Reviewer's report

Title: A Novel Multiplex Polymerase Chain Reaction Assay for Profile Analyses of Gene Expression in Peripheral Blood

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Reviewer: Tomasz Dziedzic

Reviewer's report:

I would like to express my respect to authors’ effort to improve their manuscript. I realize that (1) manuscript deals with important problem of non-invasive diagnosis of asymptomatic coronary artery disease and (2) the authors made technical progress by optimizing multiplex RT-PCR protocol.

Unfortunately I still doubt if this protocol could be useful in clinical practice for identifying subjects with asymptomatic coronary artery disease.

In my opinion it is meaningless to measure 15 cytokines whereas only 4 of them differ between studied groups. The authors should justify why they think that test measuring 15 cytokines will be better than one measuring 4 cytokines.

The authors should calculate positive and negative predictive values of the test using the cohort which already has been studied and confirm the results in independent sample. Without this kind of information, the only advantage of the manuscript is technical protocol to measure simultaneously the expression of 15 cytokines in blood. Such a protocol is publishable and suitable for journal focused on molecular methods, but without data about predictive values, the manuscript seems to be not feasible for journal dedicated to clinicians.

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'