The tremendous progress achieved in the omics field has successfully provided new insights into the pathogenesis of common diseases. Molecular phenotyping of the disease status has become feasible by novel, robust, and fast high-throughput analytic platforms, and global gene expression profiling offers a novel opportunity for molecular disease profiling and biomarker identification.

A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention, and the overall expectation of a biomarker is to enhance the ability of the clinician to optimally manage the patient. To evaluate a biomarker, important measures are its sensitivity (ability to detect the disease, i.e., identification of true-positives) and specificity (ability to detect absence of disease, i.e., identification of true-negatives), as well as its positive and negative predictive values.

Expression pattern of defined genes can also be viewed as a biomarker in which the expression levels of multiple genes are combined in a defined manner to provide an expression signature, an expression score, or a classifier. A precise gene expression signature or score has the promise to diagnose diseases, classify them further, and potentially guide personalized decisions for individual patients. Because of their easy accessibility, whole blood and peripheral blood mononuclear cells provide an optimal source for RNA and subsequent gene expression analyses. Gene expression profiling has already been shown to predict cardiomyopathy pathogenesis in heart failure and to be useful in monitoring clinically significant allograft rejection.

Recent advances in the field of oncology have already translated gene expression signatures in medical applications, including the identification of breast cancer disease subgroups and the prognosis of breast cancer.

However, the use of gene expression data as a biomarker to improve diagnosis and prognosis of diseases is dependent on a number of factors, including an appropriate study design and adequate sample size, robust statistical derivation, and validation of initial results in independent studies to support the validity of the new assay. In addition, technical factors, such as the appropriate technology for gene expression analysis and preanalytical conditions (e.g., time of the day of blood sampling, single- or multicenter sample processing, cellular composition of the blood, RNA quality, sample processing in batches), are further important contributors to the performance of gene expression as a biomarker.

In this issue of Circulation: Cardiovascular Genetics, Thomas and colleagues report on a recently developed peripheral blood-based gene expression score (GES), which includes gene expression levels of 23 selected genes, age, and sex to assess obstructive coronary artery disease (CAD) likelihood in patients without diabetes mellitus. This report is an extension of this group’s previous work in developing, validating, and evaluating outcome of the GES using a now commercially available assay (Corus CAD, CardioDx, Palo Alto, CA).

In this multicenter (19 sites involved) prospective study, Thomas and colleagues assessed the diagnostic accuracy of the GES to discriminate obstructive CAD in symptomatic patients without diabetes mellitus clinically referred for myocardial perfusion imaging. The investigators found that the GES was a significant predictor of obstructive CAD by receiver operating characteristic and net reclassification analyses, superior to clinical estimation scores, and showed proportional correlation to maximum percent stenosis. Furthermore, the GES at a predefined threshold resulted in a high sensitivity and high negative predictive value. Finally, the authors present an outline of a clinical algorithm for the sequential use of the GES and myocardial perfusion imaging to stratify patients based on a defined threshold into a rule-out group where no further work-up is proposed, and a group that should undergo myocardial perfusion imaging subsequently—depending on myocardial perfusion imaging results—being referred to invasive coronary angiography.

Although the concept to explore and to validate gene expression profiling for diagnosis and prognosis of cardiovascular disease is attractive, various limitations need to be overcome.

To reach clinically meaningful results, adequate clinical phenotyping and handling of gene expression results are mandatory. The current study addresses the phenotype obstructive CAD. To clinically assess obstructive CAD, modern stress test techniques have to be applied to ensure the best possible clinical information. Stress-echocardiography or other modern state-of-the-art techniques might provide higher diagnostic sensitivity. Furthermore, inclusion of coronary stenosis <70% into the phenotype obstructive CAD might introduce a bias. Caution is also needed because a high percentage of the general CAD patient population is not addressed as the study is limited to patients with non–diabetes mellitus without known CAD, prior revascularization or myocardial infarction, and known chronic inflammatory or autoimmune disorders. Consequently, a GES as described by Thomas and Kawanishi must be rigorously

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tested against existing noninvasive standards and explored in a broader population to define its clinical utility. Scores and biomarkers tested in small selected populations often do not hold promises in adequate real-world sample sizes.

Beyond these clinical limitations, biological and technical considerations need to be taken into account. In contrast to genomic data, where a specific variant is present or not, gene expression data will vary interindividually, temporally, and between different disease states. In addition, whole blood represents a heterogeneous pool of distinct cells with putatively variable patterns of mRNA expression. Therefore, cell composition and subsequently gene expression might be altered depending on the disease state. Furthermore, variation caused by preanalytical (technical) factors can substantially influence gene expression data and should be considered in the GES. As shown by Schurmann et al., factors such as RNA quality, storage time of whole blood, and the RNA processing and amplification batch have a high influence on gene expression data. This is in particular challenging in the clinical setting, as differences in sample collection, sample processing, and assay performance in the different clinical centers are expected and will influence the accuracy of the GES. Although Thomas et al. included 19 centers in their multicenter study, automated RNA purification, RNA processing, and reverse transcriptase polymerase chain reaction analysis were performed in a central reference laboratory at CardioDx. Ideally, the GES will be validated in a multicenter real-world study, including decentralized processing of RNA and polymerase chain reaction analysis and optimization of (decentralized) clinical laboratory testing procedures.

Another crucial consideration is the influence of cardiovascular risk factors, ethnicity, and medication on gene expression. Recent studies showed that age, sex, body mass index, inflammatory status, and smoking influence gene expression. Any such influence needs to be considered and thoroughly validated for each single gene within the GES. For clinical application, the question arises whether to use a GES differently, eg, in smokers versus nonsmokers or between men and women. Hence, as already discussed, an independent validation study in a real-world scenario should be performed. In addition, common gene variants (eg, single-nucleotide polymorphisms) and epigenetic pattern can influence gene expression and thereby may influence transcript abundance differently in certain individuals.

In aggregate, despite its high potential for clinical purposes, the available evidence currently does not support the clinical benefit of GES for obstructive CAD. The GES should be tested more rigorously against state-of-the-art clinical tests. The impact of factors such as smoking, medication, and RNA quality on expression should be taken into account adequately. If these factors are considered and validated, the current concept is of high potential. Independent of these purely scientific/clinical aspects, research is also required to address other relevant questions concerning the translation of the GES into clinical practice, such as clinical applicability, and feasible time frame for expression analysis before patient treatment, ethical issues as well as optimal cost–benefit analysis.

Disclosures

None.

References


Key Words: Editorials • biomarker • blood-based gene expression • coronary artery disease • gene expression analysis • gene expression score
Blood-Based Gene Expression Tests: Promises and Limitations
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In the article by Zeller and Blankenberg, “Blood-Based Gene Expression Tests: Promises and Limitations,” which appeared in the April 2013 issue of the journal (Circulation: Cardiovascular Genetics. 2013;6:139–140), there was an error with an author name in the text on p 139, column 2, first full paragraph, second sentence.

The current sentence reads: “In this issue of Circulation: Cardiovascular Genetics, Thomas and Kawanishi10 report on a recently developed peripheral blood-based gene expression score (GES), which includes gene expression levels of 23 selected genes, age, and sex to assess obstructive coronary artery disease (CAD) likelihood in patients without diabetes mellitus.” It should read: “In this issue of Circulation: Cardiovascular Genetics, Thomas and colleagues10 report on a recently developed peripheral blood-based gene expression score (GES), which includes gene expression levels of 23 selected genes, age, and sex to assess obstructive coronary artery disease (CAD) likelihood in patients without diabetes mellitus.”

This has been corrected online.

The publisher regrets this error.