Final Research Report
Title: The Relationship between Osteoporosis and Aortic Calcification in Postmenopausal Women
Ref: 08/H0802/9
CI: Professor Ignac Fogelman

Overall aim of the research

Evidence has shown age-independent associations between osteoporosis and cardiovascular disease (CVD), which may be explained in part by an increase in vascular calcification (VC). It has also been suggested that bisphosphonates (BPs), the most commonly used treatment for osteoporosis, have the potential to decrease aortic calcification (AC) as well as reduce the risk of fracture. This research project was designed to provide preliminary data on the effects of BPs on VC and arterial stiffness and was divided into 3 parts:

Part 1 – The precision study determined whether lateral DXA-VFA scans – primarily used for the assessment of vertebral deformity – could be used to estimate VC within the aorta compared with the 'gold standard' method of computed tomography (CT). This part of the study also investigated whether moderate dose, non-diagnostic, non-contrast CT could provide a reproducible alternative to higher dose diagnostic CT methods that are routinely used in clinical practice for quantifying VC.

Part 2 – The cross-sectional study investigated the relationship between bone mineral density (BMD) and AC in over 400 postmenopausal women.

Part 3 – The prospective study (randomised controlled trial, RCT) evaluated the effects of alendronic acid (an oral BP) on AC in 26 postmenopausal women with osteoporosis who were randomised to either alendronic acid (70 mg weekly), or the calcium and vitamin D supplement Adcal D3 (once daily), and followed-up over a 2-year period.

Main findings

A summary of the findings from parts 1 to 3 of the study are described below. However, the most exciting findings from the research project came from Part 3 (the RCT) and we will describe the findings in reverse order as follows:

Part 3 - The relationship between VC, aortic stiffness and BP use was investigated in both the prospective RCT and the cross-sectional study. The primary outcome in the RCT was the change in aortic pulse wave velocity (PWV) between baseline and 24-months. The secondary outcomes were:

- Measuring AC by CT between baseline and 24-months
- Estimating abdominal aortic calcification (AAC) using lateral DXA scans of the spine
- Changes in bone turnover markers and Endothelial Progenitor Cell (EPC) levels

Twenty-two subjects completed the RCT and the study population characteristics are shown below:

	Treatment group (n = 12)	Control group (n=10)
Age	58 (4.1)	59 (3.1)
Lumbar spine T-score	-2.6	-2.4
Serum Calcium	2.34 (0.1)	2.26 (0.1)
Vitamin D	61.3 (29.6)	60.2 (12.8)

12-month results (based on complete data sets):

AAC was significantly reduced over the 12 month follow-up period in the treatment group (n = 5), with mean scores at baseline, 6 and 12 months of 2.6, 1.6 and 0.4 arbitrary units, respectively ($p<0.05$). In contrast, there was no significant change in PWV between the time points after adjustment for mean arterial pressure (MAP) and heart rate (HR) (mean values at baseline, 6 and 12 months were 8.15 (confidence intervals (CI): 7.2-9.2), 8.71 (CI: 7.7-9.7) and 9.2 (CI: 8.2-10.2) m/sec respectively, P=0.419). In the control group (n = 7), there was no significant change in AAC over the follow-up period with mean scores at baseline, 6 and 12 months of 3.43, 0.42 and 2.0 arbitrary units, respectively, nor was there significant change in PWV. These results suggest that alendronic acid therapy may reduce AAC as detected by DXA imaging.

24-month results (based on complete data sets):

As shown in the table below, after 24-months, there was no significant change in PWV in either the treatment or the control group. There was no significant change in AAC measured by DXA in the treatment group but unexpectedly, in the control group, there was a significant reduction in AAC, which we are unable to explain. The results suggest that after 24-months, neither alendronic acid or calcium and vitamin D supplementation had any effect on AC as measured by PWV. However, the data is limited because of the large variability between the values and small sample size.

CT is the gold-standard method of identifying VC and all subjects had a baseline and 24-month follow-up CT scan. In this study, VC was determined by the change in the volume of calcium and in the treatment group (n=12), this was 30.8 mm^3 (p=0.12) which was not significant. However, in the control group (n=10), there was a significant increase of 66.7 mm^3, ($p<0.05$). Therefore, results from the 24 month CT data suggest that alendronic acid may indeed slow down the progression of AC.

Assessment	Baseline-mean (SD)	24-months-mean (SD)	P value
PWV-Tx group (n =6)	8.1 (0.9)	8.5 (1.3)	0.44
PWV-Controls (n =6)	7.91 (0.7)	8.9 (2.1)	0.33
DXA-IVA-Tx group (n =6)	2.1 (2.7)	0.1 (0.3)	0.10
DXA-IVA-Controls (n =6)	3.3 (2.9)	0.8 (1.7)	<0.05*

NB-* donates significant result

The analysis of the bone turnover markers and EPCs were not completed for the following reasons. The bone turnover markers have not been measured because funding is no longer available due to the considerable delay in the completion of the study. The researcher responsible for measuring the EPC levels left Kings midway through the study because her contract had expired. Therefore the 24-month samples were missed from 11 patients, the 12-month samples were missed from 3 patients due to staff cancellation and the 6-month samples were missed from 5 patients.

Part 2 – Results from the cross-sectional study showed that the use of BPs and the duration of treatment to be positively associated with PWV, which is a measure of arterial stiffness, but not AAC measured using lateral DXA scans. Possible explanations for this may be due to the small sample size compared with larger cohort studies, and that lateral DXA images can be affected by poor image quality as well as limited capability in distinguishing between calcification at different sites within the vessel wall.

The cross-sectional study results further tested the reliability of lateral DXA scan images for quantifying calcification, evaluating its sensitivity and specificity by comparing this with the gold-standard of CT. This was an important aspect of the project as there is limited information on the accuracy of lateral DXA for the prediction of VC in low risk patients, as most data is from patients with renal disease who have a greater incidence of VC. Results from this part of the project showed that lateral-DXA scan images were only able to predict high levels of AAC as detected by CT with moderately-good accuracy.

The cross-sectional data was also used to investigate the relationship between BMD and AC by evaluating the association between BMD measured at the lumbar spine, femoral neck and total hip with AC measured using lateral DXA scan images and VC at multiple sites using the gold-standard CT. However, the results failed to find any significant associations between BMD and measures of VC.

Prospective studies have shown AC to be related to bone loss and an increase in fracture risk. Therefore the association between circulating Wnt inhibitors sclerostin and Dkk1 (which are regulators of bone remodelling) with AC, VC and BMD are of particular interest. Data from the cross-sectional study demonstrated that serum Dkk1 was not associated with BMD or AC/VC but sclerostin was significantly associated with BMD but not AC or VC.

PWV is a simple, non-invasive method of measuring arterial stiffness and it is an independent predictor of CV-related events. Data from the cross-sectional study evaluated the relationship between arterial stiffness, BMD, AC/VC and regulators of bone remodelling. No significant association was found between PWV and BMD or Dkk1 or sclerostin. Furthermore, no association was found between arterial stiffness measured using PWV with AAC measured using lateral DXA scan images.

Part 1 – Research has shown that most individuals in later life will have evidence of calcium deposits within the major arteries leading to an increased risk of CV events. This part of the project demonstrated that lateral DXA imaging could offer a reproducible imaging method to quantify AAC.

The CT reproducibility data demonstrated that the modified scanning protocol was (a) suitable for quantifying VC at multiple vascular sites; (b) the scan images were interpreted and evaluated by an appropriately trained clinical scientist and (c) they were highly reproducible results suggesting a safer alternative to the high radiation dose methods currently in use for evaluating coronary calcification in clinical practice.

Patient Recruitment and issues encountered during the study

There were a number of challenges faced by the research team regarding completion of the study and they are described as follows:

1. Patient recruitment - recruitment was slow for the prospective study because volunteers were reluctant to participate due to fears surrounding the use of calcium supplementation as a result of the reported association between calcium use and CVD frequently published in the media. Furthermore, volunteers were concerned about the risk of osteonecrosis of the jaw and its association with BP therapy. The risks of both side-effects were explained in detail and patients who took part in the study were reassured. However, the recruitment target was revised from 50 to 26 and 22 subjects (10 on calcium and vitamin D supplements only and 12 on alendronic acid) completed the RCT. No SAE's, SAR's, or SUSAR's were reported during the study.

2. PWV measurements - a number of PWV measurements were unable to be obtained due in-part to the PhD student's time away from the project due to an ongoing medical condition, but also because of the difficulties experienced and inexperience with the PWV equipment by staff covering the visits for the RCT. Staff underwent training with Dr Marina Cecelja, who has experience with performing PWV measurements, but because the patient follow-up visits were very spread-out, it was difficult to maintain consistency with use of the equipment.

3. Staff redundancy – the PI, Dr Michelle Frost was made redundant in September 2014, and this had a direct effect on the study.

Implications and Future work

Considering postmenopausal women with osteopenia and/or osteoporosis have an increased risk for CVD and future CV-related events, early clinical interventions resulting from the identification of subclinical CVD using the novel imaging techniques highlighted in this research would ultimately help reduce morbidity and mortality rates in this population. Furthermore, the use of BPs to prevent the progression or even reverse the development of VC while concurrently maintaining bone density will provide a major clinical milestone – particularly as there are currently no therapies available to reduce VC. However, large-scale studies are still required to confirm their efficacy in preventing the progression of VC/AC. Dr Moore and Dr Cecelja, a research fellow in the Cardiovascular Division plan to apply for funding to confirm the results in a larger study.

Dissemination of findings

The results from the RCT were presented at the European Hypertension Society Conference in June 2015 and may be submitted as a full paper to an Osteoporosis journal in the future. The PhD student, Sylvia Edwards also plans to submit the findings from Chapter 3 of her thesis describing the CT and lateral DXA images for the quantification of AC, as a paper to a Radiology related journal.

This study was funded by the National Osteoporosis Society (NOS) and Dr Moore has recently presented the findings to the NOS patient support group meetings held in King's Lynn and Gravesend.

Chief Investigator Sign-off

Chief Investigator:

Print name: IGNAC TORLINSKI

Signature:

Date: 20/8/15