Author’s response to reviews

Title: Diagnostic accuracy of plasma NT-proBNP levels for excluding cardiac abnormalities in the very elderly

Authors:

Bert Vaes (Bert.Vaes@uclouvain.be)
Victoria Delgado (v.delgado@lumc.nl)
Jeroen Bax (j.j.bax@lumc.nl)
Jan Degryse (jan.degryse@med.kuleuven.be)
Rudi GJ Westendorp (r.g.j.westendorp@lumc.nl)
Jacobijn Gussekloo (j.gussekloo@lumc.nl)

Version: 2 Date: 30 September 2010

Author’s response to reviews: see over
To the Editor-in-Chief of BMC Geriatrics
Dr Melissa Norton

Brussels, Belgium, 30th September 2010

Dear Dr Norton,

We would like to thank you for considering our manuscript ‘Diagnostic Accuracy of Plasma NT-proBNP Levels for Excluding Cardiac Abnormalities in the Very Elderly’ for possible publication in BMC Geriatrics. Herewith we would like to submit our revised manuscript. On the following pages we addressed all the reviewers comments point by point.

This study was a diagnostic cross-sectional study embedded within the Leiden 85-plus Study. The aims of our study were first, to study the relation between NT-proBNP levels and structural and functional cardiac abnormalities in a convenience sample of well-functioning nonagenarians; second, to evaluate the use of NT-proBNP levels to exclude structural and functional cardiac abnormalities in well-functioning nonagenarians. Based on the comments of Reviewer 1 we performed a multivariate regression analysis with LogNT-proBNP as the dependent variable and all echocardiographic variables as independent variables, adjusted for known confounders, in order to account for the complexity of the situation.

As the diagnostic accuracy of a test is dependent from the prevalence of the target condition, the prevalence of echocardiographic abnormalities was changed to severe cardiac disease. The cut-off values for LV hypertrophy and indexed left atrial volume were changed to the severely abnormal cut-off values as published by the American Society of Echocardiography and the European Association of Echocardiography (Lang et al. Eur J Echocardiogr. 2006). As addressed by Reviewer 2 the diagnostic accuracy for significant valvular heart disease was changed to the diagnostic accuracy for severe valvular heart disease. Therefore, the diagnostic accuracy to exclude any echocardiographic abnormality could also be added, as asked by Reviewer 1.

We hope you will consider our revised manuscript for publication in BMC Geriatrics.

On behalf of all authors,

Yours sincerely,

Dr Vaes Bert
Answer to the reviewers:

Reviewer 1 report - Thomas Mueller

Comments:

1. It is not clear of whether the present work is a prospectively conducted sub-study of the Leiden 85-plus study or a retrospective analysis of the previously described cohort? In other words, did the authors intend to study the diagnostic accuracy of NT-proBNP for echocardiographic abnormalities in individuals with the age of 90 years before starting enrolment of patients into the Leiden 85-plus study in 1997, or was this a post hoc decision?

Answer: Every year ancillary studies, like the study of cardiologic abnormalities at age 90, are added to the Leiden 85-plus study. Blood samples were taken within the month after every participant’s 90th birthday, and were kept frozen at -80°C. In the majority of the participants, echocardiography was performed the same day. In the remaining patients, the echocardiography was performed between January and September 2004, in stable hemodynamic conditions. The NT-proBNP levels were determined in one batch for all participants aged 90 in 2006. The echocardiographic measurements were performed by one experienced observer who was blinded to the results of the NT-proBNP levels. Thus levels of NT-proBNP and echocardiographic measurements were determined independent from each other. Therefore, the authors believe this analysis of a previously described cohort is as valid as a prospectively designed sub-study of the Leiden 85-plus study. This was added in the Methods section (page 8, line 1).

2. When did the authors perform echocardiographies and NT-proBNP measurements in their patients?

Answer: Blood samples were taken within the month after every participant’s 90th birthday. In the majority of the participants, echocardiography was performed the same day. In the remaining patients, the echocardiography was performed between January and September 2004, in stable hemodynamic conditions. No cardiac events were recorded between the blood NT-proBNP sampling and the echocardiography. This was added in the Methods section (page 7, line 22).

3. The aim of this study is not appropriately defined. I do not know of whether the authors studied symptomatic or asymptomatic patients with respect to cardiac disease. I suppose the authors are aware of the recent ACC/AHA guidelines for the evaluation and management of chronic heart failure in the adult.

One possibility is that the authors aimed at detecting patients progressing from stage A to stage B of the heart failure pathway. Thus, the rationale of this study would have been to evaluate NT-proBNP as a screening test to be performed before echocardiography. As a consequence, the authors should have enrolled only patients who were asymptomatic and in whom no evidence of structural/functional heart disease was evident from the patient history. Is this true for this study? The relevant information on these issues is missing in the present manuscript. For example, the authors described a significant proportion of patients with well known cardiac diseases. Those same patients should
Another possibility is that the authors studied patients symptomatic for heart failure (e.g., patients with acute dyspnea). Thus, the rationale of this study would have been to evaluate NT-proBNP as a tool to differentiate between cardiac and non-cardiac causes for the patients’ symptoms. If this is true, asymptomatic patients must not be included in such a study.

The approach of the authors to include both symptomatic and asymptomatic patients as well as individuals with and without well known cardiac diseases is not convincing for me. Thus, the authors are advised to clarify the exact study hypothesis (null hypothesis H0 vs. alternative hypothesis H1). According to this study hypothesis the whole results section should be rearranged. The authors should stick to their proposed message (i.e., study hypothesis). Therefore, unnecessary results such as extensive description of data not being related directly to this issue should be deleted in a revised version.

Answer: We thank the Reviewer for this important remark. This study was a diagnostic cross-sectional study embedded in the Leiden 85-plus Study. The aims of our study were first, to study the relation between NT-proBNP levels and structural and functional cardiac abnormalities in a convenience sample of well-functioning nonagenarians; second, to evaluate the use of NT-proBNP levels to exclude structural and functional cardiac abnormalities in well-functioning nonagenarians. All participants were able to visit the study centre in the hospital and were clinically stable during the blood test and the echocardiography. The authors specifically chose not to further select the population based on symptomatology or medical history due to the high prevalence and heterogeneity of symptoms and comorbidities in this age category. Therefore, the authors chose to select the convenience sample based on functional and cognitive abilities rather than symptoms and comorbidities.

4. It is clear that the index test of this diagnostic study is NT-proBNP. However, the reference standard(s) of this study is(are) not clear as well. The authors state that they aimed to exclude structural and functional cardiac abnormalities by NT-proBNP measurements. In the results section, last paragraph they describe that they calculated the test performance of NT-proBNP to exclude 3 or more echocardiographic abnormalities. Does this make sense from the clinical point of view? Usually, at least in younger patients, an index test such as NT-proBNP should be used to exclude any structural or functional cardiac abnormalities (i.e. choosing a cut off value with a high negative predictive value). This is important, because even conditions with only one structural or functional cardiac abnormality should be detected by an extensive cardiac evaluation (including echocardiography) in order to initiate the adequate treatment. Therefore, I cannot understand the authors are satisfied to find 3 or more cardiac abnormalities by their approach. What is the therapeutic consequence of such a strategy?

Answer: We thank the reviewer for this important suggestion. The analysis has been modified accordingly: the performance of NT-proBNP to detect any echocardiographic abnormality was evaluated. This has been included in the Results section (page 11, line 16) and in Table 4.

5. In asymptomatic patients, a variety of structural/functional cardiac diseases can be detected by echocardiography such as systolic or diastolic dysfunction, left ventricular
hypertrophy, wall motion abnormalities, pulmonary hypertension, valvular abnormalities, left atrial dilatation and left ventricular dilatation, etc. Each of these conditions may be an indicator of increased intracardiac pressure and might thereby increase NT-proBNP plasma concentrations. Consequently, NT-proBNP plasma concentrations depend on the severity of each condition and also on the number of eventually coexisting conditions. Thus, it is not appropriate to perform correlation analyses or ROC curve analyses of NT-proBNP with each condition in patients with more than one structural-functional cardiac abnormalities (Table 3 and 4) and it is also not appropriate to describe a relationship between NT-proBNP and number of structural-functional cardiac abnormalities without adjusting for the severity of each condition (Figure 1) (e.g., severe aortic stenosis alone definitely rises NT-proBNP to a greater extent than coexisting mild left ventricular hypertrophy and wall motion abnormality). Instead, the authors should stick to multivariable analyses (i.e., logistic regression analyses with NT-proBNP as the dependent variable and echocardiographic findings as independent variables) in order to account for the complexity of the situation.

Answer: The authors agree with the reviewer that NT-proBNP concentrations depend on a variety of cardiac abnormalities plus the severity of each condition present. Therefore, a linear regression analysis was performed with LogNT-proBNP as the dependent variable and all echocardiographic variables as independent variables, adjusted for known confounders, in order to account for the complexity of the situation. Two models were used, one without the estimated pulmonary artery pressure (model 1) and one with (model 2). This strategy was followed because this variable could only be determined in patients with tricuspid regurgitation and thus drastically limited the number of patients included in the model from 56 (model 1) to 37 (model 2). The adjusted B values in Table 3 were derived from the first model without PAP, and the adjusted B value for PAP was derived from the second model with PAP included. This was also described in the Methods section (page 9, line 14) and the Results section (page 10, line 18).

In Table 4 the diagnostic accuracy of NT-proBNP to exclude severe valvular heart disease and/or systolic dysfunction was added. Also the diagnostic accuracy to exclude any echocardiographic abnormality was added. As the diagnostic accuracy of a test is dependent from the prevalence of the target condition, the prevalence was changed to severe cardiac disease. The cut-off values for LV hypertrophy and indexed left atrial volume were changed to the severely abnormal cut-off values as published by the American Society of Echocardiography and the European Association of Echocardiography (Lang et al. Eur J Echocardiogr. 2006). Also the diagnostic accuracy for significant valvular heart disease was changed to the diagnostic accuracy for severe valvular heart disease.

The figure describing the relationship between NT-proBNP and the number of structural-functional cardiac abnormalities was kept in the paper but it has been adjusted to the new chosen cut-off values for cardiac abnormalities. The hypothesis that an increase of NT-proBNP levels was related to the severity of cardiac disease was deleted. We described the relationship as a simple dose-response effect without any referral to the severity.

6. No information is given on, e.g., arterial hypertension or diabetes mellitus. These conditions are also known to be associated with increased NT-proBNP plasma concentrations. So, how did the authors correct their results for these conditions?

Answer: The prevalence of arterial hypertension and diabetes mellitus was added in Table 1. We did not correct for these conditions because we did not consider them as confounding
variables. Previous studies demonstrated a weak correlation between NT-proBNP levels and hypertension or diabetes at univariate analysis (Costello-Borrigter et al. JACC 2006; Abhayaratna et al. Am Heart J 2006). Moreover, in the entire Leiden 85-Plus cohort at age 90 no correlation was found between plasma levels of NT-proBNP and hypertension or diabetes (Vaes et al. Journal of the American Geriatrics Society 2009). Therefore, we would like to kindly request not to include corrections for hypertension and diabetes in the revised version of the manuscript.

7. The description of the patients’ medications is missing in the manuscript (especially as they are mentioned as confounding variables).
Answer: We thank the Reviewer for this important suggestion. Accordingly, information on medical treatment was included in Table 1.

8. In the description of the index test (i.e., NT-proBNP) information on how long the plasma samples were stored before analysis is missing. What about analyte stability in this context? What was the specimen type (EDTA plasma, heparin plasma)? Usually NT-proBNP is measured in serum (according to the package insert!)

Answer: Blood samples were taken within the month after every participant’s 90th birthday, and were kept frozen at -80°C. The NT-proBNP levels in citrated plasma were determined in one batch for all participants aged 90 in 2006 (Methods section, page 7, line 16). The fact that the analysis was performed after minimal 2 years might have influenced the absolute value, but it is unlikely that it has affected the ranking. This was added in the Discussion section (Page 13, line 1).

9. In the methods, the authors describe that 82 patients with 90 years of age were invited for echocardiography. All but one underwent echocardiography, this makes 81 patients. In the results the authors state that they had 80 patients for analysis. What about one missing patient?
Answer: Transthoracic echocardiography was performed in 81 patients. But in 1 patient NT-proBNP levels were not measured. Therefore we continued our analyses with 80 patients. This was added in the Results section (page 10, line 5).

10. It is unclear why the authors sometimes used gender-specific cut off values (Table 3) and sometimes not (Table 4).
Answer: We thank the Reviewer for this constructive comment. In the revision we used tertiles of NT-proBNP that were not gender-specific (Table 3). The linear regression analysis showed that sex was not an independent predictor of NT-proBNP, although it is considered as a confounding variable. Therefore we did not use gender-specific cut-off values in Table 4.

11. In the discussion, third paragraph, the authors describe: ?The present study confirms that low plasma NT-proBNP levels are most efficient in excluding echocardiographic abnormalities. Moreover our study shows NT-proBNP is related to the severity of cardiac disease and might be used to indicate who needs to be referred for further cardiovascular examination including echocardiography?. According to my previous comments, this statement is definitely overenthusiastic!
Answer: We thank the Reviewer for this important remark. Accordingly, we have tempered the conclusions of our study (Abstract and Conclusions, page 13, line 12).

Reviewer 2 report  Dr A D Ryding

Reviewer's report Minor Essential Revisions
1. Table 2. It is unnecessary to include both mean ± SD, and median ± IQR. One or other should be presented.

Answer: We thank the Reviewer for this important suggestion. The continuous data are presented as median and IQR. Mean values and SD were deleted in Table 2.

Major Compulsory Revisions
1. For pragmatic reasons the researchers have restricted the study to nonagenarians that are active and relatively independent (well-functioning?): such a population is clinically relevant. Nevertheless, the majority of subjects have cardiac abnormalities, as defined by standard reference ranges derived from younger healthy populations. The applicability of these reference ranges to the study population should be discussed.

Answer: The reviewer addressed a very interesting question. Definition of thresholds for normal values is a very controversial issue. Various statistical techniques have been proposed to determine normal cut-off values, all of which have important limitations. In the present study we followed the cut-off values proposed by the American Society of Echocardiography and the European Association of Echocardiography (Lang et al. Eur J Echocardiography 2006). As indicated in the recommendations for cardiac chambers quantification with echocardiography, the cut-off values have several limitations but represent a step forward towards the standardization of clinical echocardiography. A brief paragraph discussing the limitations of these cut-off values has been included in the manuscript (page 13, line 3). In concordance with the remarks of reviewer 1 the cut-off values for LV hypertrophy and indexed left atrial volume were changed to the severely abnormal cut-off values as proposed by Lang et al to lower the prevalence of the target condition.

2. It is debatable whether some of the abnormalities are clinically relevant (e.g. mild/moderate aortic stenosis, mild mitral stenosis). In my view all the analyses should be repeated excluding these categories.

Answer: According to the European, multicenter registry Euro Heart Survey on Valvular Heart Disease (Jung et al. Eur Heart J 2003), significant valvular heart disease, as defined by echocardiography, included:
- Aortic stenosis with a maximal jet velocity ≥2.5 m/s
- Mitral stenosis with a valve area ≤ 2 cm²
- Aortic regurgitation with grade ≥2/4
- Mitral regurgitation with grade ≥2/4

According to the ACC/AHA/ESC guidelines for the management of patients with valvular heart disease, the above mentioned definitions correspond to
- Any aortic stenosis severity: mild (maximal jet velocity < 3 m/s), moderate (maximal jet velocity 3-4 m/s) and severe (maximal jet velocity >4 m/s).
- Any mitral stenosis severity: mild (valve area >1.5 cm\(^2\)), moderate (valve area 1-1.5 cm\(^2\)) and severe (valve area <1 cm\(^2\))
- Moderate (grade 2) and severe (grades 3-4) aortic regurgitation
- Moderate (grade 2) and severe (grades 3-4) mitral regurgitation

Therefore, significant valvular heart disease was defined as any mitral or aortic stenosis severity, moderate or severe mitral regurgitation, and moderate or severe aortic regurgitation. However, the authors recognised this as an important remark. Since the test performance depends on the prevalence of the target condition we repeated the analyses for severe valvular disease. The prespecified cut-off value showed a high NPV of 92% (Table 4). When the cut-off values of echocardiographic abnormalities were limited to severe cardiac disease, we were able to calculate the diagnostic accuracy to exclude any echocardiographic abnormality.

3. The use of the Teicholz method of estimating left ventricular ejection fraction (LVEF) is sub-optimal, and the Simpson’s bi-plane method would be preferable. The study failed to show any significant correlation with LVEF (which is unusual given the plethora of studies demonstrating this) though modest correlations with other measures of left ventricular function were demonstrated. Presumably the small study size, relatively healthy population, and suboptimal method of measuring LVEF reduced the power to demonstrate this. Please discuss.

Answer: We agree with the reviewer that the Simpson’s method to estimate LV ejection fraction may be preferable. However, this method also relies on geometrical assumptions. In addition, several studies have demonstrated that linear measurements from M-mode images are reproducible and have low intra- and inter-observer variability (Ilercil et al. J Am Soc Echocardiogr 2001; Devereux et al. Hypertension 1998). We have discussed this in the Discussion section (page 13, line 6).

4. Table 4. I am not convinced that using the upper limit of NT-proBNP for the lowest tertile as the cut off point for all ROC analyses is valid. The cut off that provides the best AUC should be determined for each individual analysis. Please report specific cut off values for ROC analysis optimized to each parameter.

Answer: We understand the concerns of the Reviewer. However, the AUC in the ROC analysis is not determined by the cut-off value. Every cut-off value yields a different sensitivity and specificity depending on the prevalence of the disease in the study population. We chose to use a pre-specified cut-off value in order to increase the quality of our study as advised by the modified QUADAS tool for diagnostic research.

5. Table 4. ROC analysis is conducted for ?abnormal LV dimensions and/or LV dysfunction?: this composite category is rather broad, and some of the variables are presumably interdependent. I would prefer to see separate analyses for each component if possible ie LV dimensions, LV hypertrophy, LV systolic dysfunction.

Answer: We thank the Reviewer for this important remark. The pre-specified NT-proBNP cut-off value of 269.5 pg/mL identified LV dilatation with a sensitivity, specificity, PPV and NPV of 91%, 36%, 19% and 96%, and LV hypertrophy with a sensitivity, specificity, PPV and NPV of 84%, 42%, 51% and 79%. In order to maintain a clear message of our manuscript we have considered as well the suggestions of the Reviewer 1 and we included the diagnostic accuracy of NT-proBNP levels to exclude LV systolic dysfunction, severe valvular disease and/or LV systolic dysfunction and any echocardiographic abnormality (Results section, page
Therefore, we would like to kindly request not to include this subanalysis in the revised version of the manuscript.

6. The discussion should be expanded to include a more thorough critique of the limitations of the study, in particular the small selected sample, and how this might impact on the generalization of the findings. The potential clinical relevance of the study findings should be discussed in more detail.

Answer: We thank the Reviewer for this important suggestion. This was added in the Discussion (pages 12-13).