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EDITORIAL COMMENT

Ribonucleic Acid Biomarkers for Heart Failure

Is There a Correlation Between Heart and Blood Transcriptomics?*

Lina A. Shehadeh, PhD,†‡§ Joshua M. Hare, MD†‡
Miami, Florida

Biomarkers play a crucial and growing role in diagnosing and managing cardiovascular disease. Currently, there are several well-established biomarkers employing enzyme-linked immunosorbent assay detection of serum peptides such as troponin I, troponin T, B-type natriuretic peptide, C-reactive protein, creatine kinase-myocardial band, myeloperoxidase, lipoprotein(a), and myoglobin. While ribonucleic acid (RNA) signature-based biomarkers have become standard of care in cancer, notably breast cancer (1), this approach has yet to fully emerge in cardiovascular medicine, although studies in patient tissues (2–5), preclinical models, and recent clinical studies (6,7) support their utility.

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To date, clinical transcriptomic cardiac studies have employed gene/messenger (m)RNA arrays (2–5,8–10), exonic arrays (11,12), and microRNA arrays (13–17), and it is expected that data on sequencing of long noncoding RNAs (lncRNAs) will emerge and grow rapidly in the coming few years. Whereas RNAs in these studies were extracted either directly from heart tissue or peripheral blood, few studies have compared simultaneously global transcript profiles from heart tissue with peripheral blood to determine whether there is a sufficient correlation between heart and blood transcriptomics to support the use of RNA blood biomarkers for diseases of the myocardium.

In this issue of *JACC: Heart Failure*, Gerling et al. (18) address this important issue by comparing the global mRNA expression profiles from heart tissue to peripheral blood mononuclear cells in an aldosterone rat model of heart failure. Their findings in gene expression and molecular pathway analysis supported a correlation between the blood and heart transcriptomics. The mRNA data was also supported by similar correlation in the increase of cytosolic calcium and zinc cations and the elevation of 8-isoprotane in cardiac myocytes and peripheral blood mononuclear cells. These findings add an important data point to the discussion of whether RNA blood biomarkers can serve as an appropriate surrogate for cardiovascular disease.

Several studies have shown that expression profiles obtained from myocardium provide highly accurate biomarkers of disease etiology and prognosis. Almost a decade ago, Kittleson and colleagues performed microarray analysis on tissue obtained from explanted hearts and revealed that ischemic cardiomyopathy could be distinguished from nonischemic cardiomyopathy (NICM) and that that the hearts of patients with NICM who do not undergo left ventricular assist device implantation resemble nonfailing hearts more than those of the sicker NICM patients who require a left ventricular assist device before cardiac transplantation (2,3). Heidecker and coworkers identified a unique myocardial gene signature that distinguished patients with myocarditis with 100% sensitivity and specificity among a broad range of secondary cardiomyopathies, including stress-induced cardiomyopathy, sarcoidosis, peripartum cardiomyopathy, arrhythmogenic right ventricular dysplasia, giant-cell myocarditis, and systemic lupus erythematosus (5). Other investigators have shown the value of transcriptomic biomarkers for a variety of other cardiovascular disorders, including atherosclerotic coronary artery disease (10) and asymptomatic left ventricular dysfunction (9). The question remains, however, whether blood-based transcriptomic biomarkers can accurately substitute for those obtained directly by the affected tissue. Due to its amorphous nature, blood is rarely referred to as tissue. In reality, blood is tissue that is in direct physical contact with all organs (except the brain). Unsurprisingly, in a thoughtful study by Liew et al. (19) who queried the absolute transcript levels of global mRNAs from 248 human blood samples on 248 microarray chips, and compared the results with publicly available microarray data from different human tissues, blood was shown to express tissue-specific transcripts. For example, the Beta-myosin heavy chain (β-MHC) transcript, which is heart specific, was found to be expressed in the blood (Fig. 1). Similarly, Adachi et al. (20) reported based on global microRNA profiling of various human tissue and microRNA quantitative polymerase chain reaction (qPCR) of cardiac patient sera, that miR-499 is heart specific and is up-regulated in the plasma of myocardial infarction patients, respectively.

In order for RNA transcripts to become clinically useful blood biomarkers in the future (Fig. 2), there are several important studies that need to be performed.

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From the †Department of Medicine, Division of Cardiology, University of Miami Leonard M. Miller School of Medicine, Miami, Florida; ‡Interdisciplinary Stem Cell Institute, University of Miami Leonard M. Miller School of Medicine, Miami, Florida; and the §Vascular Biology Institute, University of Miami Leonard M. Miller School of Medicine, Miami, Florida. Dr. Hare is funded by the National Institutes of Health (NIH) grants (RO1 HL094849, RO1 HL084275, RO1 HL107110, RO1 HL110737, and UM1HL113460); and discloses equity in Heart Genomics. Dr. Shehadeh is funded by the NIH (K01 AG040468), State of Florida (3KN05), and Florida Heart Research Institute.

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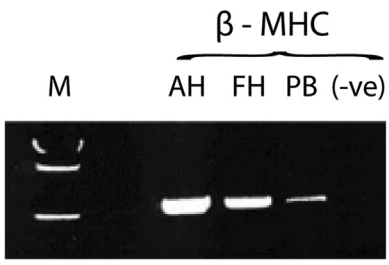

Figure 1 Heart-Specific Transcripts Expressed in Blood

β-MHC transcripts were detected in human peripheral blood (PB). Positive controls used were human adult (AH) and fetal heart (FH) tissue. No template/blank (-ve) was used as a negative control. Adapted from Liew et al. (19). M = molecular weight marker.

1) **Comparison between heart and blood transcripts in cardiac patients.**

Such studies will be ground breaking in screening for and identifying the transcripts that are potential biomarkers. It is possible that the transcripts to be identified could be previously unrelated to cardiac disease. In a study comparing the transcriptomics of brains and blood in Parkinson's disease patients, we identified an RNA splicing molecule among others to be dysregulated in both the brain and blood (21). Taken into consideration the blood–brain barrier, we anticipate that the comparison in heart disease to be much more direct and informative. In addition, these studies should not be limited to gene/mRNA expression, but rather include global microRNA expression, exonic expression/alternative splicing, and sequencing of lncRNAs.

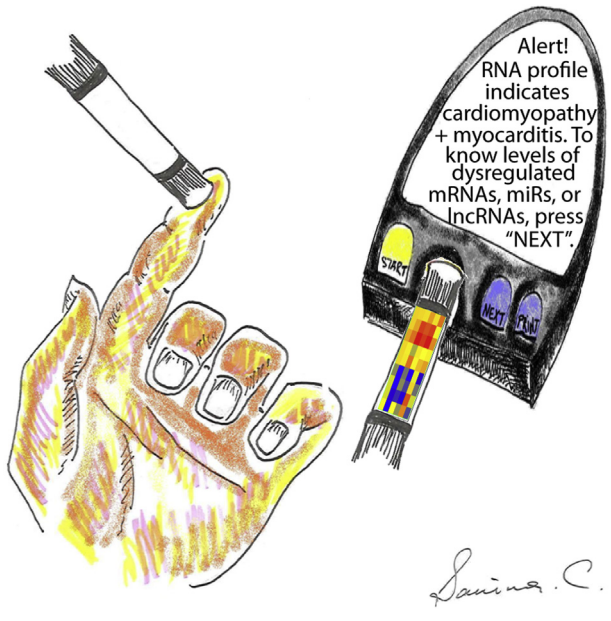

Figure 2 A Look Into the Future

RNA blood transcripts will be translated into scores that can predict the kind and degree of cardiovascular disease. lncRNA = long noncoding ribonucleid acid; miRs = microRNAs; mRNA = messenger ribonucleic acid; RNA = ribonucleic acid.

2) **The transcriptomic data from cardiac patients need to be correlated with clinical/functional measures.**

As in the informative study by Smih et al. (9) where blood transcriptomic microarray data from asymptomatic left ventricular dysfunction patients was correlated with echocardiography data to predict clinical outcome, it will be important to use clinical data as the basis for hierarchical clustering of the transcriptomic data. Otherwise, there will continue to be a gap between transcriptomic data and its clinical translation. Additionally, transcriptomic data can be correlated with the currently acceptable standard clinical assays used for cardiac diseases. For, example, Cheng et al. (22) found that plasma miR-1 transcripts were correlated with plasma creatine kinase-myocardial band levels in patients with myocardial infarction.

3) **Mathematical models need to be created to fit the blood transcriptomic data and the clinical data.**

Mathematical models need to be created integrating clinical data and the newly identified blood transcripts (which hopefully at this level would be screened down to hundreds rather than tens of thousands of genes or isoforms, and tens rather than hundreds of microRNAs and lncRNAs). Based on the combinatorial values of the absolute transcript levels, different models would be built to fit the expression data onto the clinical data. The final desired outcome is a simple readout/score of blood transcript data that, based on the built models, can predict the kind and level of cardiac disease as in Figure 2. These mathematical models will require validation in clinical populations, in a manner similar to the transcriptomic biomarkers developed using myocardial tissue (2–5,17).

In conclusion, while further studies are warranted to make blood RNA transcripts as clinical biomarkers for cardiac diseases, we agree with Liew et al. (19) that "blood cells can act as sentinels of disease," and therefore we could capitalize on this property of blood for the diagnosis/prognosis of cardiac diseases. The current study by Gerling et al. (18) provides additional supportive data for this concept. While direct sampling of myocardium might offer advantages for biomarker application, using peripheral blood has obvious benefit in terms of broader application and generalizability of transcriptomic biomarkers as they emerge in cardiovascular medicine.

Reprint requests and correspondence: Dr. Joshua M. Hare, University of Miami Miller School of Medicine, Interdisciplinary Stem Cell Institute, 1501 N.W. 10th Avenue, 9th floor, Miami, Florida 33136. E-mail: jhare@med.miami.edu.

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