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Serum Levels of Calcification Inhibition Proteins and Coronary Artery Calcium Score: Comparison between Transplantation and Dialysis

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Key Words
Fetuin · Matrix Gla protein · Osteoprotegerin · Coronary artery calcification · Kidney transplant · Dialysis

Abstract
Vascular calcifications in CKD are now linked to serum alterations of both divalent ions and calcification inhibitory proteins. Due to possible biochemical differences between dialysis (D) and transplantation (Tx), we examined the entity and severity of these biochemical modifications and of coronary artery calcium score separately in these two populations. We assayed, besides standard markers of inflammation, divalent ions and serum levels of fetuin, matrix Gla protein (MGP) and osteoprotegerin (OPG), in 51 Tx patients (age 45 ± 12 years; 30 males, 21 females; previous D duration 4.8 ± 4.2 years; Tx since 6.6 ± 5.5 years; Cr 1.8 ± 0.6 mg/dl) and in 49 D patients (age 49 ± 14 years; 30 males,19 females; D duration 5.6 ± 4.8 years). Additionally, coronary calcium score (AS) was evaluated by cardiac multi-slice CT. Compared with D patients, Tx patients had better values of divalent ions and inflammation markers, and lower prevalence (65 vs. 86%; $p < 0.02$) and severity (AS = 570 ± 1,637 vs. 1,311 ± 3,128; $p < 0.008$) of coronary calcification. In addition, a tendency toward normalization for all of the three calcification inhibitory proteins was evident. In both Tx and D, AS correlated with age and OPG (Tx: $r_s = 0.439$, $p < 0.001$, and $r_s = 0.510$, $p < 0.0001$; D: $r_s = 0.471$, $p < 0.001$, and $r_s = 0.403$, $p < 0.005$, respectively); in D patients, a correlation was present also with D duration ($r_s = 0.435$; $p < 0.002$), other markers of inflammation and, notably, fetuin ($r_s = -0.442$; $p < 0.002$). Regression analysis selected previous time on D in Tx patients ($r_m = 0.400$; $p < 0.004$), and C-reactive protein and OPG in D patients ($r_m = 0.518$; $p < 0.004$) as the most predictive parameters of AS. Discriminant analysis confirmed the major role of age and D duration in the appearance of AS and evidenced male gender as a distinct risk condition. At variance, Tx duration was never associated with AS. In conclusion, as compared to D, renal Tx patients show serum levels of calcification inhibition proteins and of divalent ions closer to normal. As this is associated with a lower prevalence and severity of AS, it is suggested that Tx antagonize the accelerating role of D in the progression of vascular calcification. Assessment of both coronary calcifications and serum levels of calcification inhibitory proteins may be of value to identify those subjects at higher risk of development and progression of vascular lesions, among whom males have the highest rate.

Introduction

Derangements of mineral metabolism [1] and vascular calcifications [2] have been recognized as relevant pathogenetic factors for reduced life expectancy of dialysis (D)

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patients [3]. In fact, increased serum levels of phosphate [4], calcium [5] and both of them synergistically [6, 7] are capable of inducing phenotypic modifications of vascular smooth muscle cells into osteoblast-like cells, which in turn support an ossification-like process in the arteries [8]. Besides divalent ions, the so-called calcification inhibition proteins seem to be involved as well [9], and in particular fetuin, osteoprotegerin (OPG) and matrix Gla protein (MGP), now assayable in circulating plasma, are of potential clinical interest. In fact, currently accepted opinions [8, 9] suggest that, in uremia, vascular calcifications may result from both an imbalance of divalent ions and a deficit of inhibitory proteins, eventually leading to the abovementioned phenotypic change and to calcium deposition.

From a clinical point of view, the possibility of halting or reverting this process by means of any therapeutic effort capable of modifying the underlying biochemical derangements is definitely exciting. Actually, clinical trials in D populations indicate that when a reduction of serum phosphate is achieved by increasing the duration of D [10], or by administering a phosphate binder that does not increase calcium balance [11, 12], it is indeed possible to hinder, but not to halt or revert, coronary calcifications. However, in this case, therapy is focused on balancing divalent ions only, while serum levels of inhibitory proteins are probably not affected.

A clinical condition characterized by a definite improvement of the uremic milieu and, thereby, with a likely correction of the abovementioned derangements, is renal transplantation (Tx). It is well known that in this situation significant changes occur in divalent ions, and that, in particular, serum phosphate levels reach normal or even hypophosphatemic values. Much less is known about changes of proteins involved in vascular calcifications, although recently Moe et al. [13] reported a tendency toward normalization for serum levels of fetuin, MGP and OPG. As a matter of fact, transplanted patients have a mortality rate significantly lower than those in D, although still higher than normal [3]. Of note, pre-existing vascular calcifications have been reported to be an independent risk factor of mortality in Tx [14].

Considering the few available reports in the literature, it seemed reasonable to us to evaluate whether renal Tx has a positive effect on serum levels of fetuin, MGP and OPG, and whether this possible outcome, being associated with an improved control of divalent ions, affects the prevalence, severity or correlations of coronary artery calcium score with calcification inhibitory proteins.

Materials and Methods

Subjects

We evaluated a total of 51 renal Tx patients followed in our outpatient unit, who had been grafted since at least 6 months, were in a stable clinical condition and were treated with any type of immunosuppressive regimen. Specifically, 25 patients received triple therapy, 22 patients double therapy and 4 patients monotherapy. Causes of renal failure included glomerulonephritis in 22, tubulointerstitial nephritis in 11, nephroangiosclerosis in 2, renal hypoplasia in 1 and unknown in 10. A fasting blood sample was obtained to determine indexes of renal function (serum creatinine), divalent ion balance (serum total calcium, phosphate, parathyroid hormone and bone alkaline phosphatase (BALP), inflammation (erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), albumin and fibrinogen) and inhibitors of calcification fetuin, MGP and OPG. Additionally, all patients accepted to undergo cardiac multi-slice computed tomography (CT) for the assessment of coronary artery calcium content (CAC).

As a control group, we carefully selected from our outpatient unit 49 D patients who had been referred to receive a pretransplant evaluation. The two principal enrolment criteria were the presence of a favorable clinical evaluation leading to inclusion in the waiting list and no history of previous failed Tx. Then, cases were selected in order to obtain a similar number of patients in the different decades of age and gender classes. Finally, time on D and prevalence of diabetes, cardiovascular disease and previous parathyroidectomy were considered. In particular, cardiovascular disease was intended as a positive anamnestic record for stroke, myocardial infarction or surgery for coronary or peripheral ischemia. Causes of renal insufficiency in these patients were glomerulonephritis in 25, tubulointerstitial nephritis in 7, nephroangiosclerosis in 3 and unknown in 14. They received the same biochemical and radiological evaluation as transplanted patients.

To determine our reference values, we obtained a fasting blood sample from 50 normal age- and sex-matched subjects (age 47 $\pm$ 10, range 24–66 years; 30 males, 20 females) for the assay of serum levels of fetuin, MGP and OPG.

From each subject involved, an informed consent to the study was obtained.

Assays

Serum levels of creatinine (kinetic alkaline picrate method), albumin (bromcresol purple method), Ca (cresolphthalein complexone method), P (ammonium molybdate method), CRP (immunoturbidimetric with specific antibody, Abbott; n.v. <0.9 mg/dl) and fibrinogen (turbidimetric, Dade-Behring; n.v. 200–400 mg/dl) were assayed by standard, automatic techniques with a Technicon RA-500 analyzer. ESR was evaluated with standard heparin prefilled tubes (Westergren method; n.v. <20 mm/h). PTH was assayed by an immunoradiometric technique, based on a double antibody against the intact molecule; our normal values were 10–55 pg/ml, with intra- and interassay variations of 6.5 and 9.8%, respectively. BALP was determined by an immunoassay utilizing a monoclonal antibody (Metra Biosystem, USA), with normal range between 12 and 43 U/l.

Serum fetuin was assayed with a two-site sandwich ELISA technique (EDI Human ELISA kit, EPITOPE Diagnostics, Inc., USA) employing two selected goat anti-human fetuin-A polyclonal antibodies, binding to different epitopes of human fetuin-

Table 1. Clinical and biochemical parameters in the population studied

	Tx (n = 51)	D (n = 49)	
Sex, M/F	30/21	30/19	n.s.
Age, years	45.5 ± 12	49.5 ± 14	n.s.
Transplantation duration, years	6.5 ± 5.5	–	
Dialysis duration, years	4.8 ± 4.3	5.6 ± 4.8	n.s.
Diabetes	7/51	7/49	n.s
CVD	6/51	6/49	n.s
Parathyroidectomy	6/51	5/49	n.s.
Ca, mg/dl	9.8 ± 0.9	9.4 ± 0.8	0.02
P, mg/dl	3.3 ± 0.8	5.4 ± 1.7	0.0001
BALP, U/l	32 ± 21	178 ± 134	0.0001
PTH, pg/ml	165 ± 165	416 ± 385	0.0001
Albumin, g/l	4.2 ± 0.4	4.0 ± 0.5	0.05
ESR, mm/h	21 ± 18	36 ± 26	0.001
CRP, mg/dl	0.49 ± 0.74	2.2 ± 4.5	0.007
Serum fibrinogen, mg/dl	353 ± 75	406 ± 128	0.01
AS score	570 ± 1,637	1,311 ± 3,128	0.008
Volume score	498 ± 1,360	1,126 ± 2,535	0.005

CVD = Positive anamnestic record for myocardial infarction, stroke, or vascular ischemia requiring surgery.

A. The lower limit of detection is 0.025 g/l, with intra- and inter-assay coefficient of variations of 5.5 and 6.25%, respectively.

Serum MGP was assayed with competitive ELISA, where a biotinylated synthetic MGP competes with sample MGP for the binding to a monoclonal anti-human MGP coated in microtiter strips (Biomedica Gruppe, Wien). The lower limit of detection is 0.3 nmol/l, with intra- and inter-assay coefficients of variation of 5.5 and 8%, respectively.

Serum OPG was assayed with a sandwich ELISA (Biomedica Gruppe, Wien) which uses two polyclonal antibodies binding to two different epitopes of the molecule. The lower limit of detection is 0.14 pmol/l, with intra- and interassay variations of 6.0 and 7.2%, respectively.

Our normal values for the three proteins with calcification inhibition properties are reported in the 'Results' section.

Multi-Slice CT

All patients were scanned using a multi-slice CT (Somatom Volume Zoom, Siemens, Erlangen, Germany) with the following scanning parameters: 4 × 2.5 mm collimation; slice thickness 3 mm; slice increment 1.5 mm; 120 kV, 100 mAs, 0.5 s gantry rotation time and prospectively ECG triggering at 70% of R-R interval. The entire heart was covered in a single breathhold (15–20 s). The mean effective dose was for both male and female patients <1.7 mSv. All images from multi-slice CT were transferred to a dedicated workstation (Vitrea 3.2, Vital Images, Minn., USA) and Agatston (AS) and volume (VS) scores were calculated. Using dedicated cardiac software, all pixels with density >130 H were automatically highlighted in color on the images. The radiologist assigned one out of five locations to each calcified plaque: left main, left anterior descending, circumflex, right coronary artery or posterior descending artery. According to the Agatston method, as previously reported, we defined the regions of interest by vessel and slice with the threshold option for pixels >130 H to measure the area and peak density of plaques. Depending on the peak density of the plaque, an area of at least 0.52 mm^2 (2 pixels) was multiplied by one of the following cofactors: 1 for 130–199 H, 2 for 200–299 H, 3 for 300–399 H, 4 for densities >400 H. The total CAC score was calculated as the sum of the individual lesion scores in all coronary arteries, with the two specific equations [15] for AS and VS.

Statistics

Statistic evaluation was performed with dedicated software (SPSS 13.0). Besides descriptive results, the relationships of demographic data, laboratory values and calcification scores were searched for by parametric (Pearson, r) or nonparametric (Spearman, r_s) correlation coefficients, as appropriate. Differences between the two groups were analyzed by the Student t or Mann-Whitney U tests, while differences between multiple groups were analyzed by parametric or nonparametric (Kruskal-Wallis) ANOVA. Multiple stepwise regression analysis and discriminant analysis were finally applied to search for main determinants of coronary calcium score. Data are expressed as mean ± SD.

Results

Mean values of clinical and biochemical parameters observed in the two populations are reported in table 1. Transplanted patients (30 males, 21 females; age 45.5 ± 12 years), who had been grafted since 6.5 ± 5.5 years and had experienced 4.8 ± 4.3 years of maintenance D, had a serum creatinine of 1.8 ± 0.6 mg/dl, with an estimated

Table 2. Comparison of serum levels of fetuin, MGP and OPG in Tx patients, D patients and normal controls

	Tx (n = 51)	D (n = 49)	Controls (n = 50)	ANOVA
Fetuin, g/l	0.49 ± 0.14[a]	0.42 ± 0.13[b]	0.57 ± 0.1[c]	p < 0.0001
MGP, nmol/l	6.5 ± 2.8[a]	5.1 ± 2.2	6.0 ± 3.3	p < 0.04
OPG, pmol/l	3.8 ± 1.5[a]	11.5 ± 7.1[b]	2.3 ± 0.6	p < 0.0001

Bonferroni's test: [a] $p < 0.05$ vs. D; [b] $p < 0.05$ vs. N; [c] $p < 0.05$ vs. Tx. Values are expressed as mean ± SD.

Table 3. Nonparametric correlation coefficients of main clinical and biochemical parameters with AS in Tx and D patients

	AS score			
	Tx		D	
Age	0.439	p < 0.001	0.471	p < 0.001
D duration	0.396[a]	p < 0.004	0.435	p < 0.002
Transplant duration		n.s.		NA
OPG	0.510	p < 0.0001	0.403	p < 0.001
Fetuin		n.s.	–0.442	p < 0.002
MGP		n.s.		n.s.
CRP		n.s.	0.344	p < 0.01
ESR		n.s.	0.323	p < 0.02
Albumin		n.s.	–0.311	p < 0.001

NA = Not available.
[a] Pearson's correlation coefficient (see text for explanation).

clearance (Cockroft and Gault formula) of 48.3 ± 17.6 ml/min (range 18–93). Prevalence of diabetes, CV disease and parathyroidectomy were 13.7, 11.7 and 11.7%, respectively. Total calcium, phosphate and BALP values were all within normal ranges (9.8 ± 0.9, 3.3 ± 0.9 mg/dl and 32 ± 21 U/l, respectively), despite increased levels of intact PTH (165 ± 165 pg/ml). Similarly, within normal ranges were serum albumin (4.2 ± 0.4 g/l), CRP (0.49 ± 0.73 g/l) and fibrinogen (353 ± 75 mg/dl), while ESR was slightly increased (21 ± 18 mm/h). As for CAC, 18/51 patients (35%) scored zero, but mean values for the whole population were definitely high: AS = 570 ± 1,637 (range 0–9,075); VS = 498 ± 1,360 (range 0–7,557).

Compared to the Tx group, D patients were not statistically different regarding gender (30 males, 19 females), age (49.5 ± 14 years), time on D (5.6 ± 4.8 years) and prevalence of diabetes (14.2%), cardiovascular disease (12.2%) and parathyroidectomy (10.2%; table 1). On average, they had slightly lower values of calcium (9.4 ± 0.8 mg/dl, $p < 0.02$), but definitely higher values of phosphate (5.4 ± 1.7 mg/dl; $p < 0.0001$), BALP (178 ± 134 U/l; $p < 0.0001$) and PTH (416 ± 385 pg/ml; $p < 0.0001$). Indexes of inflammation were significantly different as well, with lower values for albumin (4.0 ± 0.5 g/l; $p < 0.05$) and higher for ESR (36 ± 26 mm/h; $p < 0.001$); CRP (2.2 ± 4.5 g/l; $p < 0.007$) and fibrinogen (406 ± 128 mg/dl; $p < 0.01$). As for the prevalence of coronary calcifications, only 7/49 had zero score (14.2%; $\chi^2 = 4.8$; $p < 0.02$, compared to Tx), with significantly higher mean values of both AS (1,311 ± 3,128; $p < 0.008$) and VS (1,126 ± 2,535; $p < 0.005$).

Values of calcification inhibition proteins, as shown in table 2, were different between the three groups considered (patients and normal subjects). Post-hoc tests indicate that in transplanted patients serum levels of fetuin were higher than in D patients (0.49 ± 0.14 vs. 0.42 ± 0.13 g/l; $p < 0.05$), but still lower than in normal subjects (0.57 ± 0.1 g/l; $p < 0.05$). Likewise, MGP values in transplanted patients were higher than in D patients (6.5 ± 2.8 vs. 5.1 ± 2.2 nmol/l; $p < 0.05$) but not different from those in normal subjects (6.0 ± 3.3 nmol/l; p = n.s.) who had, peculiarly, values not different from D. Serum levels of OPG in Tx patients were definitely lower than those in D (3.8 ± 1.5 vs.11.5 ± 7.1 pmol/l; $p < 0.05$) and very close to those in normal subjects (2.3 ± 0.6 pmol/l; p = n.s.).

Correlation matrix analysis evidenced a very high reciprocal r value (0.999) between VS and AS, suggesting a roughly identical diagnostic importance (corresponding value for $r_s = 0.994$); therefore, considering that published data in CRF mostly employ AS, we selected only this parameter for further discussion. Table 3 shows the correlation coefficients between AS and main clinical and humoral parameters separately in the two groups. In transplanted patients, AS was positively related to age ($r_s = 0.439$; $p < 0.001$) and OPG ($r_s = 0.510$; $p < 0.0001$, fig. 1a), while in D it was similarly related to age ($r_s = 0.471$; $p < 0.001$) and OPG ($r_s = 0.403$; $p < 0.005$, fig. 1b), but also to D duration ($r_s = 0.435$; $p < 0.002$), CRP ($r_s = 0.344$; $p < 0.01$) and ESR ($r_s = 0.323$; $p < 0.02$), and negatively to

albumin (r_s = −0.311; $p < 0.001$) and fetuin (r_s = −0.442; $p < 0.002$). The correlation between AS and D duration in transplanted patients, although positive (r_s = 0.207) as in D, did not reach the level of significance (p = 0.14); a statistical correlation was nonetheless observed with the parametric test (r = 0.396; $p < 0.004$).

Multiple stepwise regression analysis aimed at selecting which of the parameters was mostly predictive of AS chose only D duration in transplanted patients (r_m = 0.400; $p < 0.004$), and CRP and OPG in D (r_m = 0.518; $p < 0.004$).

Due to the relatively low grade of correlations obtained (r values always <0.5) and with the aim of better describing the relative role played by some clinical parameters like age and sex, and, most importantly, by D or transplant duration on the occurrence of significant vascular calcifications, we also performed a discriminant analysis in which only two classes of AS were considered: values up to 10, which according to Rumberger et al. [16] are associated with minimal plaque burden and low CV risk, and values over 10. Table 4 shows that age older than 45 (OR 12.66; $p < 0.0001$), male sex (OR 10.5; $p < 0.0001$) and D duration >3 years (OR 5.6; $p < 0.004$) are each independently associated with a definitely greater risk of having an AS >10. In contrast, transplant duration of more or less than 5 years does not seem to play a role, while comparison between D and Tx (OR 3.4; $p < 0.06$) was very close to significance to indicate the latter condition as protective from the risk of having a significant CAC.

The relevant and somehow unpredicted role suggested for sex by this test, prompted us to compare mean values of AS and of fetuin, MGP and OPG separately in males and females, respectively in D and Tx. The four groups so far obtained, although not different as for age and D duration (table 5), showed remarkably different values of AS (ANOVA $p < 0.00001$; fig. 2), with male D patients (AS = 1,944 ± 3,760) and transplanted female patients (AS = 35 ± 121) being the most and the least severely affected, respectively (post-hoc test, $p < 0.0001$). Significant differences in AS values were also evident between males and females in both D ($p < 0.006$) and transplanted patients ($p < 0.0001$) and between D and Tx females ($p < 0.009$). Also serum levels of calcification inhibitory proteins were different between the four groups ($p < 0.03$ for fetuin; $p < 0.04$ for MGP; $p < 0.0001$ for OPG; table 5), with male D patients having significantly lower values of fetuin (0.41 ± 0.13) and higher values of OPG (11.6 ± 7.3) as compared to transplanted females (fetuin 0.53 ± 0.14, $p < 0.02$; OPG 3.4 ± 1.6, $p < 0.0001$).

Discussion

Increments of serum levels of phosphate and calcium are definitely affirmed factors of increased mortality in uremia [1], with the most convincing evidence available for phosphate, of which minimal increments have been

Table 4. Relative risk of having an AS value >10 according to sex and classes of age and D or transplant duration

	Odds ratio	95% CI	p
Age (> vs. <45 years)	12.6	3.6–44.5	<0.0001
Sex (M vs. F)	10.5	3.2–34.4	<0.0001
D (> vs. <3 years)	5.6	1.7–18.2	<0.004
Transplant (> vs. <5 years)	2.2	0.5–9.1	n.s.
D vs. transplant	3.4	0.91–12.5	<0.06

Table 5. Comparison of clinical parameters, AS and calcification inhibition proteins between male and female patients in D or Tx

	Male-D (n = 30)	Male-Tx (n = 30)	Female-D (n = 19)	Female-Tx (n = 21)	p
Age, years	49.7 ± 14.4	46.7 ± 12.5	51.4 ± 14	43.8 ± 12.3	n.s.
D, years	6.0 ± 5.2	5.5 ± 5.1	4.8 ± 4.1	3.7 ± 2.2	n.s.
AS	1,944 ± 3,760[a]	945 ± 2,064[b]	157 ± 217[c]	35 ± 121	<0.02
Fetuin, g/l	0.41 ± 0.13[d]	0.46 ± 0.13	0.45 ± 0.14	0.53 ± 0.14	<0.03
MGP, nmol/l	4.9 ± 2.2	6.5 ± 3.1	5.6 ± 2.2	6.7 ± 2.2	<0.04
OPG, pmol/l	11.6 ± 7.3[e]	4.1 ± 1.4	11.3 ± 6.9[e]	3.4 ± 1.6	<0.0001

Kruskal-Wallis: [a] $p < 0.006$ vs. F-D and F-Tx; [b] $p < 0.0001$ vs. F-Tx; [c] $p < 0.009$ vs. F-Tx.
Bonferroni's test: [d] $p < 0.02$ vs. F-Tx; [e] $p < 0.0001$ vs. M-Tx and F-Tx.

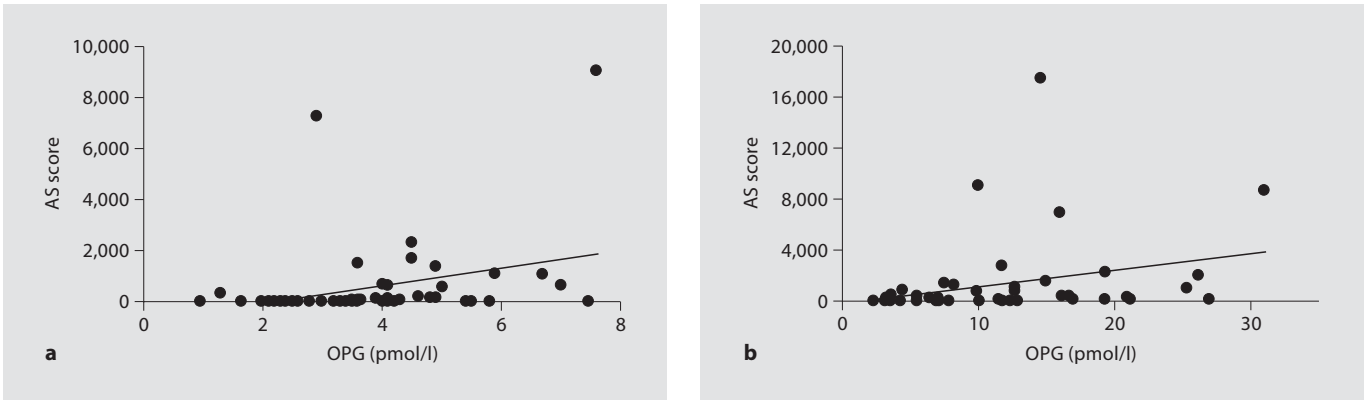

Fig. 1. Scatter plots of OPG and AS values in transplanted (**a**; r = 0.51, p < 0.0001) and D (**b**; r = 0.40, p < 0.001) patients.

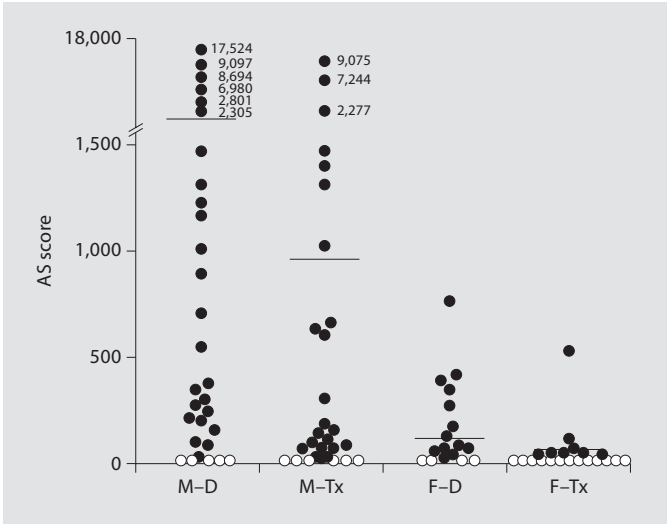

Fig. 2. Comparison of AS values between males (M) and females (F), in D and Tx. ○ = Cases with AS <10; ● = cases with AS >10. See table 5 for statistical differences.

reputed harmful even in early CRF [17] and in subjects with normal renal function [18]. Phosphate and calcium seem to influence mortality through the induction of vascular calcification, which is especially severe and growing at coronary level in D patients, as highlighted by cardiac electron beam CT (EBCT), [19] or multi-slice CT [20]. It is well known that renal Tx patients experience a significant improvement in serum levels of divalent ions which could favorably affect the occurrence of CAC; nonetheless, available data in the literature are very limited [21] and suggest no significant effect.

Compared to D patients, our Tx patients had improved values of divalent ions and PTH serum levels. Additionally, a lower prevalence and severity of coronary calcifications were evident. Time on D, a reputed key risk factor for vascular calcification progression, was not different between the two populations, but Tx patients can be considered to have a longer CKD duration (same period on D plus posttransplant follow-up). Therefore, the degree of CAC should be worse or in the best hypothesis not different from that in D. Accordingly, we suggest that in Tx there is no further increment of the calcification rate typical of D, and theoretically the hypothesis of a possible vascular disease regression could be raised. In agreement with these suggestions are also the differences we observed between D and Tx patients in serum levels of calcification inhibitory proteins. In fact, as for fetuin, we know that its serum levels are responsible for approximately 50% of the calcification inhibitory capacity of normal human plasma [22], that knockout animals become prone to calcifications [23], and that uremic patients in the lowest quartiles of serum levels are characterized by the highest mortality rate [24]. Accordingly, higher values are expected to be protective for calcifications. In our study, Tx patients had serum levels of fetuin significantly increased as compared to D, even though still lower than normal. This finding is in keeping with the data by Moe et al. [13] reporting a similar increment of serum fetuin in a small sample of patients assayed before and after surgery. A possible explanation for this result could be the presence of a lower degree of inflammation in Tx. As a matter of fact, all biochemical markers of flogosis assayed in our study appear to support such a possibility.

At variance with systematically acting fetuin, MGP puts forth its powerful inhibitory action at tissue level, after vitamin K-dependent γ-carboxylation activation [25], and similarly to fetuin, the MGP-null mice are characterized by vessel calcification [26]. Due to the local action and the possible discrepancy between carboxylated (active) and undercarboxylated (inactive) forms (both assayed with the available commercial kits), the diagnostic value of circulating levels of MGP is still unsettled. In fact, in subjects with suspected coronary artery disease and normal renal function serum MGP levels are inversely correlated with the severity of CAC detected by EBCT [27], but patients in D may have MGP values higher than normal [28]. In our study, Tx patients had serum MGP levels not different from normal and significantly higher than those in D, but values in normal subjects were not statistically higher than in D. Other data in CKD describe an increment in the same range as we observed, from very low values during D, after successful Tx [13]. As a whole, despite some possible discrepancies with other reports in the literature, the increased values of MGP in our transplanted patients fit well with the lower severity of coronary calcifications, as compared to D.

In more recent years, also OPG has been mustered into the cardiovascular disease. In fact, OPG-null mice, besides osteoporosis resulting from excessive osteoclast activity, develop arterial calcifications of the elastic vessels [29], and OPG and its mRNA have been detected in vitro in calcified but not in normal human arteries [30]. Therefore, similarly to MGP, OPG is expected to exert its action at tissue level. As for its serum levels, clinical data from nonuremic subjects indicate a positive association with the presence and severity of coronary disease [31], as well as with progressive atherosclerosis [32]. In renal patients, increased levels of OPG are associated with all-cause and cardiovascular mortality in both D [33] and Tx [34]. This increment of serum OPG along with increased CV risk seems contradictory to the calcification inhibitory properties of this protein. However, increased OPG may result from enhanced synthesis in vessel walls by activated osteoblast-like cells, committed to calcification. In our study, differences in serum levels of OPG were unequivocal, with no difference between normal and Tx patients, and increased values (three- to fourfold) in D. While this clean difference could be referred in part to renal clearance [35], or to other reputed factors like age [36], bone turnover [37, 38], and sex hormones [39], the positive correlation with vascular calcification observed in our study confirms available data in subjects in D or with normal renal function [31, 40–42].

As a whole, our results on calcification inhibitory proteins suggest that in Tx a tendency towards normalization exists for all the three proteins evaluated. These changes, associated with an improved control of divalent ions, fit well with the observed reduced prevalence and severity of CAC, as compared to D.

Correlation matrix analysis in our study indicates that AS increases along with age and OPG levels in both D and Tx patients. Similar findings have already been reported in normal [43, 44] and D subjects [13, 40, 45], but are original in Tx. In our study, AS correlated also with several markers of inflammation, including a negative correlation with albumin and, notably, fetuin, but only in D. In agreement, regression analysis selected CRP and OPG as the two most powerful predictors of AS in this group of patients. Notably, in our transplanted patients, with a lower degree of chronic inflammation, these markers were not associated with AS. Our study confirms, especially in D, the significant correlation of AS with the duration of renal replacement therapy. In Tx patients, this relationship was evidenced only by the less accurate parametric test; nonetheless, regression analysis indicated previous D duration as the most powerful predictive factor of AS. Remarkably, no correlation was present between AS and transplant duration, suggesting that Tx does not increase the calcification rate typical for D patients.

The relevant and independent role played by D duration in AS was substantiated also by discriminant analysis. In fact, according to this test, a D time of more than 3 years increases five- to sixfold the risk of having a pathologic AS. In addition, transplant duration was not associated with increased calcification, and direct comparison between the two clinical situations was very close to statistical significance to indicate D as the unfavorable condition. Accordingly, the potential for Tx condition or duration as a pathogenetic factor for increased rate of coronary calcification does not seem probable.

Discriminant analysis also evidenced that male subjects have a tenfold higher risk of coronary calcification compared with females, with strikingly different values of AS observed in the four groups obtained according to sex and therapy. Actually, the influence of gender on cardiac calcifications has already been reported both in subjects undergoing EBCT for clinical requirements [43, 44] and in an unselected population of D patients [45]. However, patients in our study are definitely younger than those reported in the former studies, allowing us to better recognize the role played by other clinical and humoral parameters, independent of age. In fact, the last remark

in our study is the finding of significantly different levels of the calcification inhibition proteins in the four groups obtained according to gender and therapy. In particular, differences in serum levels of fetuin and OPG between cases with highest or lowest AS values seem convincing and suggestive of a potential diagnostic value.

In conclusion, our study suggest a favorable effect of renal Tx on serum levels of calcification inhibition proteins. The more favorable humoral condition is associated with a lower prevalence and severity of coronary calcification that could well represent a possible explanation for the reduced mortality rate in these patients, as compared to D. Although we cannot affirm that Tx can reverse coronary calcifications, our data allow to hypothesize that it may antagonize the accelerating role played by D in the progression of vascular calcification. While prospective studies are necessary to confirm this hypothesis, assessment of both CAC and serum levels of calcification inhibitory proteins may be useful to identify those subjects at increased risk for development and progression of vascular calcifications.

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