Reviewer's report

Title: The relationship between N-terminal prosomatostatin, all-cause and cardiovascular mortality in patients with type 2 diabetes mellitus (ZODIAC-35)

Version: 1 Date: 26 October 2014

Reviewer: Thomas Mueller

Reviewer's report:

COMMENTS TO THE AUTHORS:

GENERAL STATEMENT:
It was a very good idea of the authors to look at the predictive value of N-terminal prosomatostatin (NT-proSST) in patients with type 2 diabetes. Although this is a negative study because NR-proSST was not independently associated with all-cause mortality and with cardiovascular mortality, this work is of importance in its field. The strength of this study is that this is the first description of the predictive capability of NT-proSST in patients with diabetes. However, there are some criticisms that should be addressed in a revised manuscript.

SPECIFIC COMMENTS:

1) The present patient sample should be better described in a revised version (not only referring to references 19-21). In PubMed, I can find articles on several ZODIAC “studies” (up to ZODIAC-41) including those evaluating several other BRAHMS biomarkers. At least for me, the study population of the present work is unclear. If I correctly understand, approx. 3400 diabetic individuals at baseline in 1998 and 4500 diabetic individuals at 2-year follow up in 2000 were described in reference 20, obviously being the primary description of the ZODIAC trial (Eur J Epidemiol 2003;18:793-800). However, in the present manuscript the authors describe 1143 individuals from ZODIAC-1. In reference 19 (Neth J Med 2005;63:103-10), a target population consisting of 2660 patients with type 2 diabetes is described within the ZODIAC trial. How do the respective numbers fit together? Why was an additional number of 546 patients “enrolled” in 2001? Reference 21 (Diabetologia 2009;52:789-97) is related to the UK Prospective Diabetes Study (UKPDS); this publication refers to 973 individuals with diabetes (but not 546 as stated). Is the present manuscript a study reporting on a combined sample consisting of individuals from the Netherlands and the UK? Which kind of study did the local medical ethics committee approve? In the title of the manuscript, the authors use the acronym ZODIAC-35; what does “35” mean?

2) Is this (i.e., the data of the present manuscript) a prospectively or retrospectively conducted study? Was it a post hoc decision to measure NT-proSST plasma concentrations in the present study sample? The nature of this study should be clarified in a revised manuscript.

3) The setting of this study is not clearly described in the present manuscript. I
suppose it was a primary care setting in both patient samples!?

4) It is unclear if the study sample is a consecutive patient series or not. Inclusion and exclusion criteria should be stated explicitly in the revised manuscript.

5) The study was approved by the local ethics committee. What was the a priori study hypothesis in the respective study protocol? The study hypothesis (H1 vs. H0) should be provided in a revised manuscript. The authors should provide their a priori sample size calculation according to the study hypothesis.

6) Is there a trial number at ClinicalTrials.gov?

7) What were exact diagnostic criteria for type 2 diabetes? Did the study participants suffer any comorbidity besides cardiovascular disease?

8) The authors state that endpoints were all-cause mortality and with cardiovascular mortality, and endpoint ascertainment was made in 2009. Why did the authors choose to obtain mortality data in 2009? This is 5 years ago. What was the exact time point of outcome ascertainment?

9) The authors state that the causes of death were determined according to International Code of Diseases Version 9 (ICD9). Why didn’t they use the ICD10? How did they define cardiovascular mortality - ICD9/ICD10 codes 390-459/I00-I99?

10) How was follow up data obtained? Were the hospital records of all hospitals of the Netherlands and the UK studied? Were all practitioners in the Netherlands and the UK surveyed for endpoint ascertainment of each study participant? What about individuals who died outside the Netherlands and the UK?

11) Were outcome data available for all patients? No missing data? What about censored data in the Kaplan-Meier curves including number at risk tables?

12) The authors state that NT-proSST plasma concentrations were measured with a chemiluminescence assay. Where were the measurements done? How were the plasma aliquots stored? How many freeze and thaw cycles were evident in the study samples? What was the time interval between freezing the samples and performing NT-proSST measurements? What about in vitro analyte stability? Were the samples assayed in duplicate? Where from did the authors derive the imprecision data and the stability data at 22°C? The limit of detection is claimed to be 4 pmol/L. What is the measurement range of this assay? This information is essential for interpreting the authors’ data.

13) NT-proSST plasma concentrations were measured in 1327 patients only (as stated in the materials section, 1689 individuals were studied). Why?

14) In the Zodiac study other analytes were measured (Table 1). How were the other clinical and biochemical measures obtained?

15) For several reasons, all continuous data should be expressed as median
(interquartile range), and only non-parametric tests should be used. How did the authors address the multiple testing problems in this work?

16) At least to my knowledge, the authors performed interventions in the ZODIAC trials. Does this hold true for the individuals enrolled into the present work as well? Were the results on the predictive value of NT-proSST independent of the intervention groups or did the have an impact on the results provided?

17) The authors cite 25 references. Of them only 6 were published after 2000 and only one was published after 2010 (an abstract on the AACC 2013). The authors are recommended to update the literature and, thus, to include more recently published references.

18) The authors could use different measurements of performance (discrimination, calibration and reclassification by IDI and NRI) to test the potential incremental prognostic value of NT-proSST in the setting evaluated.

19) The authors use terms such as “serum levels” and “plasma values” throughout the entire manuscript. The authors should instead use the term “NT-proSST plasma concentrations” consistently.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.