Evolution of coronary artery calcification in patients with chronic kidney disease Stages 3 and 4, with and without diabetes

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Abstract

Background. The purpose of this study was to report the evolution of coronary artery calcification (CAC) in subjects with chronic kidney disease Stages 3 and 4 comparing those with and without diabetes. We previously reported prevalence in the same population.

Methods. CAC was measured using multi-slice computer tomography. We prospectively followed up 103 patients for 2 years, 49 with diabetes and 54 without diabetes. Demographic, routine biochemistry, calcification inhibitors and bone mineral density data were collected and analysed. Evolution of CAC was defined as those with a difference of ≥2.5 U between baseline and final square root CAC scores.

Results. There were more progressors in the group with diabetes, 24 compared to 12 in the group without diabetes (P = 0.004). When diabetes was present, CAC progressed equally in men and women. Risk factors for evolution of CAC included age, baseline CAC score and serum phosphate levels. Baseline CAC score, phosphate and body mass index were independent predictors for the increase of CAC score during the study period. Severity of CAC was greater in the diabetes group (median CAC score at baseline in the group with diabetes 154 increased to 258 2 years later, P < 0.001).

Conclusions. Evolution of CAC is greater in older patients and those with diabetes, where the gender advantage of being female is lost. Serum phosphate level, despite being within the normal range and virtually no use of phosphate binders, was also a risk factor. Further studies are required to determine the levels of serum phosphate required to minimize cardiovascular risk.

Keywords: bone mineral density; fetuin-a; osteoprotegerin; phosphate; progression; vascular calcification

Introduction

We have previously published that coronary artery calcification (CAC) is common in patients with chronic kidney disease (CKD) Stages 3 and 4, particularly in subjects with diabetes where it affects men and women to the same degree [1]. In this study, we report the evolution of CAC and factors that may influence this in the same group of patients.

Vascular calcification is a common complication of CKD in dialysis patients and CKD is a significant
risk factor for cardiovascular disease (CVD) [2]. Electron beam computer tomography and multi-slice computer tomography are the most accurate techniques available for detecting CAC and quantification. Recent data demonstrate excellent correlation between the two techniques [3, 4]. The severity of CAC may have prognostic significance and has been linked to all-cause mortality [5].

The high prevalence and rapid evolution of vascular calcification in dialysis populations compared to the general population is well recognized [6]. Evolution of CAC in asymptomatic subjects without renal disease has been reported [7]. However, little is published on the evolution of CAC in CKD patients not on dialysis. Mehrotra et al. [8] reviewed a summary of published data where nearly all reports were on end-stage renal disease (ESRD) subjects. In non-dialysis patients, Bursztyn et al. [9] reported a 2-fold increase in CAC in subjects with a creatinine clearance of <60 ml/min compared to ≥60 ml/min, but this was a small study of 53 CKD subjects. Provisional data on another group of 50 subjects from the Renal Research Institute (RRI)-CKD study (Dellegrottaglie S, Saran R, Gillespie B et al.) suggest a high rate of progression (unpublished results). Russo et al. [10] has recently reported progression of CAC in normal renal function subjects compared to CKD Stages 3–5 subjects but excluded subjects with diabetes. Sigrist et al. [11] recently reported CAC progression in dialysis patients, and the study also included a small group of CKD Stage 4 subjects (n = 16).

The aims of this study were to determine evolution of CAC in patients with CKD Stages 3 and 4, with and without diabetes and without symptoms of ischemic heart disease and to assess what factors play a role in this.

Subjects and methods

We have previously reported a cross-sectional study in both male and female CKD Stages 3 and 4 subjects aged between 18 and 65 years, n = 112 [1]. Two groups were recruited according to diabetes status. This study reports a 2-year prospective follow-up of the same group of patients, from a single centre, namely Nottingham University Hospitals, Transplant and Renal Unit. At 2 years, a detailed history and physical examination was reperformed on all participants. All patients had previously given written informed consent, and the local Ethics and Research and Development Committees approved the study protocol. All investigations were performed as previously reported, on the day of the CT scan and included routine biochemistry testing on a single fasting serum sample, collected in the morning and included intact parathyroid hormone levels (iPTH), lipid profile [total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein cholesterol (HDL-C) levels, triglycerides], calcium (adjusted for albumin), phosphate, bicarbonate, albumin and glycated haemoglobin (diabetes subjects only). Urinary protein to creatinine ratio was also determined. The four variable Modification of Diet in Renal Disease (MDRD) equation was used for the estimation of glomerular filtration rate (eGFR). Vitamin D insufficiency (defined as serum 25-hydroxy-vitamin D 25OHD levels ≤75 nmol/l) was treated with ergocalciferol for 6 months according to a defined protocol, and elevated phosphate levels (defined as >1.5 mmol/l) were treated with dietary advice and if no correction, then with phosphate binders. To examine the role of calcification inhibitors, we also measured with enzyme-linked immunosorbent assay kits: fetuin-A (Epitope Diagnostics, San Diego, CA), osteoprotegerin (OPG; Biovendor, Heidelberg, Germany), soluble receptor activator of nuclear factor-κB ligand (Biomedica Medizinprodukte, Wien, Austria) and matrix-gla protein (Biomedica Medizinprodukte) serum levels. In addition, bone mineral density (BMD) was measured at the left femur and radius by dual energy X-ray absorptometry using a Lunar Prodigy densitometer (General Electric Medicine Inc., Bucks, UK), in order to evaluate its relationship with CAC.

Measurement of CAC

CAC was measured using multi-slice CT scanning of the thorax on a GE (General Electric) Medical Systems Lightspeed 16 scanner, as previously described [1], the same scanner as in the previously reported study [10]. A single radiologist, blinded to the patients’ clinical status, reported all the scans. Median inter-scan variability has been reported as 8–10% for the Agatston scoring system [13–15].

Definition of evolution

Evaluating changes in CAC over time, we have to consider inter-observer and inter-scan reproducibility that both can bias the readings and result in wrong estimates of rates and risk factors associated with evolution [16, 17]. Therefore, the method for determining evolution needs careful consideration.

To reduce the potential variability in CAC scoring that is related to the reviewers of the CT scans, a single experienced radiologist (the same as in the previously reported study) performed all the readings. However, even intra-reviewer agreement can also present a problem, and this is another limitation that we have to take in account in the evaluation of the results [16]. To reduce the inter-scan variability, we used the same equipment, the same highly experienced technician, and we defined evolution using the method of Hokanson et al. [17], where CAC scores are square root transformed to eliminate residual scan variability. Two categories of subjects were used ‘progressors’ versus ‘non-progressors’. ‘Progressors’ were defined as those with a difference of ≥2.5 U between baseline and final square root CAC scores. Those with a difference of <2.5 U between baseline and final square root CAC scores were considered to be within the error of estimation and were considered ‘non-progressors’. Many report annualized percent change but this can present a problem, as it is highly influenced by small baseline CAC scores, for which a small increase in follow-up CAC may result in a huge percentage increase. Because most reports have used this definition, we also examined our data using annualized percent change (>33% per annum = progressor).

Statistics

All data are presented as mean ± standard deviation (SD), medians with (minimum and maximum values) and proportions as appropriate. Paired comparisons were performed using paired Student’s t-test for continuous variables and McNemar test for binary variables. For comparisons between independent groups, Student’s unpaired t-test was used for continuous variables and chi-square test for binary variables. The presence or absence of CAC was analysed as a discrete trait. Bivariate correlations were performed using Pearson’s product moment correlation analysis. Significant variables identified by univariate analysis were entered into binary logistic regression analyses models (to identify variables associated with the incidence or the evolution of CAC) and multivariate linear regression analyses models (to identify variables associated with the increase or the extent of CAC). Significant multico-linearity was identified between diabetes and body mass index (BMI), total cholesterol and 25OH vitamin D level, age and BMI and phosphorus and 25OH Vitamin D level. This was corrected for in the analyses. Odds ratio and 95% confidence interval are reported for logistic regression (goodness of fit evaluated by Hosmer and Lemeshow test). Adjusted R² is reported for multivariate linear analysis (model evaluated by inspecting the normal P–P plot of the regression standardized residuals). A significant difference is assumed with P < 0.05 for all the analyses. All analyses were performed using SPSS for windows version 16 (SPSS Inc, Chicago, IL).

Results

In the original study, 112 CKD Stages 3 and 4 patients were reported, 54 and 58, respectively, with and without
diabetes, 99% Caucasian. At 2 years, $n = 105$, 49 and 56 patients, respectively, with and without diabetes remained in the study (three withdrew, three moved away and one deceased). Two non-diabetes subjects developed diabetes and were excluded from the analysis leaving a total follow-up group of 103 patients (49 with and 54 without diabetes). During the 2-year follow-up, 10 patients (7 with diabetes) progressed to ESRD and dialysis. Of the remaining 93 patients, 13 progressed from CKD stage 3 to CKD stage 4 (9 and 4 with and without diabetes, respectively) and 9 progressed from CKD stage 4 to CKD stage 5 but were not on dialysis (5 and 4 with and without diabetes, respectively).

Baseline and follow-up clinical characteristics and biochemical parameters for the two groups are shown in Table 1 (biochemistry values represent a single fasting measurement at baseline and follow-up). Mean follow-up time in months was $23.5 \pm 2.2$ and $22.4 \pm 5.1$ months for those with and without diabetes, respectively. Age and gender ratio in the two groups were not different. At baseline, patients with diabetes had significantly higher BMI ($P < 0.001$), systolic blood pressure ($P = 0.049$) and lower total and LDL cholesterol (LDL-C) levels ($P = 0.039$ and $P = 0.033$, respectively) than those without diabetes; these differences remained throughout the study. Baseline serum phosphate ($P = 0.004$) and calcium phosphate product ($P = 0.006$) were higher and 25OH vitamin D levels were lower ($P = 0.026$) in those with diabetes compared to without diabetes, but at the follow-up, the differences were not statistically significant. Fetuin-A levels were higher ($P = 0.003$) and OPG levels were lower ($P = 0.009$) in those without diabetes throughout the study. Patients with diabetes had also lower ultradistal radius BMD ($P = 0.015$) at baseline and follow-up.

There was a small rise in the median iPTH in the non-diabetes group over the 2 years, whereas iPTH remained stable in the group with diabetes. Both groups had worsening proteinuria after 2 years and progression of renal dysfunction (defined as >5 ml/min/year change in MDRD glomerular filtration rate) was not significantly different between the two groups, 14/50 in those with diabetes compared with 12/50 in those without diabetes ($P = 0.532$). Despite treatment of 25OH vitamin D insufficiency in 19 patients at baseline (13 with diabetes), mean 25OH vitamin D levels did not significantly change over the 2 years in either group. Changes in cholesterol, HDL-C and LDL-C levels were consistent with the wider prescribing of lipid lowering therapy. At baseline, 12 patients (8 with diabetes) were prescribed calcium-based phosphate binders and at follow-up, the number had decreased to 3 (2 with diabetes), and in the remaining patients, treatment had been changed to sevelamer. Use of statins was higher in the diabetes group compared to those without diabetes at both time points ($P < 0.001$). Anti-hypertensive drugs were modified during the 2 years to optimize blood pressure control, and subjects with diabetes continued to be prescribed more anti-hypertensive medications after 2 years.

### Table 1. Summary of patients’ characteristics

<table>
<thead>
<tr>
<th></th>
<th>CKD only baseline</th>
<th>CKD only 2 years later</th>
<th>CKD and DM baseline</th>
<th>CKD and DM 2 years later</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>51 (26–60)</td>
<td>53 (28–62)</td>
<td>54 (33–65)</td>
<td>56 (34–67)</td>
<td></td>
</tr>
<tr>
<td>Male/female (n)</td>
<td>33/21</td>
<td>33/21</td>
<td>31/18</td>
<td>31/18</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.6 ± 4.8</td>
<td>27.6 ± 4.9</td>
<td>31.5 ± 5.6</td>
<td>31.5 ± 6.1</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>130 ± 16</td>
<td>128 ± 17</td>
<td>136 ± 32</td>
<td>141 ± 26</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>79 ± 10</td>
<td>78 ± 11</td>
<td>76 ± 16</td>
<td>76 ± 10</td>
<td></td>
</tr>
<tr>
<td>Hypertensive medications (n)</td>
<td>1.8</td>
<td>1.85</td>
<td>2.8</td>
<td>2.82</td>
<td></td>
</tr>
<tr>
<td>Calcium-based binders (yes/no)</td>
<td>4/50</td>
<td>4/50</td>
<td>8/41</td>
<td>2/47</td>
<td></td>
</tr>
<tr>
<td>Prescribed statins (yes/no)</td>
<td>21/33</td>
<td>23/40</td>
<td>40/9</td>
<td>41/8</td>
<td></td>
</tr>
<tr>
<td>eGFR (ml/min/1.73m²)</td>
<td>35 ± 11</td>
<td>29 ± 13</td>
<td>&lt;0.001</td>
<td>37 ± 13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adjusted calcium (mmol/l)</td>
<td>2.46 ± 0.09</td>
<td>2.46 ± 0.10</td>
<td>2.44 ± 0.09</td>
<td>2.42 ± 0.13</td>
<td>0.349</td>
</tr>
<tr>
<td>Phosphate (mmol/l)</td>
<td>1.11 ± 0.26</td>
<td>1.17 ± 0.34</td>
<td>1.25 ± 0.23</td>
<td>1.28 ± 0.34</td>
<td>0.643</td>
</tr>
<tr>
<td>Ca × P product (mmol²/l²)</td>
<td>2.74 ± 0.68</td>
<td>2.89 ± 0.92</td>
<td>3.07 ± 0.59</td>
<td>3.11 ± 0.84</td>
<td>0.685</td>
</tr>
<tr>
<td>Bicarbonate (mmol/l)</td>
<td>24.8 ± 4.4</td>
<td>25.3 ± 3.9</td>
<td>26 ± 3</td>
<td>27 ± 3</td>
<td>0.102</td>
</tr>
<tr>
<td>Intact parathyroid hormone (ng/l)</td>
<td>77 (12–211)</td>
<td>91 (13–511)</td>
<td>82 (9–451)</td>
<td>86 (14–444)</td>
<td>0.215</td>
</tr>
<tr>
<td>25OH vitamin D (nmol/l)</td>
<td>59 ± 30</td>
<td>53 ± 30</td>
<td>45 ± 30</td>
<td>43 ± 28</td>
<td>0.576</td>
</tr>
<tr>
<td>Urine PCR (mg/mmol)</td>
<td>70 (10–940)</td>
<td>330 (30–4410)</td>
<td>73 (10–1034)</td>
<td>450 (29–6350)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.05 ± 0.96</td>
<td>4.69 ± 1.0</td>
<td>4.53 ± 1.49</td>
<td>4.12 ± 1.01</td>
<td>0.023</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.42 ± 0.32</td>
<td>1.52 ± 0.39</td>
<td>1.29 ± 0.37</td>
<td>1.42 ± 0.41</td>
<td>0.010</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>2.81 ± 0.89</td>
<td>2.22 ± 0.73</td>
<td>2.31 ± 1.30</td>
<td>1.91 ± 0.63</td>
<td>0.014</td>
</tr>
<tr>
<td>Fetuin-A (g/l)</td>
<td>0.85 ± 0.18</td>
<td>0.86 ± 0.19</td>
<td>0.73 ± 0.20</td>
<td>0.71 ± 0.20</td>
<td>0.582</td>
</tr>
<tr>
<td>OPG (pmol/l)</td>
<td>5.70 ± 1.8</td>
<td>6.11 ± 2.55</td>
<td>6.69 ± 1.95</td>
<td>7.41 ± 2.56</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sRANKL (pmol/l)</td>
<td>0.51 ± 0.54</td>
<td>0.53 ± 0.42</td>
<td>0.40 ± 0.09</td>
<td>0.42 ± 0.11</td>
<td>0.133</td>
</tr>
<tr>
<td>Matrix gla protein (mmol/l)</td>
<td>5.20 ± 3.67</td>
<td>4.45 ± 2.51</td>
<td>6.11 ± 3.55</td>
<td>5.31 ± 2.16</td>
<td>0.081</td>
</tr>
<tr>
<td>Total radius BMD (g/cm²)</td>
<td>0.599 ± 0.08</td>
<td>0.589 ± 0.07</td>
<td>0.568 ± 0.07</td>
<td>0.564 ± 0.07</td>
<td>0.104</td>
</tr>
<tr>
<td>Ultradistal radius BMD (g/cm²)</td>
<td>0.429 ± 0.09</td>
<td>0.417 ± 0.07</td>
<td>0.390 ± 0.07</td>
<td>0.383 ± 0.07</td>
<td>0.197</td>
</tr>
<tr>
<td>Total lip BMD (g/cm³)</td>
<td>1.030 ± 0.15</td>
<td>1.031 ± 0.14</td>
<td>1.018 ± 0.19</td>
<td>1.004 ± 0.21</td>
<td>0.020</td>
</tr>
<tr>
<td>Femoral neck BMD (g/cm³)</td>
<td>0.975 ± 0.15</td>
<td>0.968 ± 0.14</td>
<td>0.942 ± 0.17</td>
<td>0.924 ± 0.19</td>
<td>0.003</td>
</tr>
</tbody>
</table>

*Ca, serum adjusted calcium; DM, diabetes mellitus; n, number; P, serum phosphate; PCR, protein-to-creatine ratio; sRANKL, soluble receptor activator of nuclear factor-κB ligand. P values compare baseline with follow-up 2 years later, statistically significant values are in bold.*
Coronary artery calcification

Prevalence. Prevalence at baseline measurement and after 2 years of follow-up is shown in Table 2. Subjects with diabetes had a much greater prevalence of CAC compared with those without diabetes (baseline 73 versus 46%, \( P = 0.005 \), and at 2 years, 80 versus 50%, \( P = 0.002 \)).

Cumulative incidence. Ten patients developed incident CAC (i.e. previous baseline CAC score of zero) over the 2-year follow-up, 5 out of the remaining 13 subjects in the group with diabetes and 5 from the remaining 29 subjects in the group without diabetes. Eight of them were males and two females (\( P = 0.030 \)). Of note was that the amount of incident CAC detected in all patients was generally very small median of 5, range 1–43. The binary logistic model that gave the best prediction for new-onset CAC, for the whole group, identified advanced age and lower HDL-C levels as the explanatory variables for CAC incidence (Table 3).

Severity. CAC scores both at baseline and after 2 years were greater in the diabetes group; median CAC scores 154 and 258 compared to 47 and 34 in the group without diabetes (\( P = 0.013 \) comparing diabetes with non-diabetes). The number of arteries involved and the sites of CAC identified did not differ at baseline and after 2 years (data not shown). CAC progressed more rapidly in the group with diabetes, see Table 2. A substantial increase in the CAC score (defined as >100 Agatston units over the 2 years) was noted in the diabetic group 23/39 (59%) compared to 7/27 (26%) in the non-diabetic group (\( P = 0.004 \), see Figure 1). Men presented the largest measurable increases in both groups; however, women with diabetes had increases similar to the men.

In the multiple linear regression analysis for the whole group, independent predictors for the increase in CAC score were baseline CAC score (beta = 0.63, \( P < 0.001 \)), BMI (beta = 0.22, \( P = 0.002 \)) and serum phosphate levels (beta = 0.20, \( P = 0.006 \)) (for the model: adjusted \( R^2 = 0.510, P < 0.001 \)). Figure 2 shows the correlation between serum phosphate levels and the increase in CAC score. The same factors (baseline CAC score, BMI and serum phosphate levels) predicted also, significantly and independently, the extent of coronary calcification (CAC score) at follow-up (data not shown).

Evolution. Using the definition of evolution as a difference of \( \geq 2.5 \) U between baseline and final square root CAC scores [17], there were 36 progressors (24 with diabetes). Patients with diabetes were more likely to progress compared to those without diabetes 24/49 (49%) compared to 12/54 (22%), \( P = 0.004 \) (Figure 3a). In the group with diabetes, the number of years with a diagnosis of diabetes did not differ between progressors versus non-progressors 19.0/16 versus 19.7/19.8, respectively. Additionally, the level of glycated haemoglobin did not differ (8.5/1.7 versus 8.9/1.8%, \( P = 0.340 \)). Although the majority of progressors were males, 26 males compared to 10 females for the whole group,

### Table 2. CAC scores over the 2-year follow-up period

<table>
<thead>
<tr>
<th></th>
<th>CKD only</th>
<th>CKD and Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 2 years</td>
<td>Baseline 2 years</td>
</tr>
<tr>
<td>Prevalence (%)</td>
<td>25/54 (46%)</td>
<td>27/54 (50%)</td>
</tr>
<tr>
<td>Median CAC score</td>
<td>47</td>
<td>154</td>
</tr>
<tr>
<td>Range</td>
<td>2–1196</td>
<td>1–3678</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>9–156</td>
<td>45–926</td>
</tr>
</tbody>
</table>

*\( P = 0.008 \) and **\( P < 0.001 \) compared to baseline.

### Table 3. Binary logistic regression models between parameters of the study and dependent variables

<table>
<thead>
<tr>
<th>Parameter</th>
<th>B</th>
<th>Standard error</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dependent variable: new-onset CAC</td>
<td>Age</td>
<td>0.167</td>
<td>0.080</td>
<td>1.181</td>
<td>1.009–1.382</td>
</tr>
<tr>
<td></td>
<td>HDL-C levels( ^a )</td>
<td>-0.726</td>
<td>0.309</td>
<td>0.484</td>
<td>0.264–0.887</td>
</tr>
<tr>
<td>Dependent variable: CAC evolution in the whole group</td>
<td>Age</td>
<td>0.098</td>
<td>0.035</td>
<td>1.102</td>
<td>1.028–1.182</td>
</tr>
<tr>
<td></td>
<td>Baseline CAC score (SQRT)</td>
<td>0.058</td>
<td>0.024</td>
<td>1.060</td>
<td>1.010–1.112</td>
</tr>
<tr>
<td></td>
<td>Serum phosphate levels( ^a )</td>
<td>0.209</td>
<td>0.081</td>
<td>1.232</td>
<td>1.052–1.443</td>
</tr>
<tr>
<td>Dependent variable: CAC evolution in the CKD group</td>
<td>BMI</td>
<td>0.451</td>
<td>0.167</td>
<td>1.570</td>
<td>1.131–2.180</td>
</tr>
<tr>
<td></td>
<td>Male gender</td>
<td>3.325</td>
<td>1.449</td>
<td>27.808</td>
<td>1.625–475.97</td>
</tr>
<tr>
<td></td>
<td>Serum phosphate levels( ^a )</td>
<td>0.620</td>
<td>0.234</td>
<td>1.858</td>
<td>1.174–2.941</td>
</tr>
<tr>
<td>Dependent variable: CAC evolution in the group with CKD and diabetes</td>
<td>Age</td>
<td>0.113</td>
<td>0.036</td>
<td>1.119</td>
<td>1.042–1.202</td>
</tr>
</tbody>
</table>

\( ^a \)For every 0.1 U change. CI, confidence interval; SQRT, square root transformed.
within the diabetes group females progressed the same as males, 9/18 females (50% of females with diabetes and CKD), the same proportion as male with diabetes (Figure 3b). This was in sharp contrast to the group without diabetes, where 11/12 of the progressors were men compared to only one female progressor.

We additionally examined our data using annualized percent change, the more commonly reported measure, and for this data, >33% per annum is used to define a progressor. Using this measure, there were 19 with diabetes defined as progressors compared to 8 in the group without diabetes ($P = 0.008$). No significant differences were noted between the two methods (data not shown).

Number of progressors increased significantly across increasing quartiles of serum phosphate levels (Figure 4). The binary logistic model that gave the best prediction for evolution, for the whole group, identified age, baseline CAC score and phosphate as the explanatory variables for CAC evolution. In the group with diabetes, age was the unique explanatory variable for evolution of CAC. In the group without diabetes, the significant predictors for evolution were BMI, male gender and serum phosphate levels (Table 3).

Discussion

Our study demonstrates that patients with CKD and diabetes have higher probability of progression and a greater increase in CAC scores than those without diabetes. This is not surprising; however, is the first time that is reported in CKD patients not in dialysis. The RRI-CKD study ongoing by Dellegrottaglie et al. [18] hints at high rates of progression but is unreported. Mehrotra et al. [8] reported a probability of progression rate of 47% in subjects with diabetic nephropathy. Rates of evolution in our study are similar, but we differ in the magnitude of CAC scores and the overall distribution of CAC. In our study, the group with CKD and diabetes has much greater levels of CAC, with nearly 60% demonstrating detectable CAC scores >100 Agatston units and ~1/3 within the >400 CAC range at the final measurement. Much of this was due to a substantial evolution during the 2 years when diabetic men and women equally acquired CAC. The number of years with a diagnosis of diabetes and diabetes control did not influence evolution in this study. In the non-diabetic group, 20% had detectable CAC scores >100 Agatston units at the final measurement and nearly all were men.

Age is consistently a risk factor for the presence and severity of CAC in all populations [5, 19–23] and in our study. Obesity, another well-established CVD factor [5, 19, 23, 24], correlated with diabetes in this study; however, BMI was an independent predictor of evolution in the non-diabetes group and also independently associated with the increase and the severity of CAC in the whole group. Lipid profile’s alterations (primarily low HDL-C, elevated...
triglycerides, elevated LDL-C and elevated total cholesterol) are important factors in the calcification process [25, 26] and in our study, low HDL-C was an independent predictor of new-onset CAC.

It has been reported by Natarajan et al. [27, 28] that women with diabetes are at greater risk for coronary heart disease mortality and it has been suggested that female advantage occurs in pre-menopausal women [29]. In our study, median ages for the women at the end of the study were 52 years for those with CKD and diabetes and 49 years for those with CKD only, (P = 0.278). We did not measure follicle-stimulating hormone or oestrogen levels to evaluate their menstrual status; however, we did ask if they were still menstruating, before attendance for X-rays, and only four ladies were (all from the non-diabetes group).

Our data suggest that men and women with diabetes are equally likely to exhibit high CAC scores and that they progresses equally, whereas in the absence of diabetes, it mainly affects men.

Although phosphate levels were found to be an independent predictor of evolution and severity of CAC in our study, the majority of values were within the recommended normal range and our population had not been treated with calcium-containing medications. Russo et al. [10] reported phosphate as the only variable associated with CAC evolution in a group of CKD Stages 3–5 patients (patients with diabetes were excluded). The Spokane Heart Study, a long-term observational study of community-dwelling adults, showed that higher serum phosphorus levels are independent predictors of CAC over time with a risk comparable to traditional CVD risk factors [30]. The potential mechanism for the association between serum phosphate levels and CAC in the present study is unclear, especially if we consider that serum phosphate is not always independently associated with vascular calcification even in studies with haemodialysed patients where phosphate levels are more elevated [31]. Vascular smooth muscle cells respond to elevated phosphate levels by undergoing an osteochondrogenic phenotype change and mineralizing their extracellular matrix leading to calcification. Although most experimental models have used exogenous phosphate concentrations >6 mg/dl to obtain a robust calcification response, it remains possible that lower phosphate concentrations that are in the normal range might be sufficient to initiate a calcification response in vivo in the presence of additional synergistic factors [32–34]. Studies have shown that higher serum phosphate concentrations, although still within the normal range, are associated with a greater prevalence of vascular and valvular calcification and with adverse cardiovascular outcomes not only in subjects with moderate CKD but also in subjects with normal renal function [35–38]. Another explanation for the association between phosphate levels and CAC found in our study could be that higher levels of serum phosphate might identify patients with more severe secondary hyperparathyroidism, lower vitamin D status or more severe renal disease, factors that are associated with
increased vascular calcification. In fact, in our report, phosphate levels were significantly inversely correlated with eGFR and 25OHD levels and positively correlated with iPTH levels; however, adjustment for these factors did not alter the strength of association between phosphate concentrations and calcification progression. Finally, the cross-sectional design of our study leaves open the possibility that phosphate levels may increase in response to other processes linked with arterial calcification or that other factors like dietary habits might confound the association between phosphate and CAC.

Progression and extent of CAC were also related to baseline CAC score, indicating that once the calcification is established, it follows a progressive course and that factors associated with the presence of CAC at baseline-like diabetes and others may continue to exert their detrimental effect [1]. Baseline CAC score is a strong determinant of progressive calcification in studies in the general population and in patients with advanced CKD [11, 39–41]. However, it is interesting to note that there were subjects without CAC at baseline and 2 years later. Current knowledge suggests they may have genetically controlled protection and/or produce inhibitors of calcification [42]. The list of possible inhibitors is still growing; however, Fetuin-A is considered one of the most important and Russo et al. [10] reported lower levels in calcified patients but without predictive value. Although diabetes subjects had significantly lower Fetuin-A and higher OPG serum levels compared to non-diabetes subjects in our report, these levels were not different between zero CAC score subjects and those with significant calcification, or in progressors, in agreement with other studies [11].

There is an increasing interest for the association between vascular calcification and bone histomorphometric parameters. London et al. [43] found that arterial calcifications were increased in haemodialyzed patients with adynamic bone disease. Asci et al. [44] found a U-shaped relationship between CAC and bone turnover in a recently published study in haemodialyzed patients. CAC was also associated with lower bone formation rate in another study of CKD patients not yet on dialysis [45]. We did not perform bone biopsies in our patients, but we measured BMD as an association between bone loss and vascular calcification has been reported in the general population [46]. However, in our study in CKD patients, no association was found between BMD and CAC prevalence, incidence or progression. This maybe due to the complicated pathophysiology of renal osteodystrophy in CKD patients since BMD does not reflect bone turnover in these patients.

Our study has certain limitations: the small cohort that may have prevented us from identifying additional risk factors for progression of CAC, the cross-sectional design, the lack of bone biopsies and recall bias; however, this would affect both groups equally; a number of confounders were identified but in each case, the variable with the strongest relationship was used in analysis and most importantly, the lack of a control population without CKD or diabetes. On the contrary, the strengths of this study are the method for determining evolution of CAC in this study is reliable and uses the appropriate mathematics allowing elimination of errors due to inter-scan variability. In addition, the follow-up rate is extremely high (92%) and 2 years is a reasonable follow-up time [17, 47–49].

In conclusion, CKD patients with diabetes demonstrate a higher burden of CAC, and this burden is severe, evolution is more rapid and there is a loss of male gender predominance compared to those without diabetes. It is reasonable to assume that detection of CAC is part of a long-term causal pathway for the evolution of calcification; further studies are needed to establish when CAC develops within the course of CKD and diabetes and if the process is reversible. Factors such as age and diabetes status are not reversible and although not interventional, our study shows the potential deleterious effect of obesity and it would be interesting to see if weight intervention strategies could slow evolution of CAC. Phosphate control, cardiovascular risk and mortality are now recognized as factors which are linked in CKD, and prospective investigations are needed to determine if there is a pertinent level at which serum phosphate control affects cardiovascular risk.

Acknowledgements. We wish to dedicate this paper to the memory of Dr Mike Cassidy who we lost on Sunday 11 January 2009. Mike was a caring and conscientious doctor much respected by his patients, staff and colleagues. He contributed immensely to the understanding of renal bone disease and had an impressive publication history. He was a valuable colleague and beloved friend with an infectious love for life, which continued even though he was very sick. His sad and untimely death leaves behind a wife and son.

Conflict of interest statement. None declared.

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