Reviewer's report

Title: A Novel Multiplex Polymerase Chain Reaction Assay for Profiles Analysis of Peripheral Blood Gene Expression

Version: 1 Date: 28 December 2011

Reviewer: Tomasz Dziedzic

Reviewer's report:

The authors have developed the multiplex chain reaction assay for analysis of peripheral blood gene expression and tried to use it as a tool to diagnose coronary artery disease (CAD). Although I have no major concern regarding the technical aspects of the multiple gene expression analysis system, I’m not convinced that described method will be helpful in CAD detection.

Major Compulsory Revisions.

1. The studied group is not well characterized. Were the patients with CAD symptomatic? Which criteria were used to diagnose CAD on CT? What was a distribution of vascular risk factors (hypertension, diabetes mellitus, hypercholesterolemia, smoking etc.) in patients and controls? What about drugs that could change inflammatory markers expression (aspirin, statins, ACE-I etc.)? Of note, the increased expression of inflammatory genes could be related to diseases such as diabetes or hypertension. Thus, CAD group and control group should be matched not only for age and sex, but also for major vascular risk factors. Alternatively, 2 control groups should be used: healthy subjects without vascular risk factors and persons with vascular risk factors, but without CAD.

2. The choice of analysed genes should be justified. It seems reasonable that before planning multiplex experiments, separate PCR studies should be done to find the appropriate genes (selected from previously published data or chosen due to their role in pathogenesis of CAD) whose expression is significantly changed in blood cells of patients with CAD. It is important, because the expression of only 4 from 15 analysed genes was different between groups. Thus, it is questionable if it makes sense to measure the expression of 15 molecules.

3. When one looks at Figure 5, it is clear that although there is a significant difference in expression of 4 genes between groups, the values from CAD patients and controls are overlapped. Thus, in clinical practice it will be difficult to differentiate persons with and without CAD. The authors should used the cut-off point (for example values below 2 standard deviation of mean obtained in control group) to assess if the results of test correctly classify participants into 2 groups. Does the combination of 4 markers better discriminate patients from controls than single markers?

To sum up, much more effort is needed to validate and optimize this new analytic
system for CAD diagnosis.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**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