Author's response to reviews

Title: Information encoded in a network of inflammation proteins predicts clinical outcome after myocardial infarction

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Author's response to reviews: see over
Dear Editor,

Thanks for further considering our paper for publication.

We have addressed all the reviewers’ comments and suggestions. A point-by-point response is included below.

We look forward to receiving news from you.

Sincerely,

Francisco Azuaje.
We thank the reviewers for their constructive feedback. In the manuscript, text modifications are highlighted in blue.

REVIEWER 1

1. “Throughout the manuscript, it was not clear whether the authors focused on MI itself or complications of MI, specifically left ventricular dysfunction and/or heart failure. The authors should clarify the point and make necessary revisions in the title, abstract and throughout the manuscript accordingly.”

Response:

The main focus is to predict clinical outcome after MI, in particular left ventricular dysfunction. We have clarified this in title, abstract and Introduction. For example, the new title is: “Information encoded in a network of inflammation proteins predicts clinical outcome after myocardial infarction”.

2. “It may be important to discuss whether inflammation markers studied by the authors are more likely to be causes of MI or consequences of MI.”

Response:

Inflammation can cause plaque rupture in the coronary arteries, which leads to MI. However, this type of inflammation is highly localized in the heart. Therefore, we cannot confer putative causative roles to our proposed circulating biomarkers.

This has been added to Discussions.

3. “If a high-traffic node is more likely to be a biomarker for post-MI, it was surprising that none of the best known inflammation biomarkers for post-MI were contained in the list of top-10 high-traffic nodes. Furthermore, the known inflammation biomarkers appeared to be in low (or medium)-traffic nodes. The question is which group of nodes is more likely to be the biomarkers. Can authors search public gene expression databases or collaborate with other investigators who have performed microarray analyses to provide some experimental support to their predictions?”

Response:

Despite their established use, standard inflammation markers are poor predictors of clinical outcome after MI. Prior to this investigation we did not have evidence to suggest that known biomarkers would appear as top high-traffic nodes. Our research indicates that indeed standard biomarkers do not necessarily act as network bottlenecks in the inflammation network. This confirms the known little predictive power of widely-applied inflammation biomarkers as shown by others and our previous research. Thus, our results offer a possible explanation for the lack of predictive capability of traditional markers: They have limited roles as central mediators or coordinators of inflammation responses in MI.
This has been added to Discussions.

We are currently open to consider potential partnerships to expand evaluation of our predictions, which have not been reported in the literature to date.

REVIEWER 2

1. “Page 12-13: Why the SF3A1 was chosen as the housekeeping gene and not the ACTB, GAPDH, 18S et al which commonly used as the housekeeping genes in Q-PCR? Delta-delta Ct method was used for calculate the relative expression level of target genes using Taqman assay, have the authors performed the standard curve analysis to determin if all the primers have the similar PCR amplification efficiency?”

Response:

As the reviewer indicates, ACTB, GAPDH and 18S rRNAs are often used as housekeeping genes in qPCR experiments. However, it is also known that their expression is not always stable and may vary depending on experimental conditions.

In order to select the best housekeeping gene for this experiment, we measured the expression of 12 different known housekeeping genes: ACTB, GAPDH, UBC, B2M, YWHAZ, SF3A1, 18S rRNA, CYC1, EIF4A2, SDHA, TOP1 and ATP5B, using the geNorm™ Housekeeping Gene Selection Kit (PrimerDesign). Results indicated that the expression of SF3A1 was the most stable between samples.

We indeed determined the amplification efficiency of each primer pair during the set up of this multiplex TaqMan qPCR essay: efficiencies varied from 93.1% to 102.8%, depending on the primer pair studied. This indicated high amplification efficiency for each gene. Moreover, we compared the expression of each gene in the multiplex reaction to its expression in a singleplex reaction. Ct values obtained for each gene were identical between multiplex and singleplex PCR, indicating that the multiplex reaction was not rate limiting.

This information has been added to Methods.

2. “Page 16: "The highest classification performance (AUC = 0.84) was obtained when using the individual expression values measured in M11 and M17 (6 genes with available microarray data: CD5, PIK3C3, FBXL2, MIA3, SHKB1 and ZC3H7A)."

Since the gene expression level from microarray is not accurate enough, so the Area under the curve (AUC) should be estimated by Q-PCR data which is more accurate. The authors should make it clear that how to combine the genes expression level to calculate the AUC?”

Response:

The reviewer points out a critical limitation often observed in biomarker studies: the difficulty in specifying an unambiguous connection between observed expression values and the AUC estimates. We avoid this pitfall by:
1. First, standardizing all gene expression values on a common numerical scale. This was done by re-scaling the input genes so that their means and variances were equal to 0 and 1 respectively.

2. And using the standardised outputs of the logistic regression classifiers to calculate the AUC.

Thus, the AUC values do not depend on the original scales of gene expression data. This has been clarified in Methods.