Author’s response to reviews

Title: Plasma calprotectin and its association with cardiovascular disease manifestations, obesity and the metabolic syndrome in type 2 diabetes mellitus patients

Authors:

Lise LP Pedersen (lise-pedersen@rsyd.dk)
Mads MN Nybo (mads.nybo@rsyd.dk)
Mikael MKP Kjær Poulsen (mikael.kjaer.poulsen@rsyd.dk)
Jan Erik JEH Henriksen (jan.erik.henriksen@rsyd.dk)
Jordi JD Dahl (jordi.dahl@gmail.com)
Lars LMR Melholt Rasmussen (lars.melholt.rasmussen@rsyd.dk)

Version: 3 Date: 17 November 2014

Author’s response to reviews:

To the Editorial board of Cardiovascular Disorder

We hereby submit the revised edition of the manuscript “Plasma Calprotectin and its association with cardiovascular disease manifestations, obesity and the metabolic syndrome in type 2 diabetes mellitus patients”.

Below is a point-by-point description of the changes made and answers to the questions of the reviewers:

Reviewer 1:

1) Why do you have chosen type 2 diabetic patients not in therapy? There are 2 reasons for this. First, when characterizing a new biomarker the logical course of action is to first characterize the biomarker in a reference population, then characterize the biomarker in a diseased population of interest and finally, the biomarker can be characterized in intervention studies. We have included in the discussion that characterization of calprotectin levels in diabetic patients in therapy is a future perspective. Second, in this study we wished to see whether we could confirm previous studies that indicated that calprotectin can be used to assess the risk of diabetic patients with cardiovascular disease. We have emphasized this aim of the study in the abstract (p. 3, line 10).

2) The use of fecal test on plasma for detection of Calprotectin has already been validated? What is the cost of this test compared to conventional markers of necrosis for example? There are a few ELISA kits available, that are validated for use on both fecal and plasma samples. The automated ELIA calprotectin assay used in this work is only validated on fecal samples and in order to use the assay on plasma samples we had to verify the usefulness of assay with this matrix. The ELIA calprotectin test is approximately twice as expensive as the traditional markers myocardial necrosis such as myoglobin, creatine kinase–MB, and troponin.
3) Therefore, we can define Calprotectin as a new validated marker of chronic inflammation rather than of cardiovascular disease or glucose homeostasis? Yes, the data in this study indicates that levels of plasma Calprotectin in a diabetic cohort are not associated with either cardiovascular disease or glucose homeostasis. Our data indicates that high levels of plasma Calprotectin reflect the chronic low grade inflammation, which is associated with obesity. We have emphasized these findings in the discussion section.

4) So, in non-diabetic patients with hyperglycemia for manifestations of inflammation, for example in sepsis, we expect high levels of calprotectin even if insulin resistance is absent? Yes, a high level of plasma calprotectin is expected in non-diabetic patients with for instance sepsis. An example of this is higher levels of plasma calprotectin found in full-term neonates with late onset neonatal sepsis. In this regard plasma calprotectin can be grouped with acute-phase proteins such as CRP, Procalcitonin and IL6.

Reviewer 2:

1) A brief introduction to the inclusion and exclusion criterias for the diabetic cohort would be helpful. Blood donors must always be healthy. This should be clarified. A short description of the inclusion and exclusion criterias for the diabetic cohort has been added to the methods section.

2) The system is serum and plasma from donors and patients respectively. Why was this chosen? Comparison data on serum and plasma should be included. The available material from donors and patients are from previous separate projects and were originally not collected with the aim of measuring calprotectin. Therefore, only serum was available from the blood donors and only plasma was available from the patients. As requested, we have included comparative data in the methods section (p. 8 line, 8-13).

3) Results are presented in both the main text and in tables and figures. The discussion could probably be shortened and sharpened. Page 11, line 12, to page 12, line 14 have been deleted and instead references to tables and figures are used. We have shortened the discussion and tried to emphasize the individual points.

4) Do 266 of the 305 patients have metabolic syndrome? Or 243 as stated in table 2? 243 patients have metabolic syndrome. P. 10, line 15 has been revised.

   a. Page 11, line 14. Data have been removed from the text as they appear in table 2.
   b. Page 11, line 20. The analysis was performed on all 305 T2DM2 patients and height was only associated with calprotectin in the METs positive sub-group. Therefore it is not included. The list of parameters has been removed as they appear in table 3.
   c. Page 11, line 26, and page 12, line 1. Not all variables are shown in figure 1. Text has been changed to match the figure.

Grammatical errors pluralis/singularis have been edited.
Reviewer 3:
Major revisions: Personally, I find it hard to understand the study design and outcomes from the abstract, while methods are well exposed. The abstract has been revised in order to clarify the study design and outcomes. In the background section we have added that calprotectin was measured in order to evaluate whether it can be used to assess the risk of cardiovascular disease in diabetic patients without known CVD (p. 3, line 10).

Minor revisions: The acronym CAD is both used for Carotid artery disease and coronary artery disease leading to misunderstanding. The acronym CAD here is for Coronary artery disease. Acronyms have been revised accordingly in the abstract (p. 3, line 13. The definition of CAD has likewise been edited in the abbreviations list (p. 16, line 21).