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Cardiac Calcifications: Fetuin–A and Other Risk Factors in Hemodialysis Patients

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Cardiac calcifications are a frequent finding in hemodialysis for chronic renal failure. Several factors may play a role in the intimal and medial calcification of coronary arteries such as age and some known atherogenetic factors. In addition, Fetuin-A has been proposed as a protective agent through solubilization of calcium phosphate salt. Fetuin-A is also a marker of inflammatory-nutritional state, and its changes could be an expression of this condition. The aim of this cross-sectional study is to evaluate the relative importance of risk factors of calcifications with special regard to Fetuin-A.

The study was conducted with 132 hemodialysis patients. They were subjected to multislice computed tomography for evaluation of calcium deposits in the heart. In addition, the patients were sampled for evaluation of calcium-phosphate parameters, lipid profile, nutritional and inflammatory markers, and also Fetuin-A.

There was a wide variability of the extent of calcium deposits expressed as Agatston score, with only 9.3% of patients without calcifications. Age, hemodialysis age, sex, calcium-phosphate parameters, and lipid profile were important risk factors, together with nutritional and inflammatory status of the patients. An inverse correlation between coronary calcium score and Fetuin-A emerged from a multiple regression analysis. However, there was no significant difference in serum Fetuin-A among different grades of calcium score. By dividing the patients in tertiles of serum Fetuin-A, an association between low levels of Fetuin-A and high calcification score was found. Fetuin-A as dependent variable was strictly linked to prealbumin serum levels. In addition, there was a clear link between cardiac calcification scores and inflammatory-nutritional markers. Serum calcium and treatment with calcitriol emerged as predictive variables of coronary score.

Fetuin-A could be involved in the process of calcification both in the case of markedly low serum levels, due to decreased prevention of calcium phosphate precipitation, and also as a marker of inflammation, a well-known risk factor of atherogenesis. Treatment with intravenous calcitriol could marginally enhance cardiac calcifications, probably through its hypercalcemic effect. *ASAIO Journal* 2006; 52:150–156.

Cardiac calcifications are a frequent occurrence in chronic renal failure, especially in dialysis patients.[1–5] Calcifications are found mainly in the coronary arteries and in the valves.[6] In the coronary arteries, calcifications are located either in the atherosclerotic intimal plaques or, especially in uremic and diabetic patients, in the medial arterial layer.[7,8] Both types of calcification are responsible for cardiac events.[9] An important advancement in this field of clinical research has been achieved by the introduction of radiologic techniques that are able to localize and quantify the calcium deposits in the heart. This progress has been made through the introduction of electron beam computerized tomography[2] and also with fast multislice computed tomography (MSCT),[10] a now more widespread technique. Both techniques are able to locate the calcified lesions in the heart and provide a quantitative evaluation of the calcium deposits. Most patients on dialysis are affected by calcium deposits in the heart.[11,12] The accumulation process increases with the age of the patients and with their hemodialysis age. Many experimental studies have demonstrated that the process of calcification should not be considered a mere passive phenomenon, but the result of an active local process whereby the phenotype of smooth muscle cells changes and differentiates into osteoblast-like cells, which produce typical protein factors responsible for calcification in the vascular tissue.[13] In this process of structural change of the arteries, high levels of serum calcium and phosphate and of the CaxP product play an important role.[14,15] Other factors have theoretically been identified as able to counteract the vascular calcification process, probably through solubilization of the calcium phosphate salt.[16,17] The main factor, which has been linked to this process is a well-known serum protein, α_2 Heremans-Schmid glycoprotein or Fetuin-A, usually present in relatively large concentrations in the serum. Fetuin-A is a multifunctional molecule and acts as an antagonist of TGF-β, regulating cytokine-dependent osteogenesis and inhibiting insulin receptor and some proteases. It has been demonstrated that Fetuin-A levels in hemodialysis chronic kidney disease (stage 5) are generally lower than normal.[16] The tertile of patients with lower serum levels of the protein have a decreased general and cardiac survival.[16] In addition, Fetuin-A, when added to the serum of dialysis patients, was able to keep in solution calcium phosphate salts.[16] Therefore, it has been concluded that prevention of calcium salt precipitation in the

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arteries, due to the presence of normal Fetuin-A serum levels, was responsible for better survival of hemodialysis patients. However, it is well known that Fetuin-A is a negative acute reactive protein, synthesized by the liver, which varies in conditions of inflammatory stress, similar to C-reactive protein (CRP) and α_1-acid glycoprotein, which, quite the reverse, are positive acute reactive proteins. The same factors inducing an increase in CRP and α_1-glycoprotein, bring about a decrease in serum Fetuin A levels.[18] Serum levels of this protein are also influenced by nutritional status, which is frequently impaired in dialysis patients. It is known that inflammation-malnutrition is also a factor of atherogenesis,[19] a process strongly enhanced in dialysis patients.[20] Therefore, it is still necessary to determine whether the decreased Fetuin-A levels are unsafe due to a fall of a protective factor against calcium-phosphate vascular deposits or are mainly a marker of the inflammatory-malnutritional state of the patients and its consequences on mortality in general.

Materials and Methods

The study has been carried out on 132 patients on maintenance hemodialysis from six dialysis units in Rome. Patients were considered eligible for the study if the dialysis duration was >6 months, their age was >18 years, the vascular access performance was satisfactory with a blood flow of at least 300 ml/min and a Kt/V >1.0, the heart rate <80 bpm, the voluntary apnea was of at least 20 seconds, and a written informed consent to participate in the study was provided. The patients did not receive steroids or nonsteroidal inflammatory drug therapy. Parathyroidectomy and previous renal transplantation were exclusion criteria.

Of the 132 patients, 86 were male and 46 were female; 13 patients had diabetes mellitus and 62% were affected by arterial hypertension and were treated with calcium channel blockers, angiotensin converting enzyme inhibitors, AII receptor antagonists, and α- and β-blockers, achieving a satisfactory blood pressure control.

All patients were treated with phosphate-binding agents, generally, sevelamer in 61 patients (4.7 ± 3 g/die), calcium carbonate in 22 patients (2.5 ± 1 g/die) and both drugs in 49; 47 patients received aluminium-containing agents, usually associated with other phosphate-binding agents, in limited doses (≤1.5 g/die) and for restricted periods of time in the case of persistently elevated serum phosphate levels. Thirty-nine patients were under treatment with IV calcitriol, 2 to 8 μg/week, which had been started at least 8 to 12 months before the enrollment.

Forty-seven percent of the patients were treated with standard bicarbonate dialysis, using Cuprophan or Low-Flux-PolySulfone (LF-PS) membranes, 18% with acetate-free biofiltration using Polyacrylonitrile-AN69 (PAN-AN69 ST), and the remaining patients with hemodiafiltration using Helixone (FX80) or Polycarbonate (Spiraflo SG 8 Plus). All patients underwent hemodialysis 3 times per week. The dialysis sessions lasted 4 hours and were delivered at constant blood and dialysate flow rate values of 300 and 500 ml/min, respectively. Calcium concentration in the dialysate was 1.25, 1.5, or 1.75 mmol/L, in 40%, 25%, and 35% of patients, respectively.

After their consent, all patients underwent an MSCT carried out with MSCT "LightSpeed 16" (General Electric Medical Systems, Milwaukee, WI) that uses a prospectively ECG-triggered sequential scanning. As with electron beam computerized tomography, the values obtained with the MSCT technique were transformed in terms of Agatston scores, the standard unit in reporting calcification.

The examination protocol envisaged acquiring baseline image sets without contrast medium administration; the scanning volume extended from the ascending aorta, at the level of the pulmonary bifurcation, to cardiac diaphragmatic surface. The set of images were transferred to a dedicated workstation (Advantage Windows 4.1), where an operator using special software performed the calculations of the coronary and valves calcifications.

Blood samples for the biochemical evaluation were drawn before a dialysis session. Serum samples were stored at −30°C until the assays were done. The following assays were made: serum calcium, phosphorus, intact PTH, total cholesterol, HDL and LDL cholesterol, triglycerides, Fetuin-A, serum albumin, prealbumin, α_1-acid glycoprotein and CRP.

Serum calcium and phosphorus were measured by colorimetric methods using a Roche autoanalyzer. Normal values are 8.4 to 10.2 mg/dl and 2.5 to 4.5 mg/dl, respectively. Serum intact PTH was measured by an IRMA (Nichols Institute Diagnostic, San Juan Capistrano, CA). The normal range of values is 10 to 65 pg/ml.

Serum cholesterol, HDL cholesterol, and LDL were measured by colorimetric methods using the autoanalyzer, with normal values of 50 to 200 mg/dl, 35 to 65 mg/dl, and <150 mg/dl, respectively. Triglycerides were measured by colorimetric methods, using the autoanalyzer. Normal values are 50 to 180 mg/dl. Non-HDL cholesterol was a measure of total cholesterol minus HDL cholesterol and used as recommended by the National Cholesterol Education Program.[21] Normal values are 143 ± 40 mg/dl.

Serum albumin, prealbumin, α_1-acidglycoprotein and CRP levels were assayed by nephelometric methods (BN II™ nephelometer, DADE Behring, Marburg, Germany). Normal values are 3.5 to 5.2 g/dl, 0.2 to 0.4 g/l, 0.5 to 1.2 g/l, and 0 to 3 mg/l, respectively. The Prognostic Index of Nutrition and Inflammation (PINI)[22] was calculated with the following formula: α_1-acid glycoprotein × CRP/Albumin × Prealbumin (n.v. <1). Fetuin-A was measured with an ELISA kit (Epitope Diagnostics Inc., San Diego, CA), with normal values of 0.519 ± 0.15 g/l. The ratio of weekly erythropoietin administration over Hb concentration in the long-term–treated patients (EPO weekly dose, U*dl/g Hb) was used as an index of erythropoietin resistance. In addition, the body mass index and the Kt/V of the patients were evaluated at the time of the score evaluation according with current procedures.

The statistical evaluation was carried out with the use of a personal computer equipped with the statistical package SPSS for Windows (Chicago, IL, release 9.0.). In addition to descriptive statistics for the selected variables, correlation and linear regression analysis were performed to examine the relationship between the cardiac calcium scores and clinical and biochemical parameters. Multiple regression analyses were performed as well to assess the combined influence of variables on the calcification scores or on Fetuin-A. Log transform of cardiac and coronary scores were

Table 1. Clinical, biochemical data, calcification scores and normal values

	normal values	
n°	–	132
Sex, M/F	–	87/45
age, years	–	59,41 ± 12,93
HD age, months	–	71,07 ± 68,16
diabetes	–	13
hypertension	–	80
Kt/V	–	1,33 ± 0,32
BMI	18–24	24,65 ± 4,92
Score	–	2061,24 ± 3308,72
Scorelog	–	2,93 ± 0,96
coronary score	–	1359,16 ± 2181,21
coronary scorelog	–	2,46 ± 1,11
Ca_s, mg/dL	8,5–10,5	9,61 ± 0,87
P_s, mg/dL	2,5–4,5	5,48 ± 1,37
CaxP, mg^2/dL^2	–	52,67 ± 13,87
iPTH, pg/mL	10–65	545,81 ± 542,41
Cholesterol, mg/dL	<200	158,75 ± 40,62
HDL, mg/dL	35–65	43,34 ± 14,32
non-HDL Cholesterol, mg/dL	143 ± 40	115,94 ± 41,48
LDL, mg/dL	60–150	81,97 ± 29,66
Triglycerides, mg/dL	50–180	198,78 ± 155,14
Hb, g/dL	12,5–16	11,59 ± 1,14
Fetuin A, g/L	0,51 ± 0,15	0,33 ± 0,11
Albumin g/dL	3,5–5,2	4,11 ± 0,36
α1-acid glycoprotein, g/L	0,5–1,2	1,19 ± 0,38
prealbumin, g/L	0,2–0,4	0,38 ± 0,14
CRP, mg/dL	0–3	9,42 ± 20,95
PINI, index	0–1	1,59 ± 4,72
Epo/Hb*	–	679,77 ± 635,41

* Epo, weekly units, U*dl/g Hb

used to normalize the data. Comparison of groups was evaluated by parametric tests (Student's *t* test and ANOVA with Tukey post hoc test). A probability value of <0.05 was considered statistically significant.

Results

Average values of clinical and biochemical variables and the results of calcification score evaluation of the patients are reported in **Table 1**, together with the range of values for each variable. Fetuin-A in patients with chronic kidney disease was significantly lower than in normal patients ($p < 0.001$).

Comparisons of the patients with 0, 1 to 400 (mild to moderate), 400 to 1,000 (severe), and >1,000 (very severe) calcification score values are reported in **Table 2**. Percentage of patients in the four categories were 9.3, 25.0, 15.6, and 50, respectively. Some statistical differences were found among the groups, such as age ($p < 0.0001$), serum calcium ($p < 0.05$), and serum albumin ($p < 0.005$).

In **Table 3**, patients were assembled in two groups. The variables of patients with mild to moderate 0 to 400 and severe to very severe (>400) calcification scores are reported in the table. Statistically significant difference was found only for age ($p < 0.0001$), hemodialysis age ($p < 0.01$), cholesterol ($p < 0.01$), serum albumin ($p < 0.05$) and the ratio EPO/HB ($p < 0.05$).

In **Table 4**, the variables of male and female patients, with exclusion of cases receiving intravenous calcitriol administration, are compared. A significant difference was found for score log and coronary score log, with higher levels in male patients. Male and female patients were significantly different for serum calcium, HDL, LDL cholesterol, Hb, and prealbumin, whereas no difference was found for age, hemodialysis age, and Fetuin-A.

Cardiac calcification score was found to be correlated significantly to age ($p < 0.005$), hemodialysis age ($p < 0.05$), serum calcium ($p < 0.05$), serum phosphate ($p < 0.01$), α_1-acid glycoprotein ($p < 0.01$), and iPTH ($p < 0.05$). Multiregression analysis with the cardiac score as dependent variable selected the following variables: triglycerides ($p < 0.0001$), prealbumin ($p < 0.001$), non-HDL cholesterol ($p < 0.001$,) and α_1-acid glycoprotein ($p < 0.05$).

Log transform of cardiac score (score log) was correlated to age ($p < 0.0001$), serum calcium ($p < 0.005$), sex ($p < 0.05$), with prevalence of male sex, and inversely to serum cholesterol ($p < 0.05$) and HDL cholesterol ($p < 0.01$). Multiregression analysis selected the following variables: age ($p < 0.0001$), hemodialysis age ($p < 0.001$), triglycerides ($p < 0.001$), and inversely Fetuin-A ($p < 0.05$).

Coronary arteries calcification score was correlated to age ($p < 0.05$), hemodialysis age ($p < 0.05$), serum calcium ($p < 0.01$), iPTH ($p < 0.0001$), triglycerides ($p < 0.005$), treatment with calcitriol ($p < 0.001$), and the parameter EPO/HB ($p < 0.05$). Multiregression analysis selected the following variables: triglycerides ($p < 0.0001$), age ($p < 0.001$), PTH ($p < 0.01$), non-HDL cholesterol ($p < 0.01$), and ,inversely, Fetuin-A ($p < 0.005$) (**Table 5**).

Log transform of coronary calcification scores was correlated to age ($p < 0.001$), serum calcium ($p < 0.01$), PTH ($p < 0.05$), HDL ($p < 0.01$), and calcitriol dose weekly administration ($p < 0.01$). Multiregression analysis selected age ($p < 0.0001$), hemodialysis age ($p < 0.005$), triglycerides ($p < 0.001$), and Fetuin-A ($p < 0.05$) as predictive variables and excluded all the other independent variables (**Table 5**). In a multiregression analysis with log transform of coronary score as dependent variable and a selected number of independent variables, excluding intravenous calcitriol administration, serum calcium was the predictive variable ($p < 0.005$), whereas hemodialysis age, serum P, PTH, and CaxP were excluded. Serum calcium as dependent variable was influenced by intravenous calcitriol administration ($p < 0.005$), whereas hemodialysis age, serum P, and PTH were excluded. In addition, in the patient cohort of 39 cases treated with intravenous calcitriol, excluding serum calcium as independent variable, the log transform of coronary score was predicted by the weekly intravenous dose of calcitriol administration, whereas hemodialysis age, serum P, and PTH were excluded variables. However, the correlation coefficient of the relation between the calcitriol dose and the coronary score was 0.248, explaining only 6% of the total variance.

Serum Fetuin-A levels were correlated to prealbumin ($p < 0.0001$), triglycerides ($p < 0.001$), serum cholesterol ($p < 0.01$), non-HDL cholesterol ($p < 0.05$), body mass index ($p < 0.01$), and Hb ($p < 0.05$). Multiregression analysis with Fetuin-A as the dependent variable selected the following significant variables: prealbumin ($p < 0.0001$), CaxP ($p < 0.01$), KT/V ($p < 0.05$), and Hb ($p < 0.05$) (**Table 5**).

Table 2. Clinical and biochemical values and calcification scores in the patients divided in groups of severity of cardiac calcification

	score 0–1 1	score 1–400 2	score 400–1000 3	score >1000 4	p*
n°	13	31	21	67	–
Sex, M/F	5/8	19/12	13/8	50/17	–
age, years	48,23 ± 14,19	55,09 ± 13,12	58,65 ± 12,19	63,77 ± 11,03	<0,0001[1–4;2–4]
HD age, months	64,42 ± 97,10	45,38 ± 39,79	84,7 ± 97,99	80,38 ± 59,72	NS
diabetes	–	–	1	12	–
hypertension	8	18	13	40	–
Kt/V	1,33 ± 0,30	1,38 ± 0,34	1,44 ± 0,50	1,27 ± 0,22	NS
BMI	22,11 ± 2,74	24,62 ± 4,23	23,99 ± 3,34	25,26 ± 5,76	NS
Score	0,07 ± 0,26	175,35 ± 115,36	682,28 ± 151,72	3796,71 ± 3955,7	<0,0001
Scorelog	0,0 ± 0,0	2,00 ± 0,48	2,82 ± 0,09	3,43 ± 0,32	<0,0001
coronary score	0,02 ± 0,08	139,56 ± 109,12	481,60 ± 248,31	2472,9 ± 2578,5	<0,0001
coronary scorelog	0,0 ± 0,0	2,00 ± 0,48	2,52 ± 0,63	3,19 ± 0,53	<0,0001
Ca_s, mg/dL	9,019 ± 0,52	9,61 ± 0,89	9,43 ± 1,12	9,77 ± 0,79	<0,05[1–4]
P_s, mg/dL	5,71 ± 0,99	5,44 ± 1,51	5,59 ± 1,46	5,4 ± 1,35	NS
CaxP, mg^2/dL^2	51,55 ± 9,53	51,83 ± 13,09	53,46 ± 17,44	53,03 ± 14,06	NS
iPTH, pg/mL	381,57 ± 326,30	477,6 ± 393,13	396,31 ± 394,02	650,07 ± 644,89	NS
Cholesterol, mg/dL	165,69 ± 41,19	173,90 ± 44,76	158,38 ± 41,15	150,49 ± 36,85	NS
HDL, mg/dL	52,53 ± 20,64	43,51 ± 12,59	41,23 ± 14,21	42 ± 13,28	NS
non-HDL Cholesterol, mg/dL	113,15 ± 40,39	130,38 ± 46,40	119,44 ± 45,20	108,85 ± 37,16	NS
LDL, mg/dL	88,18 ± 25,04	84,31 ± 38,65	82,41 ± 19,82	79,5 ± 29,80	NS
Triglycerides, mg/dL	146,61 ± 84,08	208,64 ± 157,87	189,55 ± 145,02	206,70 ± 167,08	NS
Hb, g/dL	11,38 ± 0,93	11,73 ± 0,93	11,81 ± 0,93	11,19 ± 1,31	NS
Fetuin A, g/L	0,38 ± 0,07	0,34 ± 0,11	0,31 ± 0,08	0,31 ± 0,11	NS
Albumin, g/dL	4,28 ± 0,43	3,95 ± 0,32	3,96 ± 0,61	3,94 ± 0,52	<0,005[3–4;2–3]
α1-acid glycoprotein, g/L	1,07 ± 0,31	1,21 ± 0,39	1,17 ± 0,36	1,21 ± 0,38	NS
prealbumin, g/L	0,37 ± 0,07	0,42 ± 0,17	0,32 ± 0,07	0,38 ± 0,13	NS
CRP, mg/dL	3,88 ± 4,92	13,04 ± 33,86	5,98 ± 9,39	9,75 ± 17,31	NS
PINI index	0,38 ± 0,65	2,17 ± 7,83	0,91 ± 1,77	1,75 ± 3,64	NS
Epo/Hb**	575,06 ± 369,42	522,53 ± 440,22	788,79 ± 936,46	742,6 ± 650,2	NS

* post hoc test
** Epo, weekly units, U*dl/g Hb

Multiregression analysis with EPO/Hb as the dependent variable selected the following variables: triglycerides ($p < 0.0001$), prealbumin ($p < 0.005$), and CaxP ($p < 0.05$).

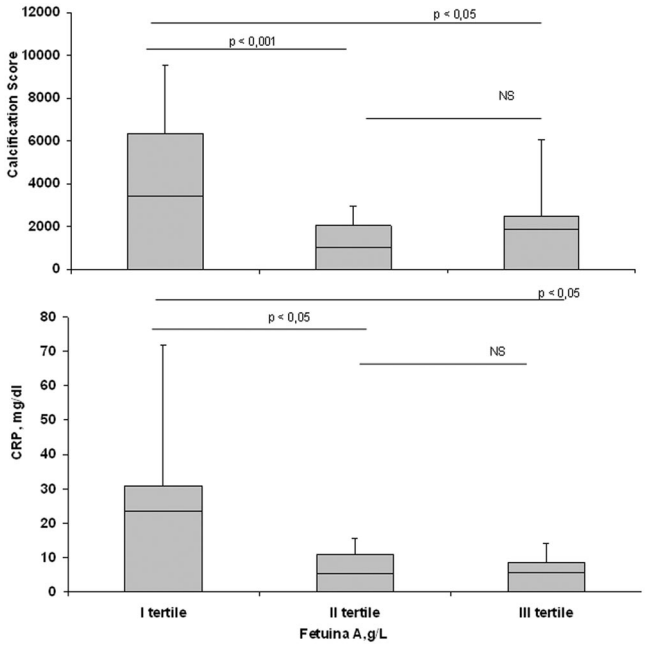

Figure 1. Calcification score and CRP average levels (±SD) at different tertiles of serum Fetuin-A.

By dividing the Fetuin-A levels in tertiles, it was found that the average calcium score was significantly more elevated in the first tertile, with lower levels of Fetuin-A. There was no difference in score levels between the second and third tertile. CRP had a similar pattern, with higher levels at the lower tertile of Fetuin-A levels (**Figure 1**).

A cardiac score of <400 was present in 28.4% of male patients and 45.5% of female patients ($p < 0.05$). A score of <400 was found in 25.5% of patients with a dialysate calcium concentration of 1.75 mmol/l, whereas it was found in 43.9% with a concentration value of 1.25 mmol/l ($p < 0.05$).

Discussion

This cross-sectional study carried out on patients on maintenance hemodialysis confirms the high occurrence of cardiac calcifications in this population, as already reported by other studies.[1,23,24] In addition, the data demonstrate the presence of a wide variability in the magnitude of calcium deposits, ranging from nil to several grams. Although investigation of supposed additional factors able to prevent the cardiac calcifications was not the object of this study, a number of risk factors became apparent. Sex, with a prevalence of calcification severity in male patients, as evidenced by comparing male and female patients (**Table 4**), age and hemodialysis age also emerged as important risk factors, as shown by the multiregression analysis of score log values and coronary scores as dependent variables.

Table 3. Clinical, biochemical data of the patients divided in <400 and >400 cardiac calcification score value

	0–400	>400	p*
n°	45	87	–
Sex, M/F	25/20	63/24	–
age, years	53,06 ± 13,65	62,61 ± 11,44	<0,0001
HD age, months	51,01 ± 61,75	81,36 ± 69,62	<0,01
diabetes	–	13	–
hypertension	26	53	–
BMI	23,96 ± 4,02	24,98 ± 5,34	NS
Score	120,82 ± 125,73	3053,5 ± 3695,7	<0,001
Scorelog	1,43 ± 1,04	3,28 ± 0,39	<0,001
coronary score	100,23 ± 112,21	1996,02 ± 2442,35	<0,001
coronary scorelog	1,35 ± 1,02	3,02 ± 0,62	<0,001
Ca_s, mg/dL	9,43 ± 0,84	9,70 ± 0,88	NS
P_s, mg/dL	5,52 ± 1,37	5,45 ± 1,37	NS
CaxP	51,75 ± 12,04	53,12 ± 14,75	NS
iPTH, pg/mL	449,23 ± 373,50	594,75 ± 606,45	NS
Cholesterol, mg/dL	171,47 ± 43,42	152,35 ± 37,71	<0,01
HDL, mg/dL	46,18 ± 15,71	41,84 ± 13,39	NS
non-HDL Cholesterol, mg/dL	125,29 ± 44,95	111,09 ± 38,95	NS
LDL, mg/dL	85,64 ± 34,14	82,25 ± 27,44	NS
Triglycerides, mg/dL	190,31 ± 142,06	203,11 ± 162,05	NS
Hb, g/dL	11,63 ± 0,93	11,55 ± 1,24	NS
Kt/V	1,37 ± 0,33	1,31 ± 0,31	NS
Fetuin-A, g/L	0,35 ± 0,10	0,31 ± 0,11	NS
Albumin, g/dL	4,20 ± 0,29	4,06 ± 0,37	<0,05
α1-acid glycoprotein, g/L	1,17 ± 0,37	1,20 ± 0,38	NS
prealbumin, g/L	0,40 ± 0,15	0,36 ± 0,12	NS
CRP, mg/dL	10,46 ± 28,95	8,91 ± 15,92	NS
PINI index	1,61 ± 6,50	1,57 ± 3,33	NS
Epo/Hb**	538,05 ± 416,98	752,27 ± 713,48	<0,05

* post hoc test
** Epo, weekly units, U*dl/g Hb

Serum phosphate, CaxP and PTH levels were also relevant factors, even if apparently of lower importance, within the range of values of this patient cohort, at least as resulting from the correlation analysis. However, increased calcium concentration of the dialysis bath (1.75 mmol/l) was associated with a higher percentage of patients with severe to very severe calcification scores, compared with patients treated with a calcium concentration of dialysis bath of 1.25 mmol/l. In addition, serum calcium was a risk factor in a multiregression analysis with coronary score as dependent variable with a selected number of independent variables. Other risk factors of importance were some common lipid parameters, such as triglycerides, HDL cholesterol and non-HDL cholesterol, and inflammatory and nutritional parameters, such as prealbumin, α_1-acidglycoprotein and also Fetuin-A. HDL serum levels in female patients was significantly higher than in male patients, probably an important factor responsible for the relatively lower degree of calcification scores compared with male patients. An inverse correlation between coronary score values and Fetuin-A emerged from the multiple regression analysis (**Table 5**). Fetuin-A, as a dependent variable, was strongly linked to prealbumin (**Table 5**). However, there was no significant difference in serum Fetuin-A levels among different grades of calcium scores (**Tables 2 and 3**). By dividing the patients in tertiles of serum Fetuin-A levels, an association between low levels of Fetuin-A and severely increased calcium score levels was evident only in the lower tertile, whereas second and third tertiles had similar average cardiac calcium scores (**Figure 1**). This finding is in favor of the conclusion that low levels of Fetuin-A may enhance arterial and heart calcium deposition, whereas at higher levels of Fetuin-A but still lower than normal, the effect is no longer visible. In the same lower tertile of Fetuin-A, CRP was significantly higher than in the second and third tertile. Therefore Fetuin-A and CRP were associated markers. The strict association between Fetuin-A and inflammatory and nutritional markers, and of these markers with calcification scores, suggests that there is an association between inflammation-malnutrition and the calcification process, probably through the known enhancement of atherogenesis.[25] Therefore, Fetuin-A could be involved in the process of calcification in two ways: in the case of markedly low levels, due to a lower prevention of calcium phosphate precipitation, and as a marker of inflammation, a well-known risk factor of atherogenesis. The association between coronary calcification score and the parameter EPO/Hb, an expression of relative resistance to erythropoietin, may suggest that the state of inflammation and malnutrition is a common factor of atherogenesis and erythropoietin resistance, as already proposed for the malnutrition, inflammation and atherosclerosis (MIA) syndrome.[19] In the patient cohort receiving intravenous calcitriol, the correlation found between the dose of calcitriol administration and the log transform of coronary calcification score suggests a possible risk effect of calcitriol treatment, which could be direct or mediated by increased serum calcium levels. However, the effect of calcitriol appears to be of marginal importance.

This study has intrinsic limitations as the result of its cross-sectional structure and the correlation analysis be-

Table 4. Clinical, biochemical data and calcification scores in male and female patients, not treated with i.v. calcitriol

	Male	Female	T-Test
n°	56	37	–
age, years	59,83 ± 12,29	61,06 ± 13,09	NS
HD age, months	59,51 ± 79,97	71,10 ± 73,50	NS
diabetes	6	5	–
hypertension	28	21	–
Kt/V	1,30 ± 0,39	1,37 ± 0,27	NS
BMI	24,62 ± 3,38	25,20 ± 7,70	NS
Score	1861,30 ± 2891,73	1916,64 ± 3083,06	NS
Scorelog	2,82 ± 0,82	2,40 ± 1,34	<0,05
Coronary Score	1039,34 ± 1235,83	1060,40 ± 2025,44	NS
Coronary Scorelog	2,6 ± 0,84	2,11 ± 1,27	0,01
Ca_s, mg/dL	9,53 ± 0,82	9,38 ± 0,87	<0,05
P_s, mg/dL	5,38 ± 1,44	5,65 ± 1,46	NS
CaxP	51,16 ± 13,67	53,10 ± 14,49	NS
iPTH, pg/mL	432,38 ± 480,44	502,86 ± 534,48	NS
Cholesterol, mg/dL	158,35 ± 40,13	172,39 ± 41,05	NS
HDL, mg/dL	40,67 ± 13,82	47,75 ± 15,05	<0,01
non-HDL Cholesterol, mg/dL	118,13 ± 41,76	120,09 ± 44,96	NS
LDL, mg/dL	74,31 ± 21,55	94,79 ± 29,71	<0,005
Triglycerides, mg/dL	208,94 ± 164,25	200,48 ± 137,27	NS
Hb, g/dL	11,77 ± 1,06	11,24 ± 1,17	<0,01
Fetuin-A, g/L	0,31 ± 0,10	0,32 ± 0,13	NS
Albumin, g/dL	4,14 ± 0,39	4,09 ± 0,34	NS
α1-acid glycoprotein, g/L	1,22 ± 0,35	1,17 ± 0,40	NS
prealbumin, g/L	0,40 ± 0,17	0,33 ± 0,09	<0,05
CRP, mg/dL	10,60 ± 25,97	9,05 ± 11,35	NS
PINI index	1,86 ± 6,20	1,78 ± 4,09	NS
Epo/Hb*	606,65 ± 582,40	692,74 ± 573,29	NS

* Epo, weekly units, U*dl/g Hb

Table 5. Factors affecting cardiac score (A), coronary score (log) (B) and Fetuin A. Multiple regression analysis

A, variables

Dependent	Independent	Beta	p	95% Confidence interval	
Coronary score	Triglycerides	11,18	<0,0001	7,98	14,38
	Age	84,58	<0,001	41,89	127,28
	PTH	1,73	<0,01	0,158	3,307
	non-HDL Cholesterol	−20,15	<0,005	−33,53	−6,77
	Fetuina-A	−6509,80	<0,003	−10515,8	−2485,9
excluded variables	Sex, CRP, Ca, P, prealbumin				
r^2 = 0,643					

B, variables

Dependent	Independent	Beta	p	95% Confidence interval	
Coronary score log	Age	0,04	<0,0001	0,024	0,063
	HD age	0,053	<0,003	0,002	0,009
	Triglycerides	0,003	<0,0001	0,001	0,004
	Fetuina-A	−3,11	<0,003	−5,14	−1,07
excluded variables	Sex, CRP, Ca, P, prealbumin, PTH				
r^2 = 0,380					

C, variables

Dependent	Independent	Beta	p	95% Confidence interval	
Fetuina-A	Prealbumin	0,366	<0,0001	0,201	0,532
	CaxP	−0,003	<0,03	−0,005	0,0001
	Kt/V	−0,156	<0,02	−0,288	−0,024
	Hb	0,027	<0,03	0,003	0,05
excluded variables	Sex, CRP, Ca, P, prealbumin, PTH, α1 acid-glycoprotein, HD Age				
r^2 = 0,412					

Due to missing data, the analysis was carried out on 92 subjects.

tween relatively stable parameters, such as calcification scores, and humoral biochemical parameters values deriving from serum samples obtained in a limited time period. A prospective study with monitoring of calcification scores and biochemical parameters could provide more reliable interrelationships.

In conclusion, the cardiac calcification process is a complex phenomenon with multiple risk factors, acting separately or in association, at different steps of the arterial wall degenerative process, from the atherogenetic development to the calcification of atherosclerotic plaques and media arterial wall. The role of Fetuin-A is probably not only linked to an active preventive action of calcium phosphate precipitation but also is a sign of the severity of the MIA syndrome. The role of Fetuin-A as a factor of insulin resistance and ensuing altered lipid profile, suggested by Mehrotra *et al.*,[26] deserves particular ad hoc evaluation.

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