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ORIGINAL ARTICLE

Plasma Complement Protein C3a Level Was Associated with Abdominal Aortic Calcification in Patients on Hemodialysis

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Abstract

The complement system plays an important role in cardiovascular disease in patients on hemodialysis. Vascular calcification is also one of the major causes of cardiovascular disease. We want to investigate the relationship between complement activation and vascular calcification in dialyzed patients. One hundred eight hemodialysis patients and 65 heathy controls were enrolled prospectively. Plasma C3a, C5a, mannose-binding lectin (MBL), and membrane attack complex (MAC or C5b-9) levels were detected using ELISA. Plasma C3c, fB, fH, C1q, and C4 levels were measured by immunity transmission turbidity. Abdominal aortic calcification (AAC) was measured by abdomen lateral plain radiograph, and the AAC score was calculated. We identified increased level of MBL and decreased level of C3c and complement factor B compared with normal control. However, C1q, complement factor H, and C4 levels remained at a similar level compared with individuals with normal renal function. The C3a and C5a levels increased, without change of MAC. Forty two of 108 HD patients had the AAC score. C3a levels were correlated with AAC score ($r = 0.461$, $p = 0.002$). The median C3a concentration was 238.72 (196.96, 323.41) ng/mL. When evaluated as AAC categories (≤ 4, > 5) with ordinal logistic regression, univariate analyses revealed that higher C3a levels were associated with severe AAC, while multivariate analyses adjusted for age, sex, and calcium level showed that higher C3a levels (OR, 6.28 (1.25–31.69); $p = 0.03$) were associated with severe AAC. The areas under the curve (AUC) for C3a to diagnose severe abdominal aortic calcification were 0.75(0.58–0.92, 0.01). The complement system was activated in patients on hemodialysis. Higher C3a levels are independently associated with severe AAC. Plasma C3a might have a diagnostic value for the severe AAC in HD patients.

Keywords Complement factors · C3a · Abdominal aortic calcification · Hemodialysis

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Introduction

Cardiovascular morbidity and mortality are extremely high in end-stage renal disease, especially in hemodialysis patients [1]. To lower the morbidity and mortality rates in dialysis patients, both traditional and non-traditional risk factors contributing to the high cardiovascular risk have been thoroughly investigated. Chronic inflammation induced by intravascular innate immune and vascular calcification are strongly associated with cardiovascular disease in patients on hemodialysis [2, 3]. It is well-known that complement intricate immune surveillance system to discriminate between healthy host tissue and unhealthy things, such as cellular debris, apoptotic cells, and foreign intruders and tune its response accordingly. As early as the 1970s, HD was known to influence the complement system [4]. Several studies have since then proved complement activation during hemodialysis [5–9].

On the other hand, both artery intimal and artery medial calcifications are important morbidity/mortality markers which associated with coronary atherosclerosis and arterial stiffness in ESRD patients. Osteogenic transdifferentiation of vascular smooth muscle cells (VSMC) and circulating osteoprogenitors penetrating into vascular wall plays a major role in vascular calcification [10]. The contributors and inhibitors participating in this process have not been fully understood yet. Nayana et al. found that higher serum C3 level significantly associated with arterial calcification in midlife women [11]. However, little is known about the role of complements in vascular calcification in HD patients. In this study, we investigated several complement factor levels in HD patients and observed the relationship between plasma complement factors and abdominal aortic calcification in HD patients.

Methods

Patients and Study Design

A cross-sectional study was conducted in a cohort of 108 hemodialysis patients, recruited at Peking University First Hospital. Patients were on a three times weekly dialysis and spKt/V > =1.2. General characteristics, clinical data, and biochemical data of the cohort are listed in Table 1. Blood samples were obtained before the start of a regular 4-h HD session.

Forty-two out of the 108 HD patients agreed to accept the lateral lumbar radiography of the aorta, and the AAC score were evaluated. The general characteristics of the 42 patients are listed in Table 1.

Sixty-five healthy individuals were recruited as normal controls.

Lateral Lumbar Radiography of the Aorta

The lateral lumbar radiographs were performed in standing position, as previously described [12]. Abdominal aortic calcification was graded using a semi-quantitative scoring system described by Kauppila et al. [13]. Calcification status of the abdominal aorta adjacent to each lumbar vertebra (the first to the forth) were assessed. The posterior and anterior walls of the aorta were evaluated separately using the midpoint of the intervertebral space above and below the vertebrae as the boundaries. Lesions were graded as follows: 0, no aortic calcific deposits; 1, small scattered calcific deposits filling less than 1/3 of the longitudinal wall of the aorta; 2, medium quantity of calcific deposits about 1/3 to 2/3 of the longitudinal wall of the aorta calcified; 3, severe quantity of calcifications of more than 2/3 of the longitudinal wall of the aorta calcified. The abdominal aortic calcification score (AAC score) varies from 0 to 24 with this numerical grading system. All the lateral lumbar radiographs were analyzed by two investigators, and the average of the two scores was the final AAC score of the patient. According to the CORD study, AAC was divided into none calcification group (AAC score = 0), mild calcification (1 < AAC score < 5) and severe calcification (AAC score ≥ 5) [12].

Measurement of the Complement Factors

Blood samples from the HD patients were obtained from the arterial side of the patient' s arteriovenous fistula before an anticoagulant injection and hemodialysis session. All samples were immediately centrifuged at 3000 rpm at 4 °C for 10 min, separated from the cells and stored at − 80 °C until use.

Plasma C3a, C5a, MBL, and MAC (C5b-9) levels were detected using a commercial ELISA kit from Quidel Corporation (San Diego, CA) following up the instructions.

Plasma C3c, fB, fH, C1q, and C4 immunity transmission turbidity kits were funded by the Shanghai Beijia Biochemistry Reagents Co., Ltd. (Nunc Mercial, Shanghai, China). Plasma samples were added by R1(PBS+PEG+EDTA-Na_2) at 37 °C for 5 min, then the samples were measured at OD 340 nm by fully automated biochemical analyzer (HITACHI, Tokyo, Japan), the OD was A1; then R2(PBS+PEG+EDTA-Na_2+ Antibodies) was added at 37 °C for 5 min, then the samples were measured at OD 340 nm by a fully automated biochemical analyzer (HITACHI, Tokyo, Japan), the OD was A2; the C3c, fB, fH, C1q, and C4 levels were [standards × (sample A2 − sample A1)/(standard A2 − standard A1)], respectively.

Statistical Analyses

All analyses were done with SPSS 16.0 (Chicago, IL, USA). Continuous data were reported as mean ± standard deviations or median (interquartile range (IQR)), categorical variables were performed as rations/percentages. *T* test, Mann-Whitney *U* test,

Table 1 Baseline characteristics of the HD cohort

	HD patients ($N = 108$)	HD patients with AAC score ($N = 42$)	p value
Age (years)	56 ± 12	57 ± 11	0.582
Gender (male/female)	62/34	22/20	0.578
Dialysis duration (months)	60 (29–122)	99 (46–162)	0.020
Hemoglobin (g/L)	112.69 ± 10.49	113.25 ± 10.48	0.776
Calcium (mmol/L)	2.33 ± 0.28	2.38 ± 0.34	0.397
Phosphate (mmol/L)	3.15 ± 13.64	1.75 ± 0.46	0.514
MAP (mm Hg)	101 ± 18	104 ± 16	0.292
Albumin (g/L)	40.75 (38.35–42.45)	41.50 (39.40–43.00)	0.198
HsCRP (mg/L)	1.89 (0.57–4.82)	2.18 (1.00–4.87)	0.697
PTH (pg/mL)	328.89 (169.80–487.40)	372.58 (187.70–620.51)	0.337
spKt/V	1.53 ± 0.29	1.63 ± 0.27	0.052
Primary cause of ESRD			
Primary glomerulopathy	37 (34.3%)	22(42.9%)	
Diabetes	14 (13.0%)	2 (4.7%)	
Hypertension	14 (13.0%)	6 (14.3%)	
ADPKD	10 (9.3%)	4 (9.5%)	
Tubulointerstitial nephropathy	17 (15.7%)	9 (21.4%)	
Other or unknown	16 (14.8%)	3 (7.1%)	
Comorbidity			
Hypertension	77 (71.3%)	31 (73.8%)	
Diabetes	12 (11.1%)	3 (7.1%)	
Coronary artery disease	35 (32.4%)	7 (16.7%)	
Stroke	10 (9.3%)	3 (7.1%)	
Post kidney transplantation	1 (0.9%)	0	

Data are shown as mean ± SD or median (interquartile range) for continuous variables and proportions for categorical variables. *MAP*, mean arterial blood pressure; *HsCRP*, high-sensitivity C-reactive protein; *PTH*, parathyroid hormone; *CKD*, chronic kidney disease; *ADPKD*, autosomal dominant polycystic kidney disease

one-way post hoc ANOVA using Bonferroni multiple comparison, median test, and chi-square test were used to determine differences between groups. Pearson and Spearman correlation coefficients were used to detect the correlation between plasma complement factor levels and clinical characteristics.

Logistic regression models were used to analyze the independent risk factors associated with severe abdominal aortic calcification (AACS ≥ 5). Both plasma C3a levels and C3a median were analyzed in the logistic models. ROC analysis was performed to evaluated sensitivity and specificity of C3a as a predictor of severe AAC.

Results

General Characteristics of the Patients

The characteristics of study groups are given in Table 1. In total, 108 individuals on MHD were involved in this study, and 42 accepted the lateral lumbar radiography of the aorta. Dialysis vintage of the subgroup with AAC score were different from the whole cohort. Other general characteristics, including age, gender, hemoglobulin, calcium, phosphate, blood pressure, albumin, hsCRP, iPTH, and spKt/V, were not different between the subgroup and the whole cohort.

Measurements of Complement Components

In order to understand the whole scale of complement activities, we measured several crucial points of three complement pathways in the HD cohort, as well as in the normal control. The measured complement proteins include C1q, MBL, C4, Factor B, Factor H C3a, C3c, C5a, and C5b-9. Among these factors, C3a, C5a, and MBL levels increased, complement factor B decreased, the C1q, complement factor H and MAC (C5b-9) did not change in HD patients (Table 2).

The Relationship Between Plasma Complement Factor Levels and Clinical Parameters in the Cohort

We first analyzed the correlation of increased complement factors with clinical characteristics in the dialysis cohort.

Table 2 Plasma complement factors levels between the whole HD cohort and controls

	HD patients ($N = 108$)	Normal ($N = 65$)	p value
C3c (g/L)	0.92 ± 0.17	1.02 ± 0.20	< 0.001
C1q (mg/L)	200.59 ± 41.68	191.71 ± 29.46	0.69
CFH (μg/mL)	361.77 ± 57.63	358.56 ± 71.23	0.41
CFB (mg/L)	346.15 (299.925, 388.2)	406.9 (369.6, 508.65)	< 0.001
C4 (g/L)	0.312 (0.2498, 0.3808)	0.285 (0.218, 0.3935)	0.10
MAC (ng/mL)	482.26(307.59, 783.75)	472.36 (328.03, 722.46)	0.77
C5a (ng/mL)	31.03 ± 10.80	17.22 ± 10.49	< 0.001
C3a (ng/mL)	238.71 (190.12, 318.95)	135.68 (91.86, 217.26)	< 0.001
MBL (ng/mL)	4346.38 (1415.73, 8979.95)	1171.21 (376.68, 3235.74)	< 0.001

MAC, membrane attack complex, complement C5b-9; *CFH*, complement factor H; *CFB*, complement factor B; *MBL*, mannose-binding lectin

Pearson's or Spearman's correlation analyses showed that the plasma C3a ($r = 0.336$, $p < 0.001$) and C5a ($r = 0.25$, $p = 0.01$) levels correlated with hsCRP (Fig. 1a, b). Plasma MBL level correlated with serum iron ($r = 0.22$, $p = 0.04$), phosphorus ($r = -0.23$, $p = 0.02$) (Fig. 1c, d). No relationships were found between complement factors with other clinical or biochemical traits, such as dialysis duration, spKt/V, albumin, calcium, phosphate, hemoglobin, leukocytes, platelets, blood glucose, ferritin, serum iron, total iron-binding capacity, and PTH.

Plasma C3a Level Is Associated with AAC Score

Forty-two out of the 108 HD patients agreed to accept the lateral lumbar radiography of the aorta and had the AAC score. Dialysis duration of the subgroup with AAC score were different from the whole cohort. Other general characteristics are not different between the subgroup with AAC score with the whole cohort (Table 1). Spearman's correlation analysis showed that HD patients' plasma C3a level were positively correlated with AAC score ($r = 0.461$, $p = 0.002$, supplementary figure 1). Thus, AAC was divided into none calcification group (AAC score = 0), mild calcification group (1 < AAC score < 5) and severe calcification group (AAC score ≥ 5) according to the CORD study [12]. Among all the complement factors we measured, only plasma C3a levels were different between groups ($p = 0.017$, Table 3). Meanwhile, the mean age of HD patients was different between groups ($p = 0.028$, Table 3).

The median C3a concentration was 238.72 (196.96, 323.41) ng/mL. We then divided patients to two groups by the median of C3a levels. The patients with lower level of C3a (C3a ≤ 238.72 ng/mL) were in group I, and those with higher level of C3a (C3a > 238.72 ng/mL) were in group II. Chi-square analysis demonstrated a dose response relationship between plasma C3a level and AAC categories (chi-square = 6.857, $p = 0.009$, Fig. 2). Among patients with no or mild

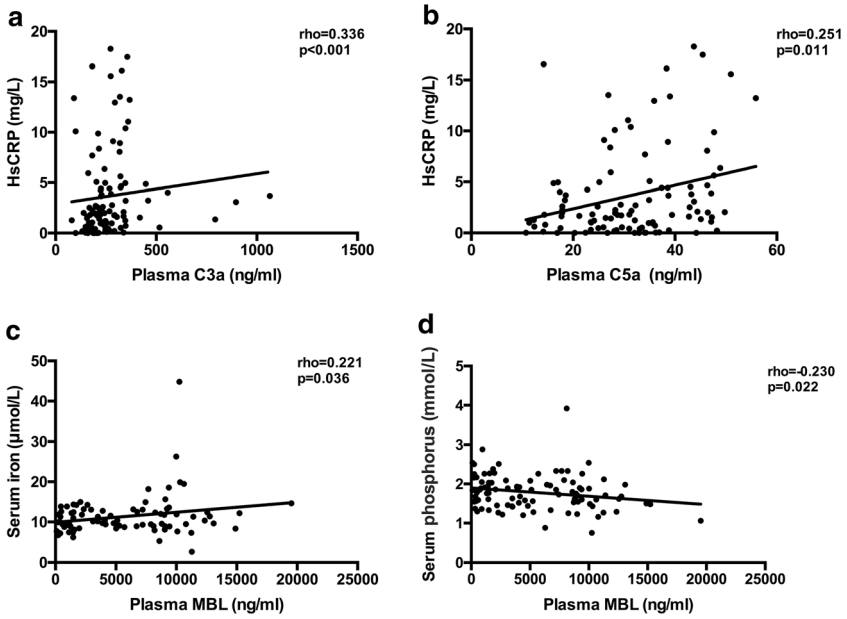

Fig. 1 Correlation between plasma complement factors and clinical parameters. **a** Correlation between plasma C3a level and serum hsCRP in HD cohort. Spearman's rho = 0.336, $p < 0.001$. **b** Correlation between plasma C5a level and serum hsCRP in HD cohort. Spearman's rho = 0.251, $p = 0.011$. **c** Correlation between plasma MBL level and serum iron in HD cohort. Spearman's rho = 0.221, $p = 0.036$. **d** Correlation between plasma MBL level and serum phosphorus in HD cohort. Spearman's rho = − 0.230, $p = 0.022$

Table 3 Characteristics in different AAC groups

AAC score				
	0 (18/42)	1 4 (10/42)	5 20 (14/42)	*p* value
Age	52.22 ± 10.67	61.80 ± 9.30	60.57 ± 10.91	0.03
Sex (M/F)	14/18	3/10	6/14	0.08
C3c (g/L)	0.79 (0.75–1.01)	0.95 (0.74–1.06)	0.97 (0.90–1.01)	0.12
C1q (mg/L)	183.15 ± 34.59	205.34 ± 41.99	200.62 ± 26.79`	0.16
CFH (μg/mL)	339.8 ± 37.54	359.4 ± 58.27	371.16 ± 44.49	0.10
CFB (mg/L)	307.3 (287.4–370.0)	360.85 (284.53–395.88)	365.10 (333.15–396.30)	0.06
C4 (g/L)	0.29 ± 0.08	0.31 ± 0.10	0.33 ± 0.08	0.28
MAC (ng/mL)	548.10 (282.51–758.05)	550.18 (331.31–919.89)	517.38 (352.42–768.23)	0.36
C5a (ng/mL)	27.28 (16.14–34.11)	34.90 (22.14–44.72)	32.13 (21.22–43.14)	0.29
C3a (ng/mL)	211.23 (177.84–240.62)	237.71 (189.99–291.57)	320.57 (236.88–374.51)	0.02
MBL (ng/mL)	6617.39 ± 4176.97	6347.58 ± 4114.01	5516.01 ± 4632.08	0.41

AAC was divided into none calcification group (AAC score = 0), mild calcification group (1 < AAC score < 5 = and severe calcification group (AAC score ≥ 5)

AAC, abdominal aortic calcification; *MAC*, membrane attack complex, complement C5b-9; *CFH*, complement factor H; *CFB*, complement factor B; *MBL*, mannose-binding lectin

calcification, 18/28 (64.3%) persons had lower C3a level, whereas10/28 (35.7%) had higher C3a level. Conversely, among those severe classification patients identified by AAC score ≥ 5, 11/14 (78.6%) had higher C3a level, whereas only 3/14 (21.4%) had lower C3a level.

We first included clinical and biochemical traits in the univariate analysis, including age, gender, dialysis duration, hemoglobin, spKt/V, primary cause of ESRD, and the presence of comorbidity. The univariate analyses revealed that higher levels of plasma C3a levels associated with severe abdominal aortic calcification (Table 4). We therefore performed unadjusted and adjusted models in further multivariate logistic regression analyses. Model I was adjusted for age and sex. Plasma Ca level was added to model II based on model I. Higher plasma C3a level was associated with severe AAC as continuous traits in unadjusted model (OR = 1.011(1.001–1.021), $p = 0.03$), model I (OR = 1.012 (1.001–1.024), $p = 0.04$), and model II (OR = 1.012 (1.000–1.024), $p = 0.04$). Higher plasma C3a level was associated with severe AAC as categorical traits in unadjusted model (OR = 6.600 (1.484–29.355), $p = 0.01$), model I (OR = 6.612 (1.433–30.509), $p = 0.02$), and model II (OR = 6.283 (1.246–31.687), $p = 0.03$) (Table 5).

The areas under the curve (AUC) for plasma C3a and calcium to diagnose severe abdominal aortic calcification were 0.747 and 0.716, respectively (Fig. 3a, b). However, the AUC increased to 0.812 after we incorporated plasma C3a concentration and calcium concentration (Fig. 3c).

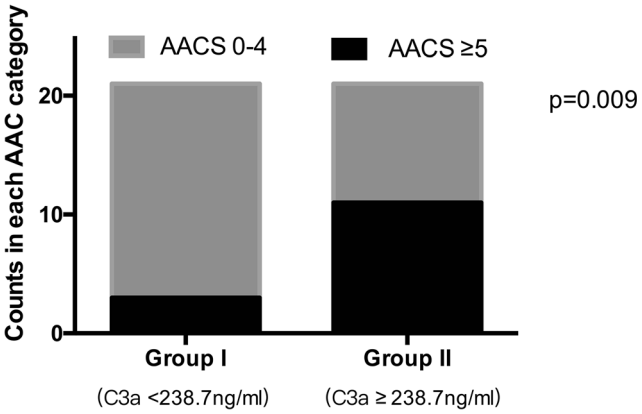

Fig. 2 Abdominal aorta calcification severity by plasma C3a median. Chi-square analysis between plasma C3a level and AAC categories. Plasma C3a was positively correlated with the AAC score. Among patients with higher plasma C3a level, the incidence of severe calcification was higher

Discussion

Patients on maintenance hemodialysis have increased risks of comorbidities, in which cardiovascular disease (CVD) is one of the major causes of mortality [14, 15]. Several CVD risk factors have been reported in HD patients. Vascular calcification and chronic inflammation are both identified to be associated with poor prognosis [16, 17]. Systemic inflammation exists in most of dialysis ESRD patients, and the causes are multiple, including disease-related and dialysis-related factors.

In patients on dialysis, contact between blood and materials used for extracorporeal circulation can induce activation of complements. Activation fragments from the complement system sustain the inflammatory states through triggering of innate immunological pathways. The early studies provided important evidence on kinetics of complement activation during one session of hemodialysis. During hemodialysis, increased level of C3a indicates C3 activation, resulting in C5a and C5b-9 formation

Table 4 Univariate logistic regression analysis of severe AAC

Associated factors	Univariate logistic regression OR (95% CI)	*p*
Age		
I < 57	1 (reference)	
II ≥ 57	1.44 (0.39–5.27)	0.59
Sex (female)	0.56 (0.15–2.06)	0.38
Plasma C3a continuous	1.01(1.00–1.02)	0.03
Plasma C3a		
I < 238.7 ng/mL	1 (reference)	
II ≥ 238.7 ng/mL	6.60 (1.48–29.36)	0.01
Ca	14.23 (1.11–181.88)	0.04
P	0.35 (0.06–2.10)	0.25
PTH	1.00 (1.00–1.00)	0.25
MAP	0.99 (0.97–0.02)	0.48
HsCRP	0.88 (0.70–1.11)	0.28
Albumin	1.06 (0.89–1.26)	0.55
Blood glucose	0.96 (0.80–1.16)	0.67
Dialysis duration	1.01 (0.99–1.02)	0.39
Hemoglobin	0.99 (0.93–1.05)	0.71
spKt/V	6.40 (0.45–90.72)	0.17
Primary causes of ESRD		
Primary glomerulopathy	0.75 (0.21–2.73)	0.66
Diabetes	1.00 (0.08–12.07)	1.00
Hypertension	5.20 (0.82–32.99)	0.08
ADPKD	4.50 (0.37–54.54)	0.24
Tubulointerstitial nephropathy	0.23 (0.03–2.10)	0.19
Comorbidity		
Hypertension	1.33 (0.29–6.15)	0.71
Diabetes	2.17 (0.22–21.46)	0.51
Coronary artery disease	0.27 (0.07–1.09)	0.07
Stroke	0.00	1.00

OR, odds ratio; *CI*, confidence interval; *AAC*, abdominal aortic calcification; *Ca*, calcium; *P*, phosphate; *MAP*, mean arterial blood pressure; *HsCRP*, high-sensitivity C-reactive protein; *PTH*, parathyroid hormone

[7]. During the past decades, although dialyzers with modern membrane were used widely, complement activation still can be detected [8, 9]. In this study, we measured the levels of factors of the three complement-activating pathways in HD patients. We found complement activation in HD patients, indicated by increased levels of C3a and C5a. It is interesting that the terminal complex MAC (C5b-9) kept at lower level, although the higher levels of C3a and C5a were identified. In this study, we also identified increased level of MBL and decreased level of C3c and complement factor B compared with normal control. However, C1q, complement factor H, and C4 levels remained at a similar level compared with individuals with normal renal function. Complement activation and the relationship with cardiovascular disease were also identified in other studies. Although different complement components were measured, most of studies got the similar results. Higher baseline C3 levels in HD patients may had a prognostic value for future CV events in HD patients [18]. Recently, a higher intradialytic C3d/C3 ratio was predominantly identified in HD patients that would develop CV-events during follow-up [19] . In our study, we did not include the prognostic data due to the short time of follow-up. Further study is needed to provide more information on prognosis. Study from other cohort also showed that functional SNP of factor H gene associated with the risk of cardiovascular disease, which indicated the balance of complement system was important [20]. However, we did not find the difference of plasma factor H level in HD patients compared with the control.

The studies on MBL showed that increased MBL levels presented a protective role in dialyzed patients, associated with lower pulse wave velocity and better prognosis [21, 22].

On the other hand, both artery intimal and artery medial calcifications constitute a significant morbidity/mortality marker which is associated with coronary atherosclerosis and arterial stiffness in ESRD patients. Multiple factors contribute to the vascular calcification in ESRD patients, and one of the risk factors is less certain vascular calcification inhibitors, such as fetuin-A and matrix Gla protein, and these proteins are inversely correlated with inflammation markers [23–25]. All the studies suggested a cross-link between inflammation and vascular calcification. Furthermore, Nayana et al. found that higher serum C3 level was significantly associated with arterial calcification in women [11]. C3, as a vital factor in complement system activating cascade, is cleaved by C3 convertase into C3a anaphylatoxin via different pathways [26, 27]. In 2014, Kazuhiko et al. indicated that osteoclast-derived C3a can stimulate osteoblast differentiation in bone remodeling [28]. Since C3a level kept at a higher level in our HD patients, we explored the association between the levels of complement factors and the vascular calcification in HD patients. Existing data indicated that abdominal aorta calcification score (AAC) is a strong predictor of cardiovascular disease-related events or death in the general population and in dialysis patients [29, 30]. Thus, we choose the abdominal aorta calcification score measurements in this study.

We found that plasma C3a levels were positively associated with the AAC score. Patients with higher plasma C3a levels had a higher risk of severe abdominal aortic calcification than those with lower C3a level, even after adjustment for age, sex, and plasma calcium. Further logistic regression analysis showed that high plasma C3a level was an independent risk factor for severe abdominal aortic calcification in the patients. Although previous study indicated that higher serum phosphate levels were associated with elevated vascular calcification scores [31], but in this study, serum phosphate was not a risk factor of severe abdominal aorta calcification, probably due to the use of phosphate binders.

Table 5 Association of plasma C3a with severe AAC

	C3a		C3a continuous
	I < 238.7 ng/mL *n* = 21	II > 238.7 ng/mL *n* = 21	
n with severe calcification (AAC score ≥ 5)/total (%)	3/21	11/21	14/42
Unadjusted Odds ratio (95%CI)	1 (reference)	6.600 (1.484–29.355)	1.011(1.001–1.021)
		$p = 0.01$	$p = 0.03$
Model 1[a] Odds ratio (95%CI)	1 (reference)	6.612 (1.433–30.509)	1.012 (1.001–1.024)
		$p = 0.02$	$p = 0.04$
Model 2[b] Odds ratio (95%CI)	1 (reference)	6.283 (1.246–31.687)	1.012 (1.000–1.024)
		$p = 0.03$	$p = 0.04$

Multivariate logistic regression analyses were used to diagnose severe abdominal aorta calcification in HD patients with AAC score. *CI*, confidence interval; *AAC*, abdominal aortic calcification

[a] Model 1 adjusted for age and sex

[b] Model 2 adjusted for covariates in model 1 plus plasma Ca level

In this study, we also identified a positive correlation between plasma C3a level and hs-CRP. C-reactive protein, as a marker of chronic inflammation status, plays important roles in initiating and promoting calcification [32, 33]. Thus, it is interesting to know whether complements involved in the calcification directly or promote calcification via inflammation. Abundance of data from atherosclerosis approved that C3/C4, C3a/C5a, and other complement fragments associated with increased risk of cardiovascular disease [34–38]. C5a associated with the incidence of restenosis after femoral artery balloon angioplasty [39]. C3a and C5a serum levels were associated with late lumen loss after implantation of drug-eluting stents [40]. Atherosclerosis is a major cause of artery intimal calcifications in dialysis patients. But gene ablation animal studies, including C3, C3aR, C5, and C5aR ablation mice, provide evidence for an important role of complement in the development of atherosclerotic disease although the results seemed contradictory [41–45]. Artery medial calcification is another essential type of calcification, resulting in artery stiffness. The mechanisms of artery medial calcification are complicated, including chronic inflammation as well as bone disorder. Kazuhiko et al. found that osteoclast-derived C3a can stimulate osteoblast differentiation in bone remodeling [28], suggesting other complements regulating calcification via different pathways.

The limitation of this study is that only 42 of the HD patients accepted the lateral lumbar radiography of the aorta and got AAC score. We did not identify blood pressure, blood glucose, dialysis duration, HDL, triglyceride, and total

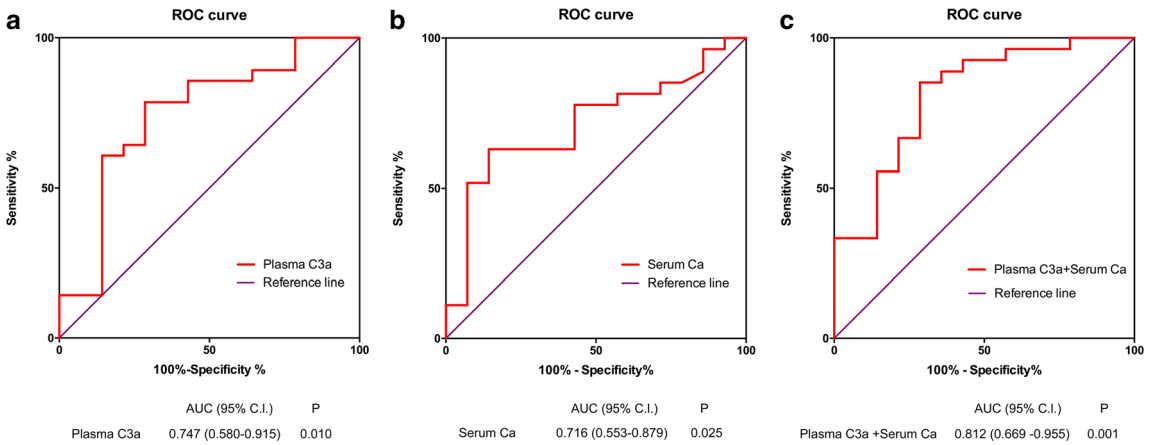

Fig. 3 ROC curve analysis of the predictive value for severe abdominal aortic calcification of plasma C3a, serum Ca, and their combination. ROC, receiver operating characteristic; AUC, area under curve. **a** ROC curve for severe abdominal aortic calcification of plasma C3a in HD patients with AAC score. The AUC was 0.747, $p = 0.010$. **b** ROC curve for severe abdominal aortic calcification of serum Ca in HD patients with AAC score. The AUC was 0.716, $p = 0.025$. **c** ROC curve for severe abdominal aortic calcification of combination of plasma C3a and serum Ca in HD patients with AAC score. The AUC was 0.812, $p = 0.001$

cholesterol as risk factors of severe calcification. Several studies demonstrated that blood pressure, HDL cholesterol, and total cholesterol were risk factors for abdominal aortic calcification [46] using several hundreds of patients from the Framingham Heart Study. Then, this study is a cross-sectional study. Although plasma C3a had a strong correlation with abdominal aortic calcification, whether plasma C3a levels are associated with CVD and mortality needs to be further observed.

CT-based abdominal aortic calcification is a better predictor of future cardiovascular events, as compared with lateral lumbar radiography of the aorta. However, the CT scan is not a routine evaluation, and patients in this study did not want to accept the CT scan, so we used the lateral lumbar radiography of the aorta.

One important character of ESRD patients is hypovitaminosis D. Vitamin D receptor-knockout mice developed cardiac hypertrophy [47]. A randomized placebo-controlled trial of vitamin D supplementation in CKD patients showed that correcting hypovitaminosis D with oral vitamin D3 yielded a decreased pulse wave velocity [48]. However, there is no clear consensus on the benefits of vitamin D in reducing cardiovascular mortality in ESRD patients [49], which probably is due to multiple functions of 1,25(OH)2 vitamin D, such as inducing more intestinal calcium and phosphate absorption. In clinical practice, 1,25(OH)2 vitamin D level is hard to evaluate, because it is usually used to treat secondary hyperparathyroidism. We did not include vitamin D level measurements in this study.

Conclusions

In summary, we found activation of the complement system in patients on hemodialysis. Higher C3a levels are independently associated with severe abdominal aortic calcification. Plasma C3a level could predict severe AAC in HD patients. Our result suggested that inhibiting complement activation might be a new therapy to target vascular calcification in dialysis patients.

Author Contributions Y.Q.W., Y.Q.C., and M.H.Z. prepared the manuscript. Y.Q.C. and Y.Q.W. designed the study and organized the coordination. Y.Q.W., Y.Y.M, K.J.G., and X.Y.C collected the specimens and basic characteristics of all the patients. Y.Q.W. performed the experiments and the data analysis. All authors read and approved the final manuscript.

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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 Peking University First Hospital and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

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

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