EDITORIAL COMMENT
Microarray Measurements of Gene Expression Before and After Left Ventricular Assist Device Placement*

Michael R. Bristow, MD, PhD, FACC
Denver, Colorado

In this issue of the Journal, Blaxall et al. (1) report broad-spectrum measurements of messenger ribonucleic acid (mRNA) abundance using microarray technology in ribonucleic acid (RNA) extracted from end-stage human left ventricles at the time of left ventricular assist device (LVAD) insertion and then removal two to four months later. No left ventricular (LV) functional or dimensional changes were made in this study, but this duration of LVAD support has been shown to produce varying degrees of improvement in chamber contractile function (2) and remodeling (2,3). Although the majority of these changes may result from unloading inasmuch as they are present at the time of acute implantation (4), LVAD treatment in end-stage cardiomyopathy has been shown to improve isolated tissue (5) and cardiac myocyte function (6) and reduce excessive cell lengthening (7). At the gene expression level mRNA measurements of selected and limited numbers of genes have demonstrated improvement towards normal in some failure-associated alterations (5,8–11), but many abnormalities are not improved by mechanical unloading (11).

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The report by Blaxall et al. (1) is the first report of the application of gene chip technology to measure broad-based gene expression in the setting of LVAD-related effects on the end-stage failing heart. These authors (1) used the Affymetrix GeneChip HuGeneFL Array (Santa Clara, California), which contains 6,800 c-deoxyribonucleic acid sequences corresponding to known human genes. That is, of the ~35,000 genes in the human genome, the mRNA expression of around 20% of them was measured. As would be expected, this approach generates a very large amount of data that needs to be vetted, winnowed, and interpreted in some meaningful way. In order to deal with these situations, an entire subspecialty, bioinformatics, has developed, which is basically a hybrid between computational biostatistics and molecular biology.

To ascribe any credence to the reported findings, the first question to be asked would be, for RNA extracted from human hearts, how good is this technology in mRNA quantitation? Direct comparison of GeneChip and quantitative reverse transcription-polymerase chain reaction measurements performed by at least two laboratories indicates that the answer is, “quite good” (12,13). In addition, the microarray-derived data of Blaxall et al. (1) found several changes previously reported by more quantitative techniques. The next question might be, given the large number of measurements, how does one know whether the observed changes are biologic or stochastic? After all, at the usual 5% setting for type I statistical error one would expect 340 changes to be randomly significantly different, compared with the 530 changes reported by the authors (1). Which of these 530 changes are a real manifestation of LVAD treatment, and which are simply statistical phenomena of large numbers? There are multiple potential solutions to this problem, which include requiring a higher level of significance for measured changes, requiring same-direction changes in most or even all examined subjects, and investigating large numbers of subjects/starting material. Blaxall et al. (1) first used a form of pattern analysis performed in multiple dimensions, and then performed standard paired-t analysis for fold changes calculated by the Affymetrix algorithm. Although it can be reasonably concluded that patterns of gene expression were truly different in the six subjects before and after LVAD placement, the conclusion of distinct changes in gene expression must be verified by more quantitative and targeted means. Nevertheless, the strength of the microarray methodology is that it allows for the detection of changes in broad categories of gene expression, as compared to precisely measuring changes in mRNA abundance of individual genes. In other words, with this technology, precision is sacrificed for the ability to broadly assess changes in a less quantitative way. So the answer to the second question is that the Blaxall et al. (1) data are probably meaningful in terms of categorical patterns and for individual changes previously reported by more quantitative mRNA measurements, but other changes are only hypothesis generating.

Finally, one should ask, what are the study design limitations? In this case there are several. First, no LV phenotypic measurements such as function or dimensions were made, and so it is not possible to ascribe molecular effects to reverse remodeling/improved function versus mechanical unloading. To this point, the majority of changes by category were in the metabolic class (1), consistent with a primary effect of mechanical unloading, because in the intact dilated cardiomyopathy heart exhibiting improvement in phenotype without alteration in loading conditions, the majority of microarray-determined gene expression changes are in other categories (14). Second, the authors found that the three patients with ischemic cardiomyopathy had fewer changes than the three patients with nonischemic.
dilated cardiomyopathy, a finding that could be explained by etiology or, more likely, by less severe LV dysfunction in the viable myocardium taken from patients with ischemic etiology. Regional contractile function measurements might have supported one interpretation over the other. Third, the number of subjects investigated is small, particularly when subdivided by etiology. In other words, caution should be exercised in interpreting specific findings in this study. Nevertheless the report of Blaxall et al. (1) demonstrates that commercially available microarray technology can be employed in studies of modification of myocardial phenotype via LVAD. This study (1) sets the stage for more definitive investigations that should provide major insights into the molecular effects of this form of therapy and for the molecular basis of myocardial failure and remodeling, as mechanical unloading effects are distinguished from true phenotypic improvement.

Reprint requests and correspondence: Dr. Michael R. Bristow, University of Colorado Health Science Center, Cardiology, 4200 East Ninth Avenue, B-139, Denver, Colorado 80262. E-mail: laurel.hunter@uchsc.edu.

REFERENCES