Author's response to reviews

Title: Diagnostic strategies for C-reactive protein

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PDF covering letter
Reviewer 1

Regression analysis
Reviewer 1 expressed concerns about using the various metabolic syndrome X parameters in a prediction based on regression. We beg to differ. The fact that these parameters can reflect the same (atherosclerotic) process as CRP is exactly why they are useful. The ultimate goal is to formulate criteria to decide whether a high CRP value is a true indicator of chronic disease. At the same time we want to take as little blood samples as possible. One way to combine these goals, is to assess as much additional informative parameters as possible in the same single blood sampling session in which CRP is obtained as well. By comparing the observed CRP to a CRP value predicted from all other information from that sample, we get an idea of the validity of that CRP value. To predict the CRP from the extra information we used a formula with parameters obtained from regression analyses in the three subpopulation, males, females-OC females non-OC. This is the standard scientific approach of prediction.

Use of tertiles
The text on tertiles is made more coherent.
1. General comments

- a. Empirical numerical data are given in 3 significant figures now in table 1. Table 2, 3, 4, 7 present non-biological data (correlation and variance) and adding extra figure does not meaningfully add to reliability.
- b. Reference range for healthy individuals for the method used.

CRP was determined using polyclonal antibody against CRP, both for catching antibodies and for conjugated antibodies (DAKO, Denmark). The reference range for healthy individuals of the method used is 2.5 – 97.5% content interval: 0.07 – 5.25 mg/L. These figures were included in the method section.
- c. Epidemiologitis

Our research question called for statistics.

2. Specific points

- a. Abstract – grammatical corrections
  - i. sentence changed.
  - ii. done
- b. Background
  - p3, grammatical correction; done
  - p4: variance of ln(CRP)

The rationale for using lnCRP in the calculations is now given more explicit in the method section, in the section ‘statistical analysis’: “we used a natural logarithm transformation of CRP (lnCRP) to meet the demands of normal frequency distribution prior statistical analyses.” And “We used lnCRP because regression analysis demands a normal frequency distribution.”
- c. Methods
  - p6 NaF instead of NAF; done
  - GC done
  - p6/7 effects of long clotting times on the results of the different analytes.

This point raised by the reviewers is relevant for the coagulation parameters e.g. fibrinogen only. The values obtained for fibrinogen were all within the detection range of the method used and therefore effects of long clotting times should be considered absent/neglectable in the current sample. The methods used for obtaining fibrinolytic system (tPA and PAI-1) do not rely on clotting times.
  - SC Friedewald formula. Non of the subjects suffered from pathologically high TG or Chol levels.
  - p7 GC; done
  - p7 more detailed description of the CRP assay and variation coefficients for the assay at different points within the reference range.

Both points are very important for the current paper and have been included in the text of the manuscript, as given below. Note that the variation coefficients hardly differ for the different points within the reference range.

C-reactive Protein (CRP) was measured using a sensitive enzyme immunoassay. Briefly, polystyrene microtiterplates were coated with a polyclonal antibody against human CRP.
Test samples were incubated in a 1000 times dilution for 2 hrs at room temperature. After a washing procedure, detecting antibody solution was added (peroxidase labelled polyclonal antibodies against human CRP, DAKO A/S, Glostrup, Denmark). After a 1 hr incubation at room temperature, the microtiterplate was washed and substrate solution was added. After a 15 minutes incubation period at room temperature the reaction with substrate was stopped with the addition of sulphuric acid. The colour development was recorded spectrophotometrically and the CRP content of the test sample was calculated from the calibration curve. Standard CRP serum (Dade behring, Marburg, Germany) was used for calibration, which was calibrated against the international standard for CRP (WHO-code 85/506).

The intra-assay coefficient of variation at different points within the reference range were 2.4% for CRP concentrations < 0.5 mg/l, 1.8% for CRP concentrations between 0.5-2.5 mg/l, 1.8% for CRP concentrations between 2.5-5.5 mg/l, and 3.1% for CRP concentrations higher than 5.5 mg/l.

- **d. behavioural risk factors**
  - We explicitly wanted to include ‘smoking’ as a predictor in the regression analyses. We must agree with the reviewer that the a ‘glass’ is not the best measure to quantify alcohol consumption in terms of ethanol-ingestion. Unfortunately this is the way this variable was measured in our population. Leaving out alcohol data alltogether seemed a worse solution. Fortunately, recent findings suggest that moderate alcohol intake unrelated to a reduction in C-reactive protein (Sierksma A et al. Ann N Y Acad Sci 2001;936:630-3.)

- **e. statistical analysis**
  - p11 ranges for CVs are now in ascending order.
  - p12 GC done

3. **Additional comments**

determination of ERS

This is a good and practical suggestion. However, the authors share the reviewer’s concern regarding the sensitivity of an ERS measure.