Author's response to reviews

Title: Longitudinal analysis of vertebral fracture and BMD in a Canadian cohort of adult cystic fibrosis patients

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Author's response to reviews: see over
July 14, 2008

Dear Dr. Norton:

Re: Response to Reviewers - Longitudinal analysis of vertebral fracture and BMD in a Canadian cohort of adult cystic fibrosis patients (Manuscript ID 1102379881173905)

A point by point response to the reviewer’s suggestions is given below. The corresponding changes to the manuscript text are highlighted in yellow.

Reviewer 1:

I have a number of specific comments that I hope will help improve the manuscript:

1. In this age range (20-30) a vertebral fracture prevalence of 20% is indeed high. Presumably there is a way to compare with a “standard” or “referent” population (without CF) to see how high is the risk.
No population based studies that we know of have radiographic vertebral fracture data for young adults. However, we did compare rates with healthy Canadians over age 50 since that is available from the population-based CaMOS study of over 9000 Canadians (only clinical reporting of vertebral fractures was available for those under 50: radiographs were taken for 50+). However, the CaMos study uses a different method (purely quantitative morphometry) and may actually provide estimates that are up to double that of the semi-quantitative method. A further discussion which makes the comparison with the CaMos group has been added in the first paragraph of the discussion section.

2. Because there was no control group, I think it is important to present the BMD data in terms of z-score for each patient group.
As per the reviewers’ comments regarding the need for z-scores, the medical physicist (author CW) has retrieved t-scores/z-scores for all the DXA readings. (T-scores were given for participants over 19 years of age, and z-scores for those under 19 years of age; See further discussion added to the methods section under ‘Bone Densitometry’). These have been analyzed and incorporated into the new text and Table 3: Bone Mineral Density (BMD) Over Study Period (formerly Table 2). Table 3 now incorporates t-scores/z-scores, absolute BMD values at all sites and for Baseline, year 2, year 3, year 4, year 5.

3. a) It is not clear how the percent change in BMD was calculated (Table 2).
Thank you for the question. A further description has been added to the methods section ‘All individuals had two to five BMD measures during the course of the study. To incorporate all BMD measurement, a regression slope was calculated using all available measurements for each patient. The slope represents the average yearly bone mineral density change over the entire follow-up period for each patient. This analysis takes into
account the differing numbers of BMD measurements and the length of follow-up for each patient.’

3.b) Was there any correlation between change in BMD and baseline BMD levels?
As stated in the manuscript, baseline BMD values and other variables that were significant in univariate analysis were included in the multi-variable regression models. The results revealed that the baseline lumbar spine BMD was negatively correlated with average yearly bone mineral density change. However, baseline proximal femur and whole body were not significantly correlated.

3.c) Given that measurement of BMD is subject of measurement error, how many patients had changed BMD beyond the measurement error?
Measurement error was not calculated for this study. However, typical measurement errors ranged from 1 to 5% (more accurate for whole body and spine). The medical physicist who read BMD for this study, has published on the precision of spine DXA measurement (Webber CE. Photon Absorptiometry, Bone Densitometry, and the Challenge of Osteoporosis. Phys Med Biol., 2006; 51: R169-R185). He cites that the precision of spine DXA measurements is typically 0.01 g/cm$^2$. This means that the least significant change is about $1.96 \times (0.01^2 + 0.01^2)^{0.5}$ or about 0.028 g cm$^2$.

Nonetheless, measurement error is not a major issue in this study given that all (yearly) data points were used to calculate average yearly bone mineral density change (slope) during the entire follow-up period. As a result, if there are extreme yearly measures in either positive or negative directions the slope calculation would blunt these extreme measures. This makes our analysis robust.

4.a) Was there any association between change in BMD and the risk of sustaining new vertebral fracture?
Please note we did not do this analysis for incident vertebral fracture as there were only 4! We have however provided a further description of some characteristics for those patients who sustained an incident fracture to better describe this group. The crude % BMD change for fracture versus non-fracture patients was already provided in the results section (see last paragraph).

4.b) It is not clear whether fracture risk was related to age and/or concomitant medication.
Re: AGE & Fracture Risk
Fracture risk was related to age. As already noted in the results and discussion section – ‘mean age of fracture patients was significantly higher than that of non-fracture patients’. Age was also significantly related to FU fracture status (i.e. presence of an incident or prevalent fracture).
An additional sentence has been added to the results: ‘The mean age at follow-up for patients with a new fracture was 35.6 years (SD 14.7) versus 28.1 years (SD 8.8) for patients without (not statistically significant but lacked power)’.
Re: Concomitant Meds and Fracture Risk
We lacked sample size to examine statistically, as few patients were taking bisphosphonate or oral steroids. However we have clarified the issue further by adding the following to the results:
Patients taking oral steroids - ‘one of these patients had a new incident fracture (cumulative corticosteroid dose of 3000 mg over 68 days) and the remainder had no prevalent or incident fractures.’
Patients taking bisphosphonate – ‘none had a prevalent or incident fracture.’

Inhaled steroid use and Vitamin D was already mentioned in the demographic table and results section.

5. I consider that the present analysis could be improved. Because this is a longitudinal study, I think the analysis should capture this important aspect of the study. At the very least, on would like to know what was the actual incidence of VF in the population. I suggest the authors present the data on the transition between baseline and follow-up period as follows:
Vertebral fracture at:
Baseline Follow-up # patients
No VF No VF n1
No VF VF n2
VF No VF n3
VF VF n4
Thank-you for your suggestion. A new table (Table 2: Vertebral Fractures in the Study Cohort) has been added to the manuscript. This table outlines the status of vertebral fractures at baseline and follow-up and also includes a clear description of the number of incident fractures and answers to the reviewers’ queries regarding fractures.

The actual incidence of new vertebral fractures was 4/47 = 8.5%. In addition to the table, this has been clearly specified in the results text as per the reviewers’ suggestions.

One patient in our study had a fracture indicated at baseline but not on the follow-up radiograph. This is a potential problem when using the semi-quantitative method we used in this study - Genant’s method (Genant HK, Li J, Wu CY, Shepherd JA: Vertebral fractures in osteoporosis: a new method for clinical assessment. J Clin Densitom 2000, 3(3):281-290). Genant’s semi-quantitative method is probably the most widely cited in osteoporosis studies. It depends on the recognition of the radiological signs of fracture by experienced observers. There is considerable debate regarding the level of deformity and accuracy of reporting, particularly that with Genant’s method the separation of the grades is not explicit. A grade 1 is, the criterion for fracture as per Genant’s; grade 0.5 deformity is observable but a reduction in height or area less than the criterion. The patient who had a ‘missing’ grade 1 fracture at follow-up was classified as a grade 0.5 instead. **We have added a paragraph and new references which discusses this issue in the discussion section.
6. Because there was 30% patients on oral corticosteroid, it would be useful to know whether there was an association between the incident VF and steroid use. Please see the answer to question 4.

7. In the Discussion (page 11) the authors state that BMD loss tends to be stable with age. I don’t think this is the case. Our study (Jones et al 1994, Nguyen et al JBMR 1998, Bone loss, physical activity, and weight change in elderly women) and the SOF study (Ensrud JBMR 1995, Hip and calcaneal bone loss increase with advancing age) showed that bone loss actually increased with advancing age.

This reference does not intend to comment on the rate of bone loss in the post-menopausal and elderly years, i.e. instead commenting on bone loss thru middle adulthood once peak bone mass has been reached, and then noting that a change occurs not until fifth (forties) or sixth (fifties) decades. It was intended to illustrate that the bone loss in these younger adults is more akin to post-menopausal or aging adults. (Our sentence reads: “In the general population, once peak bone mass has been reached, the rate of bone loss tends to be somewhat stable with age until the fifth and sixth decades [28].”)

8. Table 1: Please show actual p-values rather than “ns” or relative figures such as “<0.05”. Given the small sample sizes in the fracture and non-fracture group (n=41 vs n=8), I think the authors should show the confidence intervals for each parameter. I am confused about the “valid n” in Table 1. Why, for example, the number of “valid” men (41/8) is exactly the same as the number of “valid” women (41/8)? Please clarify!

As per the reviewer’s suggestion, exact p-values have been provided (and the term ‘ns’ removed) in the revised Table 1.

The confusion regarding the valid n has also been clarified (the proper values are now inserted for men and women) and additionally the term Nonfracture/fracture has been inserted into the column header to make additionally clear.

Also, a column providing the means and proportions for the entire cohort has also been added to revised Table 1.

Reviewer 2

1. One weak point of the study is the presence in the study group of adult and young subjects, already in the bone growth period: this fact can alter the results of BMD evaluation both at baseline and at follow-up. I suggest the authors to provide DXA measurements in absolute values (BMD) as well as in terms of T-
score and, more importantly, of Z-score, in order to correct as much as possible for this problem.

Please see the response to Reviewer 1 - Q2. We have added a Table, (Table 3: Bone Mineral Density (BMD) Over Study Period) to include T-score/Z-score information. We have also provided an additional sentence in ‘methods’ regarding need for T/Z scores.

Furthermore also the changes in BMD during the study period should also be reported in terms of Z-score.
Thank you for the comment. We now include T-score/Z-score values for each year of follow-up.

2. The particular nature of this population, the presence of both adult and young adolescents, should be mentioned clearly in the manuscript, especially in the discussion and limitation of the study.
Thank-you, this comment is well noted. The age breakdown of the study cohort has been more clearly described (added sentence to results with median and percentiles).

A sentence explaining the age span of 15-51 has also been added to the limitations in the discussion section. As noted above, the appropriate use of T and Z scores has also been reflected.

ABSTRACT
3. The characteristics of the population should be described in detail: age, sex, BMI, BMD T-score and Z-score at baseline.
These details have been added to the abstract.

RESULTS
4. Page 9: the occurrence of fractures during the study period should be more clearly stated: were all incident fractures? What was the associated trauma? How much low trauma fractures were observed?
The fractures have been more clearly presented, see previous responses to Reviewer 1 (Q5), and several changes have been made to the text and an additional table added (Table 2: Vertebral Fractures in the Study Cohort). Although the exact cause of the fractures were not known, as far as we know, these were all low-trauma fractures (we do not know of any fractures being caused by trauma).

5. How it is possible that a patient had a vertebral fracture at baseline that was not present at follow-up? What can be the reason? This should be discussed.
Please see the response to Reviewer 1, Q5. This is now fully explained in the text in the discussion section (and new references provided).

6. The rate of inhaled corticosteroid during the study period was corrected for the individual follow-up time for each patient? This should be done before testing the difference between fractured and non fractured patients.
In Table 1, the rate of inhaled steroid use is a simple yes/no – was the patient taking inhaled steroids at baseline. We did not examine inhaled steroid use over the study period; we did however collect longitudinal data on oral steroid use (quantity, length) over the study period. The importance of oral corticosteroids on bones is unequivocal; there is mixed evidence regarding the impact of inhaled steroids (“Inhaled GCs may be associated with decreased bone density and increased fracture risk, although they have much fewer adverse effects on bone than oral GCs and are probably safe in low-to-moderate doses.” {Reference: Kennedy, Papaioannou, Adachi et al. Glucocorticoid-induced osteoporosis. Women's Health (2006, Vol 2(1))}.

7. How were divided the patients on the basis of the presence of fracture? Were considered as fractured those who were already fractured at baseline, or only those who fractured during the study period, or both? Please clarify.

At baseline, vertebral fractures were prevalent. Additional fractures occurring over the study period were incident, and we have also provided the total number of fractures (prevalent and incident) at the follow-up period. We apologize for any confusion. Please note the following clarifications: In Table 1, which compares baseline characteristics between fracture groups, a foot-note has been added that these were prevalent fractures. To clarify the occurrence of new fractures, an additional table (Table 2: Vertebral fractures in Study Cohort) has been added and this tracks prevalent, incident, and total number at baseline and follow-up. Where possible in the text, the descriptors ‘incident’, ‘prevalent’, or ‘incident and prevalent’) have been added.

8. The rate of bone loss has been reported in percentage, but the standard deviation or standard error should be reported, even in percentage, if preferred. The standard deviation is now provided.

>9. The outlier evidenced in the calculation of BMD changes should be considered in light of the expression of the results in terms of Z-score.

>10. All BMD changes should also be expressed in terms of Z-score throughout the manuscript.

Response to #9 and #10:
Thank you for the suggestion. We have now included T-score/Z-score values for each year of follow-up. However, we do not believe that it is necessary to express the slope analysis as T-scores/Z-scores given that the g/cm2 unit is the more robust analysis (because the Z-score calculations depend on the manufacturers reference group). The medical physicist reviewed the file for the outlier and we had provided a reason for its occurrence and appropriate removal.

11. Last paragraph of the results: what were the BMD changes at the femur?
We have done this analysis and the BMD changes at the proximal femur are now provided in the last paragraph.

DISCUSSION
12. What was the BMI in the fractured group? Was it in the healthy range?
The BMI in the fracture group was 22.8 for men and 22.3 for women (see Table 1). Both of these were in the healthy range as were those of the men and women with no fractures. This sentence has been clarified in the discussion.

13. **Please discuss the role of PTH in the evaluation of bone quality.**
We have provided a discussion regarding bone quality and potential limitations with BMD measurement. We did not focus on PTH in this study, and the discussion of PTH and bone quality is best reserved for another paper.

14. **Please discuss the longitudinal effect of subjects already in the bone growth period (< 25 yrs of age).**
Persons with CF often have delayed growth and it is not precisely understood the pattern of bone growth in this group. However, we did examine age as a covariate in the multi-variable analysis of longitudinal change in BMD. It was a significant factor for whole body (see Table 4). As suggested by both reviewers, we have re-reviewed the files and now provided z-scores for those under 19 and t-scores for over 19.

Sincerely,

Alexandra Papaioannou, MD, MSc
On behalf of corresponding authors