Author’s response to reviews

Title: Clinical and Postmortem Measures of Cardiac Pathology in Subjects with Alzheimer’s Disease

Authors:

Thomas G Beach (thomas.beach@bannerhealth.com)
Chera L Maarouf (chera.maarouf@bannerhealth.com)
Reed G Brooks (reed.brooks@bannerhealth.com)
Scophil Shirohi (scophil.shirohi@bannerhealth.com)
Ian D Daugs (ian.daugs@bannerhealth.com)
Lucia I Sue (lucia.sue@bannerhealth.com)
Marwan N Sabbagh (marwan.sabbagh@bannerhealth.com)
Douglas G Walker (douglas.walker@bannerhealth.com)
LihFen Lue (lihfen.lue@bannerhealth.com)
Alex E Roher (alex.roher@bannerhealth.com)

Version: 2 Date: 3 December 2010

Author’s response to reviews: see over
Dear Editor(s);
Please accept our revision of the manuscript. The reviewers are thanked for their helpful and insightful comments. Our replies to each comment are listed below.

Reviewer # 1

Major points:

1) "...subjects in the study were not evaluated with prefixed timing... on most subjects every two or three years. This kind of approach could bring into the study a bias... Among the many clinical evaluations which one was chosen as the reference and why."

The program aims to examine every subject once per year but due to some subjects not being able to attend exactly as scheduled due to illness, vacations, etc, there is some variability in the frequency of clinical examinations. We chose as the reference examination the final one before death. We presented in the original Table 1 the figures for mean and standard deviation for the interval between the last examination (listed as last BMI) and noted that there was no significant difference between the groups on this measure. Therefore we do not think there is a significant bias in our group treatment in this respect.

2) "In the paper were not considered the drugs... could the authors analyze also what kind of action, if any, they had in the AD, AVD relation?"

We have a detailed current medication history for a subset of cases, i.e. 21/22 of the control group and 27/35 Alzheimer's disease cases. The missing cases are those that came to autopsy before a clinical assessment could be scheduled. We analyzed for all cardiovascular-related medications, grouped by medication class, that had been taken on the final clinical assessment. For every medication class except anti-platelet agents, a smaller proportion of AD subjects were taking the agents. The proportional differences were significant for anti-hypertensive agents (25.9% of AD subjects vs 71.4% for control subjects). These results support our data indicating that cardiovascular disease diagnoses were generally less common in the AD group and make it unlikely that observed postmortem findings of a lesser degree of cardiovascular pathology in AD subjects were due to increased anti-cardiovascular disease treatment. We have added text to the methods, results and discussion sections of the revised paper as well as a new table, Table 3.

3) "Lack also a clinical classification of AD, a TC scan or MRI of the subjects (AD and controls) enrolled."

Our program does not have funding to do imaging on our subjects in the antemortem stage. However, we do standardized neurological and neuropsychological testings annually, and it is from these tests that our subjects are clinically classified. We have added the mean MMSE scores for AD and control subjects to Table 1.

4) "Statistical Analysis: I have some concern about the statistical approach...t-test was not the correct test to compare 4 groups."
   And
5) "Conclusions section (pg 11) the authors stated that the greater statistical strength... for female may be due to the larger BMI... If it was the case, adjusting the analysis for BMI the differences between groups will disappear…"

In answer to both of these concerns, we have added a logistic regression analysis for our two major findings, i.e. decreased coronary artery stenosis and decreased heart weight in AD subjects. We have left in the t-test results as we feel that univariate statistics are always helpful for readers, in addition to more complex multivariate models. The multivariate analysis is adjusted for age, gender, apoE genotype and BMI. With these adjustments, control subjects were significantly more likely to have increased heart weights. Of the variables adjusted for, only diagnosis was significantly associated with heart weight and coronary stenosis, although BMI would likely have been significant as well with a larger sample size. Adjusting the analysis for BMI did not make the group differences disappear so there does appear, for heart weight, to be some responsible factor(s) other than BMI. However, for the adjusted analysis with coronary artery stenosis as the dependent variable, diagnostic grouping was not significant and BMI was the only significant factor (Wald p value = 0.026).

6) ".. they did not have any longitudinal data …therefore their conclusion need to be modulate."

We have modulated the conclusions somewhat, specifically stating that we do not have longitudinal data. However, we cite four longitudinal studies that have previously shown a progressive weight loss over time in demented subjects. These studies, combined with our multivariate analysis results implicating BMI, make it seem very likely that weight loss plays a major role in the reduced cardiovascular pathology seen with end-of-life AD subjects.

Minor points:

1) "…could [the authors] use a different abbreviation [for AVD].."

As reviewer #2 had the same comment and suggested ATH for the abbreviation, we have changed AVD throughout to ATH.

2) "…a strong trend is improper…"

We have instead used "… a trend…"

3) "…Table 3… could be dropped…."

We have dropped the original Table 3 and refer to the data in the text only.

4) ".. for Table 4… four columns would be better…"

We have reformatted Table 4 for four side-by-side columns.

5) "… p-value should be reported in the table… not as a footnote"

We believe that this would be awkward and would interfere with the table structure and so we have not changed as the reviewer requested, we hope this is OK.
6) "...the last paragraph of the conclusion section are not so pertinent..."

We have deleted this paragraph.
Reviewer # 2

1) "… I would include the number of subjects in the abstract…"

We have done this.

2) "I would change AVD [to] ATH…".

We have done this.

3) "….I would use CHD (for coronary heart disease) or CAD (for coronary artery disease)…"

We have done this.

4) "Table 1: I would underline the 3 times increase in apoE4 allele in AD… it is obvious the difference would be highly significant in a larger sample."

We re-examined our data and realized that we had mistakenly attributed one extra e4 allele carrier within the control group, there are actually only 4 subjects with the allele, not 5. On retesting the difference is now significant.

5) "Table 3.. is not necessary…"

The first reviewer also felt that the original Table 3 was not necessary and so we have removed it, discussing the results only in the text. There is a new Table 3 that has data on cardiovascular drug use.

6) "Table 4… suggest to compare AD with controls and not males with females…"

Reviewer 1 also wished us to change Table 4 and we think the changes make it much easier to understand.

7) Figure 1… compare some digital images coming from AD patients (e.g. in the left side of the figure) with some coming from controls (e.g. in the right side of the figure).

We originally did not do this as we did not want to give the impression that one could see the difference between groups qualitatively, which is of course not possible when the mean difference is only 4-5%. However, we have redone the figure as the reviewer suggests, with all of the frames on the left in (A) being control cases and all those on the right in (B) being AD.

8) "I would appreciate some comments [about] AD e.g. inflammation, CNS alterations, frailty syndrome, etc."

We have added a few sentences in the introduction and discussion that help inform the more general reader about AD and specifically about AD in the context of this study.