Dear Reviewer,

thank you very much for your invaluable suggestions for revising our manuscript. We introduced your comments and critical remarks in the revised manuscript.

Here, we want to explain how the manuscript was revised. The changes are indicated using blue color.

The study has been conducted exclusively on patients with established CVD. The importance of IL-10 serum levels and haplotype analysis in these patients may be seriously questioned, as these patients are already characterized by very high CVD risk.

The reviewer is absolutely right that the patients examined already are on an increased cardiovascular risk. The inclusion criterion for enrolment in the study was cardiovascular disease with the need to perform a coronary angiography.

However, the present study was not designed to determine whether an altered IL-10 level or IL-10 haplotype is associated with the occurrence of cardiovascular disease in previously healthy individuals.

In contrast, it was to be investigated whether the clinical long term outcome of patients with pre-existing cardiovascular disease is associated with the IL-10 level and/or genetic characteristics in the IL-10 gene. To examine this question, the patients were followed for 10 years with regard to the progression of their cardiovascular disease.

The study is limited by its design as a sub-study of a longitudinal cohort study designed for other reason

*Fo*r the study "Periodontitis and Coronary Heart Disease" (ClinTrials.gov identifier: NCT01045070) 1002 patients with cardiovascular disease and the need for coronary angiography were recruited from 10/2009 to 02/2011. The main aim of the study was to investigate the significance of periodontal inflammation on cardiovascular outcome. Periodontal inflammation is characterized by the complex interaction of a large number of inflammatory markers, including IL-10. For this reason, we focused in this so called sub-study on one factor of the inflammatory pathway, IL-10.

All patients included in the study "Periodontitis and Coronary Heart Disease" also represented the patient population analyzed in the sub-study reported here.

The limitations of the study were discussed in the corresponding section.

Of the 1,002 patients who met the inclusion and exclusion criteria, more than half were excluded from protein analysis. This is a high percentage. The authors mention as reason for this exclusion the determination of IL-10 levels below measurement limits. Why was IL-10 in these samples below measurement limit? Are the authors justified to exclude these patients for this reason or is this biased?

The protein expression analyses performed in this study were measured with the BD™ Cytometric Bead Array for human IL-10 (BD Bioscience, Heidelberg, Germany). Unfortunately, a high percentage of patients were below the detectable level of this kit. When we consulted an experienced statistician (Prof. A. Wienke, Institute of Med. Epidemiology, Biometrics and Informatics, Martin-Luther-University Halle-Wittenberg, Halle, Germany), he gave us the opportunity to randomly generate values between 0 and the lower detection limit of the test system. These data were used for a further calculation (see below). As suggested by the reviewer, the 25IQR (<0.45pg/ml) / 75IQR (>1.48pg/ml) was used as a basis (see also the

changes in the manuscript, Figure 4.). Here too, patients with increased IL10 expression have a higher risk of suffering an adverse cardiovascular outcome (p Breslow = 0.046).

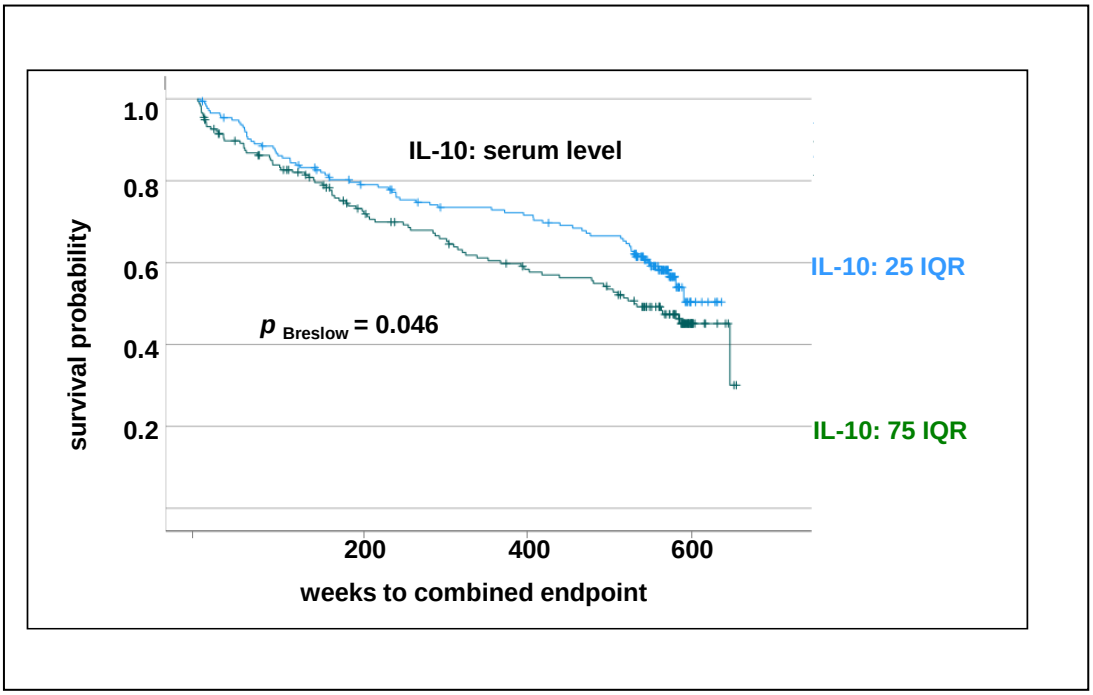

Figure a. Kaplan-Meier survival curve and Breslow test. Impact of IL-10 level (IQR25: < 0.45 pg/ml vs. IQR75: > 1.48 pg/ml) on combined endpoint (myocardial infarction, cardiovascular death, stroke/ transient ischemic attack, death from stroke) in 10-years follow-up.

Baseline characteristics of the patients not included definetely need to be presented versus those who were included for protein analysis.

The baseline data taking into account the two patient groups (IL-10 expression below the detection limit vs. IL-10 expression above the detection limit) have been added in the following table. This table was attached to the manuscript and uploaded as supplemental data.

Table a. Baseline characteristics of cardiovascular patients below and above detection limit of IL-10 serum level. Skewed variables were evaluated by Mann-Whitney U Test and presented as median (IQR: 25th/75th-interquartiles). Categorical variables are presented in percentage and compared by Chi-square test. (IQR: interquartile range; TIA: transient ischemic attack; MI: myocardial infarction; HDL: high density lipoprotein; LDL: low density lipoprotein).

* Yates correction.

Characteristics	Patients with IL-10 level below detection limit (n=455)	Patients with IL-10 serum level above detection limit (n=421)	p-value
Demographical and anamnestic parameters			
Age, years (median; 25/75 IQR)	68.7 (59.3/74.5)	68.2 (59.5/75.0)	0.542
Female gender (%)	23.5	28.1	0.139*
Current smoking (%)	11.6	12.8	0.668*
Body mass index, kg/m2 (median; 25/75 IQR)	28.3 (25.1/30.9)	28.1 (25.4/30.7)	0.743

History of			
Diabetes mellitus (%)	35.2	32.5	0.454*
Hypertension (%)	87.9	88.6	0.834*
MI (%)	35.2	41.1	0.083*
Stroke/TIA (%)	12.5	14.0	0.583*
Peripheral artery disease (%)	8.1	10.5	0.286*
Biochemical parameters (median; 25/75 IQR)			
C-reactive protein (mg/l),	7.3 (2.3/29.5)	10.8 (5.0/38.1)	0.001
Leukocytes (Gpt/l),	7.9 (6.4/9.7)	8.0 (6.4/9.6)	0.776
Interleukin 6 (pg/ml),	7.1 (3.5/14.1)	7.8 (3.8/17.1)	0.192
Uric acid (µmol/l)	5.7 (4.2/7.4)	5.7 (4.5/8.0)	0.128
Creatinine (mmol/l),	85 (72/106)	88 (73/109)	0.173
Total cholesterol (mmol/l),	4.4 (3.8/5.4)	4.3 (3.7/5.3)	0.150
HDL cholesterol (mmol/l),	1.0 (0.8/1.3)	1.0 (0.8/1.2)	0.113
LDL cholesterol (mmol/l),	2.7 (2.1/3.5)	2.6 (1.0/1.9)	0.137
Triglycerides (mmol/l),	1.4 (0.9/2.0)	1.4 (1.0/1.9)	0.618

None of the reported data differed significantly between the two patient groups except for CRP. In bivariate correlation analysis, it was shown that the level of CRP and Il-10 are positively associated (Spearman Rho: 0.113; p>0.001). For this reason, the significant dependence in the Chi^2 test can also be explained if the patients are subdivided according to the Il-10 level.

Similarly, did patients who were excluded from genetic analysis differ from those who were included?

2 patients out of 942 could not be included in the genetic analyses. The patients were a 65-year-old woman and a 73-year-old man. However, since only 0.2% (2 of 942) patients were not genetically determined, the clinical characteristics of the two patients should not confound the results of the group with respect to the genetic analyses.

To evaluate the influence of IL-10 serum levels on CVD outcome, IL-10 were dichotomized. Did the authors perform an analysis based on 25/75IQR? This would probably have been more meaningful, as upper and lower values of biomarkers are used for outcome prediction.

The authors would like to thank the reviewer for his valuable advice. A corresponding figure based on 25/75IQR has been included in the manuscript (Figure 4).

Did the authors evaluate levels of other interleukins or other markers of subclinical inflammation such as hs-CRP?

The study also analysed CRP and Il-6 as inflammatory markers. These two markers are listed in the introduction. The patient cohort showed increased values for C-reactive protein (8.6 mg/l (25/75IQR: 3.7/32.9) compared to the reference range (<5mg/l). However, Il-6 was within the internal laboratory reference range (IL-6: 7.8 pg/ml, 25/75IQR: 3.8/15.9).

Both markers are also listed in the new table included in the supplements. CRP and Il-10 correlate positively with each other as mentioned above (Spearman Rho: 0.113; p>0.001).

In the Abstract, the "conclusion" is not actually a conclusion of the study findings.

The “conclusion” of the abstract was revised: “Conclusion: In the present study, an elevated IL-10 level but not the Il-10 haplotype was linked to an adverse cardiovascular outcome (10-year follow-up) in a cohort of CVD patients.

The introduction needs to be slightly shortened, as some parts appear to fit better in the discussion section.

The introduction was restructured and revised on the helpful recommendation of the reviewer.

The discussion should start by summarizing the study findings.

The discussion section was revised as pointed out by the reviewer. This section is preceded by a brief summary of the study results.

We hope we have satisfied the concerns of the editorial board and the reviewer with regard to the points marked.

With kindest regards
Susanne Schulz on behalf of all authors