The potential for the transcriptome to serve as a clinical biomarker for cardiovascular diseases

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The ability to measure the level of expression of tens of thousands of genes simultaneously with a variety of technologies has introduced the concept of deriving molecular signatures based on specific patterns of gene expression. This notion is conceptually resonant with regard to oncogenesis, and coupled with the ready availability of tumor tissue, has led to the introduction of molecular signature–based biomarkers for several solid and hematologic tumors. Recently, molecular signature analysis (MSA), prospectively validated in large patient cohorts, has become available to physicians managing patients with breast cancer, and ongoing clinical trials suggest that MSA could be a valuable tool to predict diagnosis, prognosis, and individual responses to therapies.

The promising results of microarray technology in oncology raise the issue of whether and how this tool could be used in other areas. In the cardiovascular field, there is a need for improved tools to detect inflammatory diseases of the myocardium and to guide immunosuppressive treatment in cardiac allograft recipients. The current gold standard for detecting myocarditis or monitoring cardiac allograft rejection—histopathological examination of endomyocardial biopsy specimens—is limited by imperfect sensitivity and requires, in the case of transplant recipients, repeated invasive procedures. The availability of high-throughput genomic technology could contribute substantially to the management of inflammatory diseases of the myocardium.

From a conceptual basis, genetic susceptibility does play a role in these disorders. For example, various single nucleotide polymorphisms correlate with cardiac transplant outcomes. Therefore, the advent of high-throughput genotyping holds great promise to assess one patient’s individual risk for developing rejection episodes, and testing of pharmacogenomic markers could help to guide the complex immunosuppressive regimens. Moreover, gene expression profiles may aid in the diagnosis and management of cardiac allograft rejection and myocarditis by reflecting complex genotype–environmental interactions.

In this issue of Circulation Research, Morgun and Shulzhenko apply transcriptomic-based MSA to human myocardial biopsy specimens of cardiac allograft recipients to detect transplant rejection and Chagas disease, the latter being the number one cause of myocarditis worldwide. After establishing a classifier on a training dataset of 23 patient samples, they validated their classifier on two separate microarray test datasets, together consisting of 44 prospectively collected samples. As a result of this internal validation design, they estimated their prediction accuracy to be more than 90% for detecting rejection episodes and Chagas reactivation. The authors noted that, in certain cases, microarray analysis identified molecular changes in biopsy specimens well before any signs were apparent on conventional histopathological examination, suggesting that microarray analysis of endomyocardial biopsies may detect the onset of rejection or Chagas disease in advance of conventional histopathological techniques. Despite the fact that this proof-of-concept study did not include a sufficiently high number of grade 1R rejections to develop a classifier for addressing the clinically important discrimination between different grades of rejection, these results add to the nascent field of transcriptomic-based molecular biomarkers for cardiovascular disease. For the field to advance, several conceptual and experimental issues need to be addressed. We briefly focus on the issues of statistical analysis, validation, biologic plausibility, and measurement platform.

Statistical analysis and validation of a genomic classifier

An obvious use of microarrays is to discover in an unsupervised manner genes that are either up- or downregulated. The high-dimensionality of microarray data with examination of thousands of transcripts in a relatively small number of samples raises the potential concern of creating false-positive results. In addition to simple fold-changes which fail to account for variability of expression between groups, more advanced statistical tests based on multiple testing were established, including the concept of the false discovery rate (FDR) which offers a sensible balance between the number of true and false-positives and basically yields probability values with higher stringency. Given concerns over microarray methodology, there is a demand that results of key changes in transcript levels be confirmed by an independent molecular method. When the goal of microarray analysis is gene discovery, usually an independent method such as quantitative real-time RT-PCR has been used to reproduce mere fold-changes of up- or downregulated genes.

Classification is distinct from gene discovery as it serves to stratify a biological sample into predefined clinical categories.
based on the expression levels of component genes. Current oncology signatures range between 15 and 100 genes and rely on consistency and pattern association. In the present study by Morgun and Shulzenko which focuses on discovering genomic classifiers rather than the discovery of differentially expressed genes, there is little use in confirming individual gene expression differences. Indeed, the overall usefulness of corroborative studies for individual genes has little value in the setting where the emphasis is put on “pattern” recognition. Rather, the validation comes from testing the accuracy of the molecular biomarker to perform as a diagnostic tool in new patient populations from other centers, referred to as “external validation”. For this purpose, Morgun and colleagues applied their classifier to independent datasets, for which microarray data were publicly available and found that their 98-classifier gene set achieved similarly high prediction accuracy in detecting rejection in three studies examining tissue samples from kidney transplants and bronchoalveolar lavage samples from lung transplants. As reproducibility of microarray data remains a critical issue, the good performance of this classifier in independent datasets of different organ systems seems remarkable. Not only does it strengthen the validity of the original dataset, but it also suggests similarities of rejection processes across different organs.

Interestingly, when the classifier was applied to a fourth large microarray dataset consisting of nearly 300 blood samples of cardiac allograft recipients, no significant predictions were found. Of note, this study itself failed to identify samples of cardiac allograft recipients, no significant predictive factors. Even though it is noteworthy that 20 of the 44 prospectively collected tissue samples in the study by Morgun et al were hybridized at a different institution on a different chip batch than the samples from the training set, implying a certain robustness of their classifier, reproducibility of microarray data across different laboratories raises potential challenges. The acceptance of MSA into clinical practice could be enhanced by conversion of the classifier to a platform which is more readily available and already validated in clinical diagnostic testing, eg, automated real-time RT-PCR. In this sense, it is important to mention that Morgun and Shulzenko identified a subset of their 98 gene classifier for detecting rejection which consists of 14 genes and which had a similarly high prediction accuracy to that of the original classifier. Testing of stringent classifiers in a prospective way in a large number of patients from different centers will be important for translation of genomic research into clinical practice.

The study by Morgun and colleagues represents an important contribution toward achieving this goal and is apt to stimulate interest in future studies of transcriptome analysis in cardiovascular medicine. As evident by the increasing importance of transcriptome-based MSA in oncology, high throughput genomic technologies hold great promise to lead to individualization of pharmacological treatment and assessment of prognosis in clinical practice in general (personalized medicine), potentially providing new tools in the assessment and management of common cardiovascular syndromes such as atrial fibrillation and congestive heart failure.

### Measurement Platform

Microarray measurements can be greatly influenced by extraneous factors. Even though it is noteworthy that 20 of the 44 prospectively collected tissue samples in the study by Morgun et al were hybridized at a different institution on a different chip batch than the samples from the training set, implying a certain robustness of their classifier, reproducibility of microarray data across different laboratories raises potential challenges. The acceptance of MSA into clinical practice could be enhanced by conversion of the classifier to a platform which is more readily available and already validated in clinical diagnostic testing, eg, automated real-time RT-PCR. In this sense, it is important to mention that Morgun and Shulzenko identified a subset of their 98 gene classifier for detecting rejection which consists of 14 genes and which had a similarly high prediction accuracy to that of the original classifier. Testing of stringent classifiers in a prospective way in a large number of patients from different centers will be important for translation of genomic research into clinical practice.

### Biological Interpretation

Biological interpretation of the classifier might not always be straightforward, as the classifier may contain many genes previously not associated with rejection or infection. In fact, the lack of biological interpretable information in the classifier should not imply that the genomic results are not valid. It is not the biological plausibility that distinguishes an excellent classifier, but its robustness of classification accuracy with independent data.

However, determining biological plausibility certainly increases the value of a given microarray dataset. In this sense, Morgun and Shulzenko extended their analysis beyond class prediction and identified biological pathways in rejection and Chagas disease. As expected, transcripts encoding for immune processes were upregulated. An intriguing new finding, however, was that energy-related transcripts were predominately downregulated in rejection and T. cruzi infection. This suggests that energy metabolism processes are depressed beyond the level previously encountered in a rat model of acute cardiac allograft rejection and highlights the power of an unsupervised approach to identify new pathophysiological molecular pathways which merit further investigation.

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### Disclosures

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