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Original Article

Acute-phase Proteins as Promoters of Abdominal Aortic Calcification in Chronic Dialysis Patients

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ABSTRACT. The aim of this study was to find a correlation between acute-phase proteins (APPs) and abdominal aortic calcification (AAC) as well as the impact APPs on AAC in chronic dialysis patients (CDPs). Native lateral lumbar radiography and biochemical analysis were performed in 112 CDPs (aged 60.0 ± 5.43 years) to estimate and score AAC and biochemical values of APPs. The mean AAC score was 8.39 ± 5.43. We detected 16 (14.28%) CDPs without AAC and 96 (85.71%) CDPs with AAC (10 ± 5.43). The number of CDPs with AAC ≤4 was 34 (30.36%) with mean AAC score of 1.85 ± 1.94. By multiple regression analysis, we found positive correlation between AAC and ferritin (β = 0.004398, *P* = 0.0085) and AAC and C-reactive protein [(CRP), β = 0.1972, *P* = 0.0178]. Sensitivity/specificity pairs and criterion variables (CrVs) were as follows: for CRP: 44.21%, 100%, and CrV ≥6 and for ferritin: 83.16%, 56.25%, and CrV ≥196.32. The area under curve (AUC) for CRP and ferritin was 0.721 (*P* <0.0001) and 0.730 (*P* <0.0026), respectively. Fibrinogen and serum iron AUC in the prediction of AAC were 0.533 (*P* = 0.5749) and 0.618 (*P* = 0.0795), respectively. CRP and ferritin were the most powerful APPs involved in the promotion of AAC; serum iron and fibrinogen were shown as lower activity promoters in CDPs. Serum albumin showed inverse activity on AAC.

Introduction

Mortality of patients with end-stage renal disease (ESRD) remains a significant problem, with most deaths resulting from cardiovascular (CV) disease. Aortic calcifications, C-reactive protein (CRP), and serum albumin are strong independent predictors of overall and CV mortality in patients undergoing dialysis.[1] Anemia and malnutrition in ESRD patients share a common cause: the acute-phase inflammatory process, that is, a normal host-defense mechanism. Data from multiple studies indicate acti-

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vation of the acute-phase process in patients with kidney failure, and emerging evidence suggests that the process has a significant role in the risk for CV disease.[2]

Acute-phase proteins (APPs) are proteins whose levels fluctuate in response to tissue injury, for example, trauma, acute infection, chronic inflammation, myocardial infarction, and malignancy. Their concentrations increase (positive APPs) or decrease (negative APPs) in response to the acute-phase reaction.[3] Despite the large number of APPs, we studied CRP, serum iron, ferritin, hepcidin, and ceruloplasmin as positive APPs and serum albumin and leptin as negative APPs. The ability and financial capability of our biochemistry laboratory is limited to procuring expensive reagents for current inflammatory markers; hence, our focus will be on a narrower range of markers. We focused our attention on serum albumin, total protein, calcium × phosphorus product (Ca × P), CRP, fibrinogen, leukocytes, serum iron, ferritin, and thrombocytes.

Unlike the coronary circulation, relatively little is understood about the importance of abdominal aortic calcification (AAC). Similar to the coronary circulation, aortic calcification likely influences subsequent CV events such as aortic occlusion, aneurysm development, and distal embolization.[4] Vascular calcification (VC) is an independent predictor of all causes of CV mortality in chronic kidney disease (CKD).[5] Studies have reported increased VC in CKD patients, compared to the general population (GP), with the predominant differences being earlier age of onset and greater distribution.[6] Similar to the coronary circulation, AAC likely influences subsequent CV events such as aortic occlusion, aneurysm development, and distal embolization. The burden of atherosclerosis and calcification in the aorta was shown to correlate with the degree of atherosclerosis in other arterial beds, but the role of aortic calcific deposits as determinants of later CV risk in living individuals has received less attention.[7] Although traditional risk factor levels are often abnormal in people with calcified abdominal aorta, a host of metabolic and inflammation factors may also play a role in fostering arterial calcification. Some proteins have been identified as inhibitors of calcification, whereas others promote VC.[8] We did not pay attention on bone morphogenetic protein factors as promoters of aortic calcification, but only on APPs, because we do not have appropriate clinical laboratory tests. The APPs are associated with increased incidence of myocardial infarction, future development of hypertension, and increased incidence of diabetes in CKD and GP.[9-11]

The aims of this study are to find a correlation between APPs and AAC in chronic dialysis patients (CDPs) and to find the promoters' activity of some APPs on AAC.

Patients and Methods

Patients

This cross-sectional observational study was conducted during the nine-month period from March to November 2016. We recruited 112 CDPs (64 male, 48 female) from two dialysis centers, aged 60.0 ± 5.43 years with their mean body mass index being 23.73 ± 3.58 kg/m^2. Forty-one patients (36.6%) were smokers, 28 were diabetic patients (25%), and 61 were hypertensive patients (54.46%). The mean duration on chronic dialysis therapy was 5.3 ± 4.8 years. The patients had been on a regular hemodialysis (HD) using a low-flux synthetic membrane, and bicarbonate dialysate was used at flow rate of 500 mL/min, to achieve a Kt/V ≥1.2 (1.245 ± 0.337). The HD session was tailored, 4–5 h, three times a week. We maintained the serum phosphate <1.8 mmol/L with calcium carbonate tablets of 1 g, as an alternate phosphate binder. No patient had clinical signs of acute infection, congestive heart failure, liver cirrhosis, or malignancy at entry into the study. All participants signed an informed consent, and the study was approved by the ethics committee of our institution. Demographic and clinical data were collected from the patient's chart and included age, weight, history of diabetes mellitus, smoking habit, hypertension, and HD duration (Table 1).

Table 1.Demographic and laboratory data and their correlation with abdominal aortic calcification.

Independent variables	Dependent variable		Significance *P*
	ACC score = 8.39±5.43		
	Dependent ACC	Correlation	
	Mean±SD	*r*	
Laboratory			
Ferritin (µg/L)	443.16±333.55	0.371	0.0001
CRP (mg/L)	10.39±8.78	0.355	0.0001
Fibrinogen (mg/L)	4,54±1.79	0.256	0.006
Ca×P ($mmol^2$/L2)	3.49±0.86	0.233	0.014
Serum iron (g/L)	14.7±4.13	0.211	0.025
Albumin (g/L)	37.9±4.6	0.208	0.027
Demographics			
Age (years)	60.0±5.43	0.737	<0.0001
Dialysis duration (years)	5.3±4.8	0.279	0.003
Hypertension, *n* (%)	61 (54.46%)	0.218	0.021
Diabetes, *n* (%)	28 (25%)	0.168	0.077
Smoking, *n* (%)	41 (36.6%)	0.160	0.092
Sex (female, *n*, [%])	48 (42.85%)	0.127	0.182

Variables with *P*>0.2 were not included in the model: leukocytes, protein, thrombocytes, and body mass index. ACC: Abdominal aortic calcification, CRP: C-reactive protein, Ca×P: Calcium-phosphorus product, SD: Standard deviation.

Abdominal aortic calcification detection and scoring

We determined the AAC by lateral lumbar radiography (LLR) in standing position using radiographic equipment (Shimadzu RAD Speed 324-DK, Nishinokyo-Kuwabarachou, Nakagyo-ku. Kyoto 604-8511, Japan). We exposed all 112 patients at film distance of 1 m, with the estimated radiation dose of no more than 15 mGy. We estimated the aortic score using a previously validated system.[12-17] The measure for the unit AAC score is the linear length of aortic calcification compared with one-third of the aortic wall projected near the vertebral segment (L1 to L4) beside it: no calcific deposits in front of the vertebra = 0 score; small scattered calcific deposits filling <1/3 of the longitudinal wall of the aorta = score 1; 1/3 or more but <2/3 of the longitudinal wall of the aorta calcified = score 2, and 2/3 or more of the wall calcified = score 3. The maximum summed score for both anterior and posterior walls for 4 vertebral segments is 4,·3 and·2 = 24 (4 is total number of vertebral segment, 3 is maximal score for more than 2/3 calcification, and 2 is for anterior and posterior wall of aorta). The scoring system of AAC X-ray LLR is depicted in Figure 1.

Figure 1 presents LLR of a 48-year-old woman with HD duration of 51 month and AAC score of 9. We found linear calcification in posterior wall of aorta smaller than 1/3 of L3, localized in the first caudal 1/3 segment of L3 (score 1). The length of linear calcification in anterior wall is smaller than 2/3 of total L3 linear length (score 2) localized in the first and in the second caudal segment of L3. Both anterior and posterior wall of aorta in the L4 level presented aortic calcification bigger than 2/3 of total L4 linear length (score 3 + 3). We had not detected any calcification in anterior or posterior wall, at L1 and L2 levels of the abdominal aorta. The total AAC score – (1 + 2 + 3 + 3) = 9. Four observers (radiologists with more than 30 years' experience) performed an independent and blinded radiographic review assessing all radiographic parameters (detection of calcification, length of calcification, and scoring) and the interpretation of final scoring. Interobserver reliability was determined using Cohen's kappa coefficient (κ), for AAC detection (κ = 0.896) and AAC scoring (κ = 0.953).

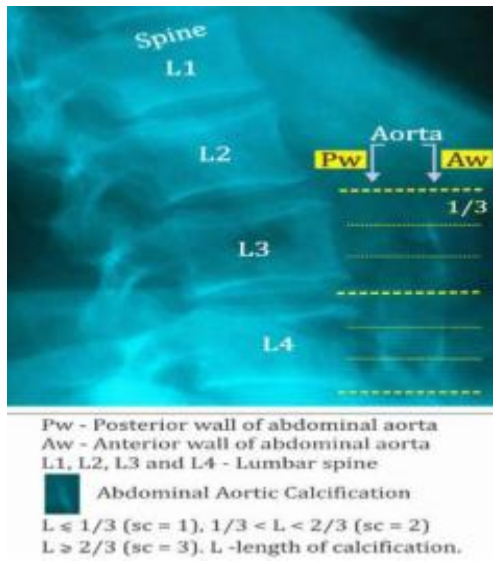

Figure 1. X-ray lateral lumbar spine in detection and scoring of calcification at the anterior and posterior walls of abdominal aorta adjacent to vertebrae L1 to L4.

Clinical and biochemical parameters

We determined biochemical parameters of APPs (albumin, total protein, Ca × P, CRP, fibrinogen, leukocytes, serum iron, ferritin, and thrombocytes) and ionized calcium, calcium, hemoglobin, erythrocytes, hematocrit, urea, and creatinine in all participants using standard laboratory procedures, performed on a Cobas Mira S Analyzer (Roche Diagnostics, Holliston, MA, USA). The blood was drawn immediately before the start of a dialysis session in a fasting state.

Statistical Analysis

Statistical analysis was performed using MedCalc version 15.8 (MedCalc Software bvba, Ostend, Belgium). The results are expressed as means ± standard deviation, or percentage. Bivariate Pearson's correlation analysis was used to find linear relationship between pairs of continuous variables (AAC and APP) and estimate the strength and direction of their relationships. Multiple regression analysis was used to show predictable values of independent variables (APP as predictors) on the dependent variable AAC. Discrimination, the ability of statistic model to distinguish between patients with or without AAC, was assessed using receiver operating characteristic (ROC) curve analysis. The ROC curves for APPs were created for CRP, ferritin, fibrinogen, and iron in the prediction of AAC. If the area under curve (AUC) was >0.5 (P <0.05), the model had discriminatory power.

Results

Lateral lumbar X-ray radiography measurements and APP biochemical tests were successfully conducted on 112 CDPs. The mean AAC score was 8.39 ± 5.43 (D = 0.1117, P = 0.0826, median = 8.5). Every blue circle in Box-and-Whisker diagram presents values of AAC score in each CDPs (Figure 2).

Descriptive and bivariate statistics

The laboratory analysis and demographic data are presented in Table 1.

Pearson's correlation revealed a significant positive correlation between AAC and ferritin, AAC and CRP, AAC and fibrinogen, AAC and Ca × P, and AAC and serum iron, but inverse correlation between AAC and serum albumin. Table 1 shows the positive value of Pearson product-moment correlation coefficient

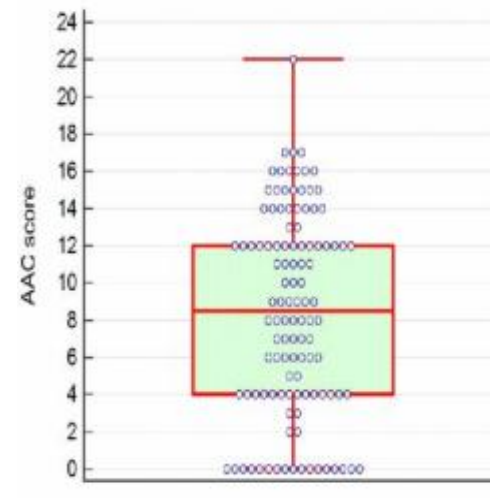

Figure 2. All data Box-and-Whisker diagram of AAC in CDPs.

AAC: Abdominal aortic calcification, CDPs: Chronic dialysis patients.

(*r*) as a measure of the strength of linear dependence between two variables indicating a significant positive correlation between AAC and age (*P* <0.0001), AAC and dialysis duration (*P* <0.003), and AAC and hypertension (*P* = 0.021). The remaining demographic independent variables such as diabetes, smoking, and sex are shown in Table 1, and they do not show a significant correlation with AAC score as a dependent variable.

Multiple regression analysis

The results from backward multiple regression analysis (standardized coefficient β, standard error of β, t and *P* value, residual, and significance level) are shown in Table 2. We used this statistical model to show predictable values of independent variables and so-called predictors (serum albumin, total protein, Ca × P, CRP, fibrinogen, leukocytes, serum iron, ferritin, and thrombocytes) on the dependent variable AAC.

Receiver operating characteristics analysis

We used the discrimination model (estimation of cutoff point) to distinguish between patients with or without abdominal aortic VC. We detected 16 (14.28%) CDPs without AAC (AAC score = 0) and 96 (85.71%) CDPs with AAC (AAC score = 10 ± 5.43). The number of CDPs with AAC ≤4 was 34 (30.36%) with mean AAC score of 1.85 ± 1.94. We assessed all CDPs to distinguish the patients with or without calcification (AAC = 0 and AAC ≤4) by ROC curve analysis, one of the best fundamental statistical tools for diagnostic test evaluation. The summary image of the two ROC curves for CRP and ferritin as a prognostic marker for AAC is shown in Figure 3.

Each point on the ROC curves (CRP and ferritin) represents a sensitivity/specificity pair corresponding to a particular decision threshold (CRP and ferritin in detection of AAC): CRP [sensitivity 44.21% and specificity 100%, the mean of criterion variable (CrV) is ≥6] and ferritin (sensitivity 83.16% and specificity 56.25%, the mean of CrV is ≥196.32). Above assigned values are calculated for the AAC criterion of zero. The sensitivity, specificity, and *P*-value are significantly improved if we suppose that AAC ≤4 is small, initial calcifications and not significant, so we can group them in CDPs without AAC. The ROC curves for CRP and ferritin in prediction of AAC are statistically significant (*P* <0.0001 and *P* <0.0026). The summary image of two ROC curves for fibrinogen and serum iron as a prognostic marker for AAC is shown in Figure 4.

Table 2. Multiple backward regression analysis of determinants of abdominal aortic calcification.

Multiple regression				
Dependent Y		AAC score	Sample size 112	
Coefficient of determination R^2				0.18242
Multiple correlation coefficient				0.4291
Residual standard deviation				49,968
Regression equation				
Independent variables	B coefficient	Standard error	T	*P*
Ferritin	0.004398	0.00164	2,681	0.0085
C-reactive protein	0.1972	0.08196	2,407	0.0178
Fibrinogen	0.1434	0.0839	1,709	0.0903
Analysis of variance				
Source	DF	Sum of Squares	Mean Square	
Regression equation	3	603.09	150.77	
Residual	108	2671.61	24.96	
F-ratio			60,386	
Significance level			*P* = 0.0002	

Variables with *P*>0.1 were not included in the model: Ca × P product, serum iron, albumin, protein, leukocytes and thrombocytes. AAC: Abdominal aortic calcification.

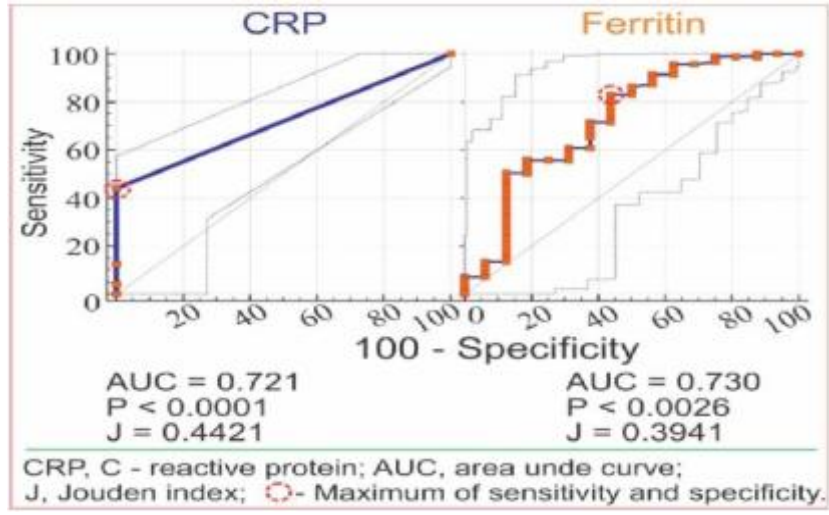

Figure 3. Receiver operating characteristics curves for C-reactive protein and ferritin as promoters of abdominal aortic calcification.

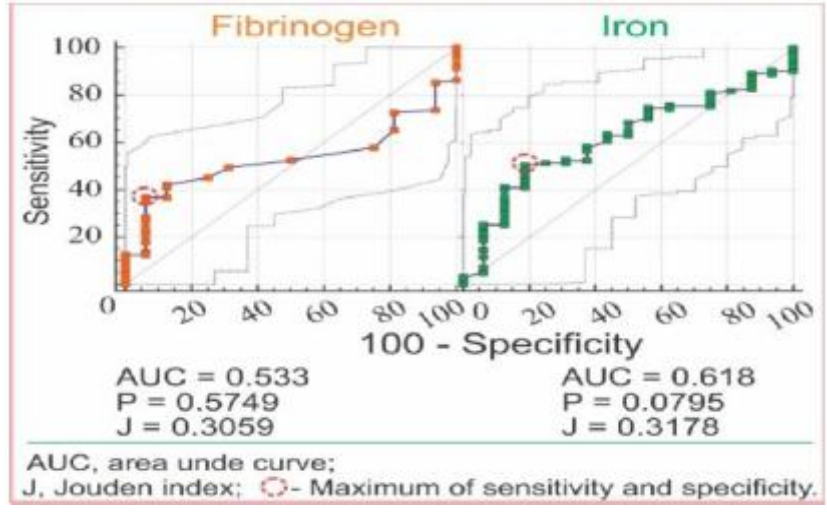

Figure 4. Receiver operating characteristics curves for fibrinogen and serum iron as promoters of abdominal aortic calcification.

Discussion

In this cross-sectional observational study, we estimated AAC and APPs in 112 CDPs by X-ray LLR and biochemical analysis, respectively. The purpose of this study was to find correlation between APPs and AAC and the impact of APPs on VC in the abdominal aorta in CDPs. Our modest financial and diagnostic capabilities allowed us to examine AAC and some of the essential APPs.

The mean AAC results in our study are very close to the AAC results of the other studies (P = 0.612) and significantly differed from AAC in the GP, P <0.0001.[11,13] The spread of aortic calcification is more pronounced in CDPs than in patients with rheumatoid arthritis, severe osteoporosis, and in the GP covered by the normal natural aging process.[18-21] While traditional risk factors predominated in the GP (resulting with normal spread of AAC during aging process), in CDPs, nontraditional risk

factors play an increasingly important role, being perhaps dominant in ESRD patients (resulting with accelerated AAC process). In this population, the chronological age is greater than biological age, expressed through increased arterial calcification. Nontraditional risk factors' (Vitamin D, CRP, hyperhomocysteinemia, plasma norepinephrine, oxidant stress…) expression are more prevalent in ESRD patients compared to the GP.[22-24] When we talk about atherosclerosis and aortic calcification, to the influence of traditional and nontraditional factors, we have to mention the influence of APPs. There are numerous studies that compare the impact of APPs with the impact of traditional risk factors for the appearance and development of atherosclerotic plaques and calcification in blood vessels.[22,25] They conclude that traditional risk factors are the main determinant of atherosclerotic plaque and calcification, but they are associated with APPs and nontraditional factors. No APP was independently associated with atherosclerotic disease.[23,25]

We found significant positive correlation between AAC and the following APPs: ferritin, CRP, fibrinogen, Ca × P, and serum iron but inverse correlation between AAC and serum albumin and between AAC and demographic parameters such as age, dialysis duration, and hypertension. Many epidemiological studies have considered the association of iron status and CV disease, and many studies have suggested that elevated serum ferritin increased the risk of atherosclerosis and aortic calcification.[26,27] Endothelial dysfunction in CDPs corresponding to uremia plays an important role in the development of AAC through well-known mechanisms of oxidative stress as one of the factors affecting endothelial dysfunction and the development of atherosclerosis. Iron (serum Fe) overloading increases oxidative stress and causes endothelial dysfunction, while ferritin preserves iron excess and counteracts the toxicity of iron and protects against oxidative stress.[28-30]

Our finding about positive association of AAC and serum Ca × P correlates with the results from other studies. Cozzolino et al concluded that in patients with chronic renal failure, the increased serum Ca × P and hyperphosphatemia are important contributors to the higher incidence of arterial calcifications and CV events.[31] Bone loss is increased by hyperphosphatemia which accelerates the progression of secondary hyperparathyroidism by increasing parathormone (PTH) levels. Phosphorus-induced and PTH-induced bone loss elevates the Ca × P and most likely the expression of factors that mediate the strong association between bone loss and arterial calcification, such as bone-associated proteins.[30,31]

Having in mind that the impact of atherogenic factors is intertwined, the boundary for novel risk factors for atherosclerosis and APPs is not clearly defined. Multiple studies have examined the effects of novel risk factors such as CRP, fibrinogen, and homocysteine as atherothrombotic biomarkers.[32,33] They analyzed data from 52 prospective studies that included more than 240 thousand participants without a history of CV disease to investigate the value of adding CRP or fibrinogen levels to conventional risk factors for the prediction of CV risk.[33] However, whether CRP and fibrinogen will be counted in novel, traditional or APPs, it is irrelevant. It is important that their effect on the development of atherosclerosis and calcification is repeatedly proven.

We must not forget the importance of serum albumin as a negative APP. Its inverse association with atherosclerotic changes, calcification, and with an increase in arterial stiffness has been confirmed in our and many other studies in CDPs.[1,6,34-36] They concluded that arterial stiffness, CRP, and serum albumin are strong predictors of overall and CV mortality in patients undergoing dialysis. Detection of aortic calcification that has a direct impact on arterial stiffness provides physicians with useful prognostic information for CV mortality, independent of traditional CV risk factors, because the traditional CV risks factors do not accurately predict survival in CDPs. Although neither the traditional CV risk factors nor the novel CV risk factors or APPs are direct prognostic indicators of CV mortality, we cannot prove their relation to true tissue

damage (aortic calcification), rather than only circulating biomarkers' risk factors.

To find the real determinants that have the greatest impact on AAC genesis, we used multiple backward regression analysis. Because of the strong intercorrelation of age and AAC (r = 0.737, P <0.0001), we did not enter age in the statistical model. In this way, we performed the elimination of independent variables (APPs) that showed only an association, but not determination for AAC as atherosclerotic process. Confirmation of the determinant effect of some of independent variables was established only for those that had a statistical significance <0.05. Only ferritin and CRP were shown as determinants of AAC. The impact of these APPs as clinical biomarkers on AAC as tissue markers of real lesion is not for underestimation. According to calculated coefficient of determination by the aforementioned statistical model, 18.42% of the AAC changes are dependent on ferritin and CRP. The residual 81.58% of AAC were dependent on other factors, which were not covered with the regression model. Despite the established correlation of fibrinogen with AAC (P = 0.006), its determinant effect on AAC by multiple regression had no statistical significance in our study (P = 0.0903). The other APPs that showed a statistically significant correlation by Pierson's correlation (Ca × P, serum iron, and albumin) were rejected by regression analysis as statistically insignificant determinants for AAC, too. For that reason, we focused our attention on ferritin and CRP, as the most potent APPs for AAC genesis and progression.

We found a high prevalence of AAC in CDPs. There are a sufficient number of studies on VC in ESRD, but the specific anatomical distribution (abdominal aorta) and severity of AAC, in contrast to coronary calcification, are less well documented.[5-7,12,21] We detected by LLR that only 14.28% of CDPs were spared from AAC. This was a small group of younger age (44.3 ± 11.4 years) with short HD duration (3.9 ± 4.8 years) and small reference values of APPs. Patients with a slightly advanced age (49.2 ± 11.6 years), decreased duration on HD (3.7 ± 4.7 years), and slightly elevated APPs (CRP = 7.02 ± 8.8, ferritin = 425.4 ± 272.1) showed initial values of AAC (AAC score ≤4). It is obvious that these minor changes in APPs, but no significant changes in age (P = 0.167) and HD duration (P = 0.889), cause the number of CDPs with mild AAC compared to CDPs without AAC, to be almost doubled. These facts give us the right, once again, to focus our attention on APPs, more accurately on the CRP and ferritin.

We confirmed the significance of APPs as promoters of AAC by estimation of cutoff point value expressed by the mean CrV for CRP (≥6) and ferritin (≥196.32). The critical point over which the AAC development and progression is significantly started is expressed by above-mentioned cutoff point value (6 for CRP and 196.32 for ferritin). The high sensitivity, high specificity, and large surface of AUC have proved important on the value of CRP and ferritin in promoter activities of AAC. The promoter activity value of APPs for AAC was more expressed when we excluded the soft low-scored AAC (AAC ≤4 treated as "no calcification"). In that case, we got high statistically significant P-value for both APPs (CRP and ferritin, P <0.0001).

The fibrinogen and serum iron did not show their promoter activity on the AAC (P > 0.05). Beside their large specificity, their small sensitivity and relatively small surface of AUC, these two APPs markers did not take their place as promoters for AAC. A slightly more promoters activities position of ferritin and iron were observed when in the construction of the ROC, the VC with relatively low score (AAC ≤4) were rejected and treated as null. In that case, we got high statistically significant P-value for both APPs (fibrinogen, P = 0.041 and serum iron, P = 0.044) and for albumin (P = 0.032).

We conclude that CRP and ferritin emerged as the most powerful APPs promoting AAC, while serum iron and fibrinogen were shown as lower activity promoters in CDPs. The positive promoter's activity of above-mentioned APPs is emphasized when we analyzed the aortic calcification with AAC score >4. In that

case, serum albumin showed inverse promoter activity on AAC. In this way, examining the level of APPs as clinical biomarkers, we get an insight into the level of atherosclerotic process expressed through calcification in the aorta among CDPs, without performing X-ray imaging. Or, in other words, we concluded that APPs are prospective risk markers for AAC in CDPs, obtained by fast, inexpensive, and noninvasive way, with the sole purpose for early prediction of the occurrence of VC.

Study Limitations

The important limitations of this study are impossible spatial assessment and density estimation of the AAC. One-dimensional plane of estimation of AAC is the main disadvantage of the LLR, whereas in fact, it may have been circumferential. Although the density of calcification may be relevant with APP association, unfortunately, the present study does not provide data on the density of calcification. The other limitation is that we were unable to distinguish between intimal and medial aortic calcifications, due to the limitation of the current LLR imaging techniques. Computed tomography (CT) is the gold standard of AAC detection and measurement despite the higher radiation dose exposure compared with radiography. Using LLR instead of CT, because of its higher accuracy, is one of the limitations of this study. The other limitation of this study includes the need for validation of the results in broader trial on CDPs and exploring a larger number of negative and positive APPs available in diagnostic centers of more developed countries.

Ethical approval

All procedures in our studies and all human participants were in accordance with the ethical standards, and our regional ethics committee approved the study. All participants give us informed and signed consent before including in the study.

Conflict of interest: None declared.

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