Reviewer's report

Title: A search for potential biomarkers of cardiac allograft rejection using expression profiling of human endomyocardial biopsies

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Reviewer: Marja Steenman

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General

The goal of the study by Karason et al. was to find serum biomarkers for diagnosis of cardiac allograft rejection. They used Affymetrix DNA microarrays to look for genes differentially expressed in cardiac biopsy material during rejection in 3 patients. They identified a list of 16 genes with an interesting gene expression profile and selected from this list 2 genes for analysis by ELISA in blood samples of a larger group of patients. Since these 2 genes had already been described in the literature as being preferentially expressed in cardiac allografts during rejection, an analysis of the literature would have provided the same result as the microarray analysis. The subject of the manuscript is important and interesting, even the negative result. However, in my opinion the data have not been explored to their full potential, and positive results can not be excluded at this point.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. The main message of the authors is that CXCL9 and CXCL10 are not serum biomarkers for cardiac allograft rejection. This is not at all reflected by the title, therefore the title should be changed.

2. ‘Data analysis’ paragraph in the ‘Methods’ section: I was not able to reproduce their analysis since the description is too succinct. “Genes were classified as detectable”: This means that they were classified as P(resent), M(arginal) or A bsent). What do the authors do with this information? Do they keep all genes for their analysis of the 9 arrays? Probably not, otherwise there would be no need to classify the genes. Do they delete Absent genes in at least one array on all arrays? Do they keep genes that are Present or Marginal on at least one of the arrays? There are many ways to use this information and without it I have no way of knowing on how many (and what) genes they performed their search for differential genes. The inclusion filters are also not clear: The minimal signal of 200: It is not specified if this concerns before, during or after rejection, or in all 3 situations. Based on Table 2 I am assuming that this only concerns during rejection. They also mention a minimal fold change of 1.6 without specifying between what situations: Between ‘during rejection’ and ‘before rejection’, between ‘after rejection’ and ‘during rejection’, for both comparisons or for at least one? It is also not explained how they predefine the 9 clusters. And finally, I think they may have missed genes: Why not look for genes that are Present during rejection but Absent before and after rejection? In this way I identified HLA-G in patients 12 and 8, CCL14-CCL15 in patient 1 and AIF1 (allograft inflammatory protein 1) in patient 8.

3. ‘Serum analysis of CXCL9 and CXCL10 concentrations’, page 11: Why didn’t the authors test BNP as a positive control (Hammerer-Lercher et al. J Heart Lung Transplant. 2005 Sep;24(9):1444)?

4. In the Discussion, the authors mention microarray studies in animal models. They should compare the results from those studies to their results. What happened to those genes in the patients in this study? Where they differentially expressed or not at all. Differences and similarities should be discussed.
5. Although the negative result (CXCL9 and CXCL10 are not serum biomarkers for cardiac allograft rejection) is in itself important, it would have been interesting to now the identities of the genes in clusters 1, 2, 8 and 9. It is not impossible to think that the absence of a marker might be linked to the diagnosis of rejection. This should have been further explored, especially since no marker was identified.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. In the ‘Background’ section, the authors cite several studies that used DNA microarrays to search for genes with altered expression in human diseases. Since their study is on heart tissue, they should cite at least 1 DNA microarray study on cardiac disease.
2. The name of PPIA should be written in full.
3. ‘Serum analysis’ paragraph, page 8: “CXC9 and CXC10” should read “CXCL9 and CXCL10”.
4. On page 10 and in Table 1 the authors mention “cardiomyopathy” when referring to DCM patients. I am assuming this should be “dilated cardiomyopathy”.
5. Last sentence on page 12: “only 3 of the subjects” should read: “only 3 of the biopsies”, since from the cited paper it is not clear whether these biopsies are from 1, 2 or 3 patients.
6. Legend to Figure 2: “The gene expression of” should read: “Gene expression levels of”. “Bars indicate the analysed time-points in the histopathological sequence” should be deleted since the function of the bars is not to indicate the time-points but the actual gene expression levels. (This comment also goes for the legend to Figure 3.) “The gene expression was related to” should read: “Gene expression levels were relative to”. “*<0.05” should read “*: p<0.05”.
7. (Legend to) Table 1: “HCMP” should read “HCM”
8. Table 1 (and not Tabel 1): “Diagnose” should read “Diagnosis”.
9. In Figure 2 commas should be replaced by dots.
10. In Figure 3 the correlation coefficient should be given.

Discretionary Revisions (which the author can choose to ignore)

1. In the ‘Methods’ section, ‘DNA microarray analysis’ paragraph the authors cite the Affymetrix manual and one of their own papers. It is not clear to me what information is in their paper that is not in the Affymetrix manual.
2. Why weren’t all patients analyzed by ELISA?

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests