, high-density lipoprotein cholesterol; iPTH, intact parathyroid hormone; Kt/V, total urea clearance; LDL-C, low-density lipoprotein cholesterol; P, phosphate; Scr, serum creatinine; TG, triglyceride; T-Chol, total cholesterol; UA, uric acid; 25-OH-VD, 25-hydroxy Vitamin D3.

and iPTH among the three groups. Serum ALP level was lower in both users than in UFH users or LMWH users (P = 0.018), Kt/V level was higher in LMWH users than UFH users or both users (P = 0.015) (Table 2).

Coronary artery calcification

Baseline CT scans showed a total of 43 patients (76.8%) with CAC. Among the 13 patients (23.2%) without CAC at baseline, 3 patients developed CAC after 2 years of follow-up. The baseline CAC scores of the UFH, LMWH and both UFH/LMWH users were 91.0 (1.0, 1052.0) (mean value 511.4), 134 (0,1292.00) (mean value 574.9) and 250.5 (27.0, 1139.0) (mean value 651.9), respectively, and no significant difference in baseline CAC scores were found among the three groups (P = 0.663, Table 3). After 2 years, the progression CAC scores in the three groups were 42.0 (0, 364.0) (mean value 351.2), 172.0 (7.0, 653.0) (mean value 349.5) and 118.5 (0, 434.0) (mean value 216.1),

Table 2 Baseline and follow-up characteristics of patients using different types of heparin substances

	UFH (n = 27)	LMWH (n = 15)	UFH + LMWH (n = 14)	P
Male (n (%))	20 (74.1%)	10 (66.7%)	7 (50.0%)	0.330
DM (n (%))	9 (33.3%)	3 (20%)	1 (7.1%)	0.160
Primary causes of ESRD (n (%))				
CGN	12 (44.4)	8 (53.3)	8 (57.1)	0.223
DN	8 (29.6)	3 (20.0)	1 (7.1)	0.199
CTIN	1 (3.7)	2 (13.3)	2 (14.3)	0.223
HN	3 (11.1)	0 (0)	1 (7.1)	0.199
Others	3 (11.1)	2 (13.3)	2 (14.3)	0.223
History cardiovascular disease, n (%)	5(18.52)	4(26.67)	5(35.71)	0.448
Age (years)	51.52 ± 13.99	51.93 ± 15.03	54.36 ± 12.58	0.874
Dialysis vintage (months)	40.00 (12.00,66.00)	68.00 (28.00,91.00)	30.50 (13.75,78.25)	0.367
BMI	21.98 ± 3.34	21.20 ± 2.54	23.57 ± 3.08	0.081
Baseline values				
cCa (mmol/L)	2.18 ± 0.13	2.25 ± 0.20	2.25 ± 0.20	0.521
P (mmol/L)	1.62 ± 0.63	1.47 ± 0.72	1.69 ± 0.40	0.515
iPTH (pg/mL)	123.30(55.40239.20)	86.70(59.40414.40)	103.35(59.10360.90)	0.958
ALP (U/L)	72.67 ± 21.34	75.47 ± 28.76	56.07 ± 12.34	0.018*[†]
Alb (g/L)	39.64 ± 3.30	40.73 ± 2.55	39.59 ± 1.93	0.348
Hb (g/L)	111.04 ± 15.03	112.40 ± 9.63	110.36 ± 13.15	0.966
Scr (mmol/L)	1047.04 ± 221.99	1059.67 ± 243.03	1047.07 ± 271.90	0.870
UA (umol/L)	422.30 ± 69.63	421.87 ± 88.23	470.14 ± 100.20	0.464
TG (mmol/L)	1.69 (1.00,2.29)	1.72 (1.46,2.41)	1.87 (1.28,3.32)	0.486
LDL-C (mmol/L)	2.09 ± 0.62	2.09 ± 0.62	2.09 ± 0.62	0.862
HDL-C (mmol/L)	1.02 ± 0.28	1.04 ± 0.28	0.89 ± 0.20	0.281
T-Chol (mmol/L)	4.06 (3.63,4.37)	4.53 (3.64,4.76)	4.23 (3.51,5.21)	0.287
25-OH-VD (nmol/L)	9.38 (5.49,11.98)	7.64 (5.29,11.53)	6.45 (4.36,9.97)	0.401
Kt/V	1.49 ± 0.22	1.62 ± 0.20	1.38 ± 0.22	0.015*[‡],
Time-average values				
cCa (mmol/L)	2.32 ± 0.16	2.41 ± 0.12	2.36 ± 0.15	0.254
P (mmol/L)	1.59 ± 0.36	1.63 ± 0.42	1.81 ± 0.30	0.269
iPTH (pg/ml)	235.52 (95.27, 351.48)	134.71 (38.02, 310.09)	130.27 (64.63, 452.12)	0.721
ALP (U/L)	80.38 ± 29.34	66.94 ± 29.04	64.89 ± 14.11	0.083
Alb (g/L)	39.50 ± 2.98	40.96 ± 1.40	40.16 ± 1.15	0.156
Hb (g/L)	113.80 ± 9.60	105.22 ± 29.56	113.37 ± 5.56	0.421
Scr (mmol/L)	1016.85 ± 220.20	1058.00 ± 238.75	1076.50 ± 238.69	0.666
UA (umol/L)	433.43 ± 54.61	438.04 ± 61.36	463.10 ± 60.87	0.372
TG (mmol/L)	1.97 (1.25,2.74)	2.18 (1.93,2.49)	2.07 (1.54,3.65)	0.636
LDL-C (mmol/L)	1.91 ± 0.52	2.29 ± 0.69	2.39 ± 0.40	0.013*[§]
HDL-C (mmol/L)	0.99 ± 0.20	1.02 ± 0.27	0.92 ± 0.16	0.641
T-Chol (mmol/L)	3.82 (3.43, 4.47)	4.41 (3.86, 5.10)	4.57 (4.10, 4.81)	0.047*[¶]
Kt/V	1.59 ± 0.25	1.56 ± 0.46	1.55 ± 0.26	0.459
Medication use				
Calcium-based phosphate binder (n, %)	27 (100)	15 (100)	14 (100)	--
Non-calcium-based phosphate binder (n, %)	0 (0)	2 (13.33)	1 (7.14)	0.204
Cinacalcet (n, %)	0	1 (6.67)	1 (7.14)	0.195
Vitamin D analogue (n, %)	14 (51.85)	9 (60.00)	6 (42.86)	0.691

*$P < 0.05$. [†]For the pairwise comparison, the ALP levels were different between the UFH and UFH + LMWH groups ($P = 0.026$), and the LMWH and UFH + LMWH groups ($P = 0.049$), and there was no difference between UFH and LMWH group ($P = 0.927$). [‡]The Kt/V levels were different between the LMWH and UFH + LMWH groups ($P = 0.014$), and no difference between other groups ($P = 0.753, 0.108$). [§]The LDL levels were different between the LMWH and UFH + LMWH groups ($P = 0.021$), and no difference between other groups ($P = 0.124, 1.000$). [¶]The T-CHO levels were different between the UFH and UFH + LMWH groups ($P = 0.027$), and no difference between other groups ($P = 0.075, 0.706$). Alb, albumin; ALP, alkaline phosphatase; BMI, body mass index; CAC: coronary artery calcification; cCa, corrected calcium; CGN, chronic glomerulonephritis; CTIN, chronic tubulointerstitial nephropathy; DM, diabetes mellitus; DN, diabetic nephropathy; Hb, haemoglobin; HDL-C, high-density lipoprotein cholesterol; HN, hypertensive nephropathy; iPTH, intact parathyroid hormone; Kt/V: total urea clearance; LDL-C, low-density lipoprotein cholesterol; P, phosphate; Scr, serum creatinine; TG, triglyceride; T-Chol, total cholesterol; UA, uric acid; 25-OH-VD, 25-hydroxy Vitamin D3.

respectively. No significant difference in CAC progression was found among the three groups ($P = 0.660$, Table 3). The detailed changes between baseline and 2 years later CAC score by group were shown in Fig 1. And the delta CAC scores of each patient in three groups were shown in Fig. 2.

Table 3 Coronary artery calcification and dose of heparin of patients using different type of heparin substance

	UFH (n = 27)	LMWH (n = 15)	UFH + LMWH (n = 14)	P
Baseline CAC scores	91.00 (1.00,1052.00)	134 (0,1292.00)	250.50 (27.00,1139.00)	0.663
2 years later CAC scores	259.00 (5.00,1399.00)	349.00 (16.00,1477.00)	391.00 (36.75,1144.75)	0.888
Progression of CAC	42.00 (0,364.00)	172.00 (7.00,653.00)	118.50 (0,434.00)	0.660
Progression of CAC				0.805
<100	14 (51.9)	7 (46.7)	6 (42.9)	
100–500	9 (33.3)	4 (26.7)	6 (42.9)	
>500	4 (14.8)	4 (26.7)	2 (14.3)	
Dosage of UFH (U)	1 064 375 (981 250,1 267 125)	7500 (0,51 563)	553 125 (429 672,674 844)	
Dosage of LMWH (IU)	0 (0,49 000)	1 236 000 (1 169 000,1 254 000)	702 500 (639 500,1 035 500)	

CAC, coronary artery calcification; LMWH, low molecular heparin; UFH, unfractionated heparin.

Influencing factors of the progression of CAC

The proportion of patients combined with diabetes in groups of CAC progression scores between 100–500 and >500 was higher than that in the group of progression scores <100 ($P = 0.025$). There were no significant differences in baseline laboratory indices among the three groups. The time-average serum P, iPTH, and ALP levels in the progression scores >500 group were higher than those with progression scores between 100–500 and <100 ($P = 0.001, 0.043, 0.025$, respectively, Table 4). Pearson and Spearman correlation analysis showed that progression of CAC was not associated with the type (correlation index: 0.086, P: 0.530) and cumulative dosage (correlation index: 0.052, P: 0.747) of heparin used (Fig. 3).

Logistic regression analysis showed that diabetes (B = 2.332, odds ratio (OR) = 10.296, 95% (confidence intervals) CI 1.877–56.483, $P = 0.007$) and higher time-average iPTH (B = 0.004, OR = 1.004, 95% CI 1.000–1.008,

$P = 0.028$) were independent risk factors of the progression CAC (Table 5). Multiple linear regression analysis showed that time-average P level (B = 0.324, T = 2.416, $P = 0.019$) and diabetes (B = 0.374, T = 2.992, $P = 0.004$) were positively correlated with progression of CAC, but not the types of heparin used (Table 5).

DISCUSSION

In this perspective cohort, we did not find the significant differences in the effects of types and cumulative dosages of heparin on the CAC progression of HD patients in our centre.

A total of 56 HD patients using UFH, LMWH or both of UFH and LMWH were finished the follow-up in the study, CT were performed twice, 2 years apart. Results showed that there was no significant difference in baseline and progression of CAC score among the three heparin type groups.
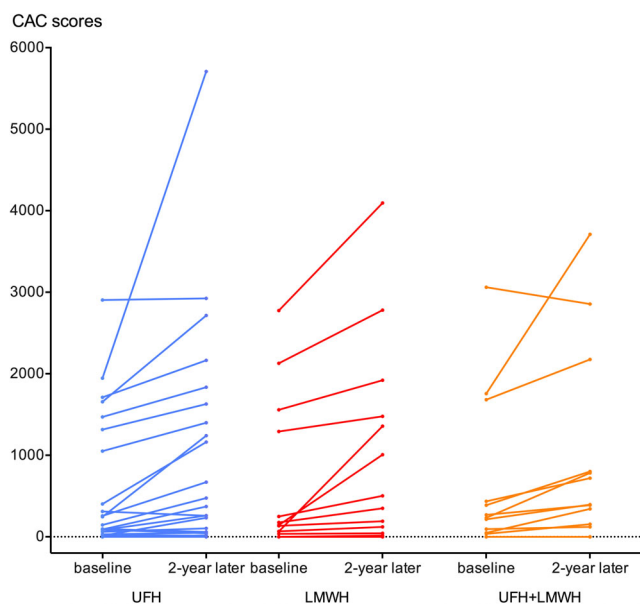

Fig. 1 The progression of coronary artery calcification (CAC) in three groups.
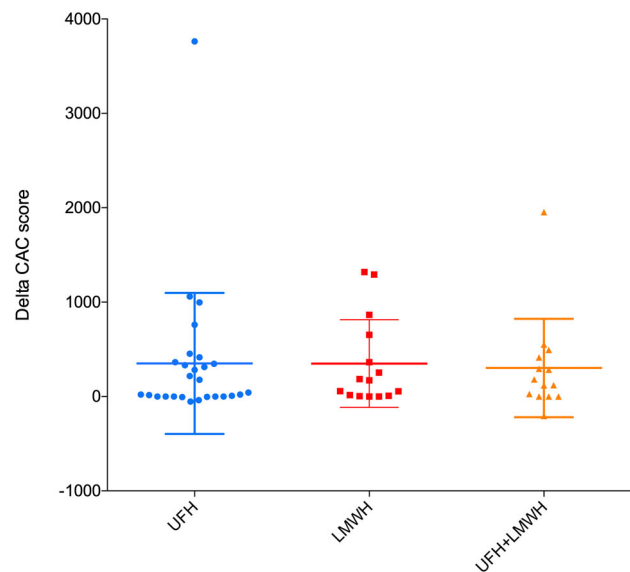

Fig. 2 The delta coronary artery calcification (CAC) scores in three groups. *Notes*: Each point represented the increased value in coronary artery calcification scores during 2-year follow-up period of a patient. (●) UFH, (■) LMWH and (▲) UFH + LMWH.

Table 4 Comparison of demographic and clinical data among patients of different CAC progression scores

	Progression score <100 (*n* = 27)	Progression score 100–500 (*n* = 19)	Progression score > 500 (*n* = 10)	*P*
Male (*n* (%))	16 (59.3)	14 (73.7)	7 (70.7)	0.572
DM (*n* (%))	2 (7.4)	7 (36.8)	4 (40.0)	0.025*,†
Age (years)	49.44 ± 16.09	53.89 ± 11.20	57.20 ± 9.93	0.439
Dialysis vintage (months)	30.00 (12.00,77.00)	32.00 (13.00,82.00)	51.50(24.00,91.00)	0.778
BMI	21.52 ± 2.51	22.93 ± 3.69	22.17 ± 3.15	0.505
Baseline values				
cCa (mmol/L)	2.22 ± 0.17	2.19 ± 0.18	2.26 ± 0.19	0.474
P (mmol/L)	1.46 ± 0.61	1.76 ± 0.66	1.67 ± 0.38	0.203
iPTH (pg/ml)	86.70(55.60277.10)	123.30(52.70340.70)	243.45(182.13354.88)	0.138
ALP (U/L)	68.19 ± 28.98	68.32 ± 17.43	74.00 ± 11.28	0.206
Alb (g/L)	39.77 ± 3.04	40.54 ± 2.29	39.13 ± 3.11	0.301
Hb (g/L)	109.70 ± 11.67	112.00 ± 15.74	113.90 ± 12.04	0.397
Scr(mmol/L)	1048.26 ± 238.32	1066.89 ± 232.71	1025.00 ± 259.87	0.664
UA (umol/L)	425.93 ± 76.74	443.68 ± 74.81	438.20 ± 121.19	0.563
TG (mmol/L)	1.56(0.82,2.14)	1.85(1.65,3.20)	2.00(0.98,3.12)	0.197
LDL-C (mmol/L)	1.94 ± 0.59	2.22 ± 0.64	2.23 ± 0.62	0.134
HDL-C (mmol/L)	1.02 ± 0.26	0.94 ± 0.23	1.03 ± 0.33	0.493
T-Chol (mmol/L)	3.82(3.42,4.34)	4.32(3.83,4.76)	4.38(4.13,5.20)	0.048*,‡
25-OH-VD (nmol/L)	9.38(6.00,12,21)	6.85(5.19,11.53)	6.38(4.99,9.51)	0.453
Baseline Kt/V	1.51 ± 0.25	1.49 ± 0.21	1.48 ± 0.22	0.983
Time-average values				
cCa (mmol/L)	2.36 ± 0.13	2.33 ± 0.16	2.36 ± 0.19	0.813
P (mmol/L)	1.60 ± 0.36	1.54 ± 0.35	2.01 ± 0.37	0.001*,§
iPTH (pg/mL)	147.17 (52.27284.41)	100.72 (58.45434.53)	300.01 (186.48534.65)	0.043*,¶
ALP (U/L)	64.48 ± 28.35	77.40 ± 27.36	87.14 ± 10.62	0.025*,††
Alb (g/L)	40.20 ± 2.66	40.09 ± 2.10	39.62 ± 1.83	0.779
Hb (g/L)	108.97 ± 23.22	113.20 ± 8.68	114.49 ± 5.51	0.801
Scr (mmol/L)	1061.74 ± 230.55	1032.58 ± 218.35	1011.00 ± 252.76	0.846
UA (umol/L)	446.12 ± 50.19	441.63 ± 61.19	432.04 ± 76.38	0.603
TG (mmol/L)	1.98(1.27,2.62)	2.33 (1.89,3.65)	1.80 (1.19,2.58)	0.077
LDL-C (mmol/L)	1.96 ± 0.65	2.09 ± 0.66	2.42 ± 0.47	0.150
HDL-C (mmol/L)	1.00 ± 0.20	0.93 ± 0.16	1.02 ± 0.31	0.466
T-Chol (mmol/L)	4.08 (3.43,4.59)	4.38 (3.64,4.78)	4.50 (3.99,4.67)	0.418
Kt/V	1.59 ± 0.39	1.58 ± 0.25	1.51 ± 0.21	0.357
Dosage of UFH (U)	770 500 (62 500,1 095 000)	910 000 (483 750,1 184 688)	590 625 (28 672,975 516)	
Dosage of LWMH (IU)	770 750 (52 500,1 221 750)	694 000 (45 000,1 084 000)	1 191 500 (1 093 000,1 289 750)	

*P < 0.05. †For the pairwise comparison, the percentage of DM were different between the progression <100 and progression >500 groups (*P* < 0.05), and the progression <100 and 100–500 groups, and no different between the progression 100–500 and > 500 groups (*P* > 0.05). ‡The baseline T-Chol were different between the progression <100 and progression >500 groups (*P* = 0.024), and no different between other groups (*P* = 0.092, *P* = 0.397). §The time-average P level were different between the progression <100 and progression >500 groups (*P* = 0.001), and the progression 100–500 and progression > 500 groups (*P* = 0.002), and there was no difference between progression <100 and 100–500 group (*P* = 0.634). ¶The time-average iPTH levels were different between the progression <100 and progression > 500 groups (*P* = 0.037), and no difference between other groups (*P* = 0.495, 0.064); ††The time-average ALP levels were different between the progression <100 and progression >500 groups (*P* = 0.021), and no difference between other groups (*P* = 0.632, 0.332). Alb, albumin; ALP, alkaline phosphatase; BMI, body mass index; CAC: coronary artery calcification; cCa, corrected calcium; DM, diabetes mellitus; Hb, haemoglobin; HDL-C, high-density lipoprotein cholesterol; iPTH, intact parathyroid hormone; Kt/V, total urea clearance; LDL-C, low-density lipoprotein cholesterol; LMWH, low molecular heparin; P, phosphate; Scr, serum creatinine; TG, triglyceride; T-Chol, total cholesterol; UA, uric acid; UFH, unfractionated heparin, 25-OH-VD, 25-hydroxy Vitamin D3.

Also, we did not find a significant association between heparin dosages and progression of CAC score. These results were consistent in our logistic model and linear model, after adjusted recognized risk factors of CAC, including diabetes and some laboratory indices. To obtain stable regression model, the time-average value of laboratory indices, which can reflect patients' actual condition, were used.

According to literatures,[6,7,14–16] it was reasonable if we confirm that UFH can promote more progression of CAC than LMWH or a high cumulative dosage of heparin substances was relative to fast progression of CAC, but our results were negative. There were several reasons that we got these negative results. (i) The shorter period of observation. Vascular calcification in CKD is a complication of slow onset and slow progression,[22] There was a meta-analysis indicated that LMWH for 3–6 months may not increase the risk of fractures in participants with venous thromboembolism and underlying CVD, but longer exposure for up to
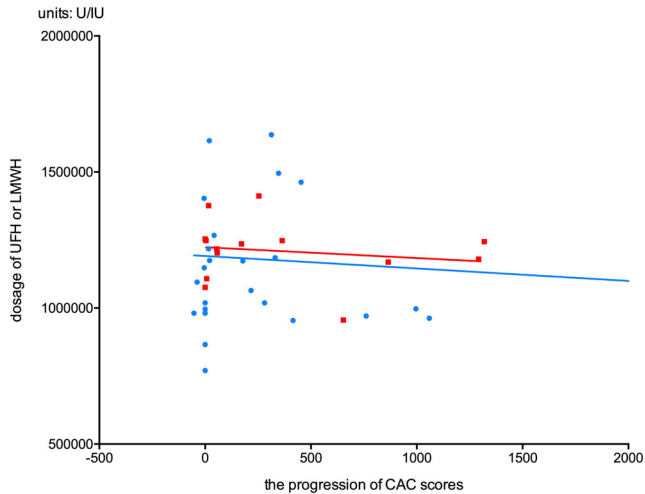
Fig. 3 Scatter plot of the relationship between the dose of UFH or LMWH and the progression of coronary artery calcification. (—●—) UFH and (—■—) LMWH.

2 years may adversely affect BMD.[20] In last year, Meng et al.[23] published another study, they divided rats into normal group, CKD control group, UFH group, and LMWH group, and they did not found the different effects of UFH and LMWH on vascular calcification in rats in 3-week observation. For the effects of UFH and LMWH on bone metabolism, some previous studies have suggested that LMWH causes less bone loss and osteoporosis than UFH.[24,25] We

Table 5 Independent risk factors of CAC progression in logistic and multiple linear regression models

Logistic regression model				
Variables	B	OR	95% CI	P
DM	2.332	10.296	1.877–56.483	0.007*
Time-average iPTH	0.004	1.004	1.000–1.008	0.028*
Time-average P	0.280	1.324	0.185–9.463	0.780
Time-average ALP	0.071	1.017	0.991–1.043	0.204
Heparin types				0.349
LWMH	Ref.	Ref.	Ref.	
UFH + LMWH	−0.380	0.684	0.121–3.869	0.667
UFH	−1.587	0.206	0.038–1.130	0.206

Multiple linear regression model			
Variables	B	T	P
DM	0.374	2.992	0.004*
Time-average iPTH	0.152	1.129	0.264
Time-average P	0.324	2.416	0.019*
Time-average ALP	0.069	0.509	0.613
Heparin types			
LMWH	Ref.	Ref.	
UFH + LMWH	0.120	0.799	0.428
UFH	0.072	0.440	0.662

*P < 0.05. ALP, alkaline phosphatase; CI, confidence intervals; DM, diabetes mellitus; iPTH, intact parathyroid hormone; P, phosphate; LMWH, low molecular heparin; OR, odds ratio; UFH, unfractionated heparin.

hypothesized that if UFH and LMWH promote vascular calcification by causing bone loss and osteoporosis, this process should be slow, 2 years observation might not long enough to find. (ii) Low dosages and narrow ranges of UFH and LMWH. In previous studies, when UFH and LMWH were used in patients with thrombosis condition, the doses of UFH used were usually more than 10 000 IU per day for continuous several months.[7,19,26,27] However, marginal dosages or the lowest effective dosages of UFH or LMWH were used in HD patients for anti-coagulation purposes during dialysis sessions. Not only the dosages were low, but also the dose range was narrow among patients. The low dose might not be enough to cause osteoporosis and CAC progression; the narrow range of dosages made it difficult to find statistically significance. (iii) heparin may prevent vascular smooth muscle transformation. Many underlying risk factors could promote transform of vascular smooth muscle cells to chondrocyte-like or osteoblast-like cells. These factors include P, Ca, iPTH, inflammatory cytokines, oxidative stress and advanced glycation end products.[28–32] In Yang's study,[33] they found that bovine aortic smooth muscle cells cultures can undergo a phenotypic transition into mature osteoblasts when cultured in the presence of beta-glycerophosphate. This cell type transformation could be inhibited by heparin. Therefore, on the one hand, the use of heparin can promote the maturation and trans-differentiation of osteoclasts to cause osteoporosis, which was considered associating with vascular calcification. On the other hand, the use of heparin can inhibit the transdifferentiation of vascular smooth muscle cells into osteoblasts and inhibit the formation of ectopic vascular calcification.

Our results also found that some factors were related to the progression of CAC, including diabetes, higher time-averaged iPTH, higher time-averaged P. These findings were consistent with previous studies.[22,32,34] Interestingly, there were 10 patients had no CAC during baseline and follow-up period. Dive into the data of these patients, most of them were younger, had a shorter dialysis vintage and without diabetes. As aging and progression of diseases, some of them might develop vascular calcification in their future days. Among them, there were a 61 years old male who had been on HD for 3 years and a 48 years old female who had been on HD for 5 years, did not have CAC. Further study was needed to elucidate why not all HD patients develop CAC in their disease course.

The limitation of our study was the relatively small sample size, which may have impact on the stability of statistical results. And the processes of osteoporosis and vascular calcification are slow, two-year follow-up period might not enough to be observed.

In conclusion, after 2 years observation, we did not find significant differences in baseline and progression of CAC scores among the three groups of UFH, LMWH and both users after adjusted by DM, time-averaged iPTH, P and ALP. In the future, studies with large sample sizes and long follow-up period are still needed to explore the effects of heparin substances on vascular calcification in HD patients.

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DISCLOSURES

We have no conflict of interest to report.

REFERENCES

1. Amann K. Media calcification and intima calcification are distinct entities in chronic kidney disease. *Clin. J. Am. Soc. Nephrol.* 2008; **3**: 1599–605.
2. Niu Q, Hong Y, Lee CH, Men C, Zhao H, Zuo L. Abdominal aortic calcification can predict all-cause mortality and CV events in dialysis patients: A systematic review and meta-analysis. *PloS One* 2018; **13**: e0204526.
3. Sigrist M, Bungay P, Taal MW, McIntyre CW. Vascular calcification and cardiovascular function in chronic kidney disease. *Nephrol. Dial. Transplant.* 2006; **21**: 707–14.
4. Block GA. Prevalence and clinical consequences of elevated Ca x P product in hemodialysis patients. *Clin. Nephrol.* 2000; **54**: 318–24.
5. Raggi P, Boulay A, Chasan-Taber S *et al*. Cardiac calcification in adult hemodialysis patients. A link between end-stage renal disease and cardiovascular disease? *J. Am. Coll. Cardiol.* 2002; **39**: 695–701.
6. Mulloy B, Hogwood J, Gray E, Lever R, Page CP. Pharmacology of heparin and related drugs. *Pharmacolog.Rev.* 2016; **68**: 76–141.
7. Monreal M, Lafoz E, Olive A, del Rio L, Vedia C. Comparison of subcutaneous unfractionated heparin with a low molecular weight heparin (Fragmin) in patients with venous thromboembolism and contraindications to coumarin. *Thromb. Haemost.* 1994; **71**: 7–11.
8. Griffith GC, Nichols G Jr, Asher JD, Flanagan B. Heparin osteoporosis. *JAMA* 1965; **193**: 91–4.
9. Muir JM, Hirsh J, Weitz JI, Andrew M, Young E, Shaughnessy SG. A histomorphometric comparison of the effects of heparin and low-molecular-weight heparin on cancellous bone in rats. *Blood* 1997; **89**: 3236–42.
10. Nelson-Piercy C, Letsky EA, de Swiet M. Low-molecular-weight heparin for obstetric thromboprophylaxis: Experience of sixty-nine pregnancies in sixty-one women at high risk. *Am. J. Obstet. Gynecol.* 1997; **176**: 1062–8.
11. Meng Y, Zhang H, Li Y, Li Q, Zuo L. Effects of unfractionated heparin on renal osteodystrophy and vascular calcification in chronic kidney disease rats. *Bone* 2014; **58**: 168–76.
12. Li B, Lu D, Chen Y, Zhao M, Zuo L. Unfractionated heparin promotes osteoclast formation in vitro by inhibiting Osteoprotegerin activity. *Int. J. Mol. Sci.* 2016; **17**(4), 613.
13. Kock HJ, Handschin AE. Osteoblast growth inhibition by unfractionated heparin and by low molecular weight heparins: An in-vitro investigation. *Clin. Appl. Thromb. Hemost.* 2002; **8**: 251–5.
14. Xiaohong Z, Caiming C, Jianxin W. Risk factors of bone density disorder and vascular calcification in non—Dialysis chronic kidney disease patients. *Chin. J. Nephrol.* 2017; **33**: 100–5.
15. Tangvoraphonkchai K, Davenport A. Aortic pulse wave velocity is greater in peritoneal dialysis patients with lower dual energy X-ray absorptiometry (DXA) femoral neck bone mineral density. *J. Nephrol.* 2019; **32**: 471–6.
16. Aleksova J, Kurniawan S, Vucak-Dzumhur M *et al*. Aortic vascular calcification is inversely associated with the trabecular bone score in patients receiving dialysis. *Bone* 2018; **113**: 118–23.
17. Cronin RE, Reilly RF. Unfractionated heparin for hemodialysis: Still the best option. *Semin. Dial.* 2010; **23**: 510–5.
18. Brunet P, Simon N, Opris A *et al*. Pharmacodynamics of unfractionated heparin during and after a hemodialysis session. *Am. J. Kidney Dis.* 2008; **51**: 789–95.
19. Barbour LA, Kick SD, Steiner JF *et al*. A prospective study of heparin-induced osteoporosis in pregnancy using bone densitometry. *Am. J. Obstet. Gynecol.* 1994; **170**: 862–9.
20. Gajic-Veljanoski O, Phua CW, Shah PS, Cheung AM. Effects of long-term low-molecular-weight heparin on fractures and bone density in non-pregnant adults: A systematic review with meta-analysis. *J. Gen. Intern. Med.* 2016; **31**: 947–57.
21. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. *J. Am. Coll. Cardiol.* 1990; **15**: 827–32.
22. Wang M, Wang M, Gan LY, Li SJ, Hong N, Zhang M. Vascular calcification in maintenance hemodialysis patients. *Blood Purif.* 2009; **28**: 15–20.
23. Meng Y, Zhang H, He N *et al*. A comparative study on effects of low molecular weight heparin and unfractionated heparin on renal osteodystrophy and vascular calcification in rats with chronic kidney disease. *Chin. J. Osteoporos.* 2018; **24**: 181–86+234.
24. Lai KN, Ho K, Cheung RC *et al*. Effect of low molecular weight heparin on bone metabolism and hyperlipidemia in patients on maintenance hemodialysis. *Int. J. Artif. Organs* 2001; **24**: 447–55.
25. Rajgopal R, Bear M, Butcher MK, Shaughnessy SG. The effects of heparin and low molecular weight heparins on bone. *Thromb. Res.* 2008; **122**: 293–8.
26. Dahlman TC. Osteoporotic fractures and the recurrence of thromboembolism during pregnancy and the puerperium in 184 women undergoing thromboprophylaxis with heparin. *Am. J. Obstet. Gynecol.* 1993; **168**: 1265–70.
27. Pettila V, Kaaja R, Leinonen P, Ekblad U, Kataja M, Ikkala E. Thromboprophylaxis with low molecular weight heparin (dalteparin) in pregnancy. *Thromb. Res.* 1999; **96**: 275–82.
28. Takeuchi K, Zhang B, Ideishi M *et al*. Influence of age and hypertension on the association between small artery compliance and coronary artery disease. *Am. J. Hypertens.* 2004; **17**: 1188–91.
29. Cozzolino M, Mazzaferro S, Pugliese F, Brancaccio D. Vascular calcification and uremia: What do we know? *Am. J. Nephrol.* 2008; **28**: 339–46.
30. Shroff RC, McNair R, Figg N *et al*. Dialysis accelerates medial vascular calcification in part by triggering smooth muscle cell apoptosis. *Circulation* 2008; **118**: 1748–57.
31. Niu Q, Zhao H, Wu B *et al*. Study on the prevalence of vascular calcification in different types of arteries and influencing factors in maintenance peritoneal dialysis patients. *Blood Purif.* 2019; **47**(Suppl 1): 8–16.
32. Chen NX, Moe SM. Vascular calcification: Pathophysiology and risk factors. *Curr. Hypertens. Rep.* 2012; **14**: 228–37.
33. Yang L, Butcher M, Simon RR, Osip SL, Shaughnessy SG. The effect of heparin on osteoblast differentiation and activity in primary cultures of bovine aortic smooth muscle cells. *Atherosclerosis* 2005; **179**: 79–86.
34. Russo D, Morrone L, Russo L. Coronary artery calcification and cardiovascular mortality in predialysis patients. *Kidney Int.* 2011; **79**: 258 author reply 58.