Author’s response to reviews

Title: Integrative model of leukocyte genomics and organ dysfunction in heart failure patients requiring mechanical circulatory support: A prospective observational study

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Author’s response to reviews:

April 28, 2017
Matteo Pasini (Handling Editor)
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Re: MGNM-D-16-00112

Integrative model of leukocyte genomics and organ dysfunction in heart failure patients requiring mechanical circulatory support: A prospective observational study
Submission of Revised Manuscript

Dear Editor,

Thank you for the opportunity to submit a revised version of our above manuscript. We have included a detailed point-by-point response to the reviewers’ comments below.

We feel that the revision has substantially improved the manuscript and hope that it may now be suitable for publication in BMC Medical Genomics.

Sincerely,

Mario Deng MD FACC FESC
Professor of Medicine

RESPONSE TO EDITOR/REVIEWER COMMENTS:

Manuela Cabiati (Reviewer 1)

Methods session "Sample processing - Sample collection and RNA isolation"

“I am somewhat unclear on how they processed their samples. The authors should better explain the extraction method used. Were leukocytes isolated from the plasma/serum samples prior to extracting RNA? How is the degree of RNA purity and integrity? The authors should better explain the principles of the kit used (Rneasy QIAamp RNA Blood Mini Kit, specific for purification of cellular RNA from fresh whole blood, I suppose) for RNA extraction”.

Response: We thank the reviewer for the important question allowing us to clarify the method that was used and have added the following paragraph: “We collected blood in Vacutainer Cell Preparation Tubes (CPT) with sodium citrate (Becton Dickinson, Franklin Lakes, NJ). Peripheral blood mononuclear cells (PBMC) were isolated using manufactual protocol. In short: Samples were processed within 2hrs after blood collection. The CPT tube was centrifuged at room temperature (22⁰C) for 20min at 3000RPM or 2000RCF. Plasma was separated without disturbing the cell layer. The cell layer was collected, washed with Phosphate Buffered Solution (PBS)( Thermo Fisher Scientific, Woodlend Hills, CA), and centrifuged again for 20 min at 1135 RMP or 300RCF at 22°C. The supernatant was aspirated, the pellet was washed with PBS, and centrifuged for 20min at 5.6 RPM at 4°C. The supernatant was discarded. The pellet was
dissolved in 0.5 ml of RNA protect (Qiagen, Valencia, CA), frozen, and stored at -80°C. The RNA was isolated from the PBMC using RNeasy Mini Kit (Qiagen, Valencia, CA). The purity and concentration of the RNA was checked by NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). The concentration was 50 ng/ml. The purity was 260/280 ~ 2.0. The integrity of the RNA was analyzed by Agilent® 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA); RIN > 9.0 and average > 9.5.”

Methods session "Leukocyte Subpopulations"

“The authors should better explain if they have quantified the percentage of leukocytes extracted. What specific cells do the authors think are the most involved in the process studied?”

Response: We did not quantify subpopulations because we believe – based on the Allomap experience – that we need an integrated prognostication measure. Therefore, we inserted the following sentence: “Based on the successful AlloMapTM test development, which was the first FDA-cleared PBMC-GEP-based In-Vitro-Diagnostic Multivariate Index Assay (IVDMIA) that maps the interaction of a transplanted heart’s allo-endothelial cells with the recipient’s leukocytes, we chose the same mixed PBMC population, refraining from isolated subpopulation analyses, and used this preliminary data for our current project and its planned clinical utility.”

Robert M. Blanton (Reviewer 2)

The main concern is that the study does not identify how the findings after mechanical support surgery differ, or not, from those after other types of surgeries.

“Ideally the study would contain control, non-MCS patients, and more importantly would perform a similar analysis on patients receiving other types of cardiac surgery or non-cardiac surgery. This would help answer the question of what gene expression patterns are unique to the MCS population. This limitation is of course difficult to address experimentally in the present study. However, increased discussion of this in the manuscript, particularly in the Discussion section, would be useful.”

Response: We agree with the reviewer’s comment. For this study, we chose a homogenous population. For comparison, we are planning comparative studies with heart failure patients on Optimal Medical Management (OMM), heart transplantation (HTx) and hi-risk coronary artery bypass surgery (CAGB). Therefore, we inserted the following sentence: ”To address to most pressing clinical problem of MCS-related perioperative MOD [Deng 2001, Deng 2005, Kirklin 2014=1], we chose to base this analysis on AdHF-patients undergoing MCS-surgery alone.”
“Control Population: While we chose to base this analysis on AdHF-patients undergoing MCS-surgery alone to address the problem of MCS-related perioperative MOD [Deng 2001, Deng 2005, Kirklin 2014=1], we acknowledge that we have not addressed aspects of the PBMC-biology related to MCS-surgery intervention versus general heart surgery.. In order to address this question, we have initiated a follow-up project examining AdHF-cohorts undergoing A) Optimal Medical Management, B) Heart transplantation, C) High Risk Coronary Artery Bypass Surgery, and D) healthy volunteers, utilizing the same study protocol.”

Background Section Model Citations of the Literature.

“The detailed model outlined in paragraph 2 of the Background section and in Figure 1 would benefit from more citations of the literature.”

Response: We agree with the reviewer that the background literature benefitted from addition of key literature. Therefore, we inserted the following sentence paragraph: “Heart failure (HF), initiated by various mechanisms, leads to compensatory chronic upregulation of sympathetic nervous system and renin-angiotensin-aldosterone system activity in an attempt to maintain blood pressure, cardiac output (CO) and oxygen delivery (O2). Further progression of myocardial injury leads to HF progression with reduced CO and O2–delivery to organs/tissues. This triggers, hypothetically via organ-specific endothelial cell /platelet()/peripheral blood mononuclear cell (PBMC) interactions, compensatory immune system activation, which is first described by elevated tumor-necrosis factor–alpha levels in patients with most severe HF [Levine 1990]. This hypothetically provides short-term compensation for the failing heart. This immune system activation unfortunately promotes organ dysfunction in the kidneys (higher creatinine), liver (higher bilirubin), bone marrow (lower platelets) and brain (worse Glasgow Coma Scale). Therapies such as MCS implantation restore normal CO and O2–delivery to the organs, yet are associated with an unpredictable inflammatory transition state, and increased risk of organ dysfunction and death [Deng 2001, Deng 2005, Kirklin 2014] (Figure 1).”.

Study Sample Size

“The n's of the study are relatively small. However, this is acknowledged appropriately in the discussion.”

Response: We agree with the reviewer’s comment. As indicated by the reviewer, we had previously incorporated the following limitation paragraph: ” Sample Size: While our model was able to accurately identify and integrate many known features of organ dysfunction following MCS surgery, we acknowledge statistical limitations due to the small sample size of our dataset. The small sample size affects the predictive modeling aspect of our study, most
particularly the Cox survival models, while the unsupervised approach based on WGCNA has been shown in previous studies to be robust even at small sample sizes (n<30) [44-46]. Furthermore, because of our repeated-measures design, the number of total samples used was sufficiently large when inferring the eigengene network, and relating it to the clinical parameters using a linear mixed-effect model. Thus, most of the statistical limitations in this experiment arise from the heterogeneity of the small patient cohort, rather than the inferential methods used in analysis. This limitation is currently being addressed and implemented; we are expanding our scope to a coordinated multi-center study to gain a much larger sample size."

Italicized Sections in the Discussion

“The italicized sections in the discussion distract the reader and should be removed.”

Response: We agree with the reviewer and have eliminated the italizations.

Figures 1 and 6 Layout

“Figures 1 and 6 could be laid out more clearly.”

Response: We agree with the reviewer and have revised the two figure layouts.

Figure 1 Legends

“Figure 1. The "A," "B," "C" in the legends are confusing.”

Response: We agree with the reviewer and have revised the figure legend.

Biological Data to Support the Gene Expression Changes

“Any biological data to support the gene expression changes would be useful. For example, measures of mitochondrial function in leukocytes, etc would be useful if they correlated with the gene expression patterns.”

Response: We agree with the reviewer and have inserted the following sentence: “This study was designed as a hypothesis-generating study. In our ongoing work, we are generating biological validation data.”