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Will Global Transcriptome Analysis Allow the Detection of Novel Prognostic Markers in Coronary Artery Disease and Heart Failure?

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Abstract: Coronary artery disease (CAD) is one of the leading causes of death in the developed countries. Myocardial infarction (MI) is an acute episode of CAD that results in myocardial injury and subsequent heart failure (HF). In the acute phase of MI several risk factors for future cardiovascular events have been found. The molecular mechanisms of these disorders are still unknown, but altered gene expression may play an important role in the development and progression of cardiovascular diseases. High-throughput techniques should greatly facilitate the elucidation of the mechanisms and provide novel insights into the pathophysiology of cardiovascular diseases. In this review we focus on the perspectives of gene-expression profiling conducted on cardiac tissues and blood for the determination of novel diagnostic and prognostic markers and therapeutic targets.

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INTRODUCTION

Coronary artery disease is one of the most frequent causes of death. It is estimated that every sixth man and every seventh woman in Europe will die from myocardial infarction [1]. Despite the continuous improvement in the management of myocardial infarction, in-hospital mortality in unselected patients is high (6-14%), and within 6 months up to an additional 12% of patients die.

Myocardial infarction (MI) is the death of cardiomyocytes due to prolonged ischemia. From the pathophysiological point of view, cell death begins ca. 20 minutes after the onset of myocardial ischemia, while complete necrosis requires 2-4 h [2]. Our understanding of the mechanisms leading to myocardial infarction (MI) are still far from satisfactory, and despite decades of vigorous research it is still unpredictable event of coronary artery disease (CAD). In most cases MI is caused by an atherosclerotic plaque rupture followed by thrombus formation and sudden closure of a coronary artery [3]. It is worth underscoring that the atherosclerotic plaque leading to myocardial infarction usually does not cause significant stenosis before that incident. Inflammatory cells and their mediators seem to play an important role in the plaque rupture [4].

Several complications of myocardial infarction are recognized, one of the most important in the long term being heart failure (HF). Heart failure is an "abnormality of cardiac structure or function leading to failure of the heart to deliver oxygen at a rate commensurate with the requirements of the metabolizing tissues" [5]. Heart failure occurs in about 1-2% of the adult population. Its main cause is coronary artery disease (in about two-thirds of all patients with heart failure, and in the vast majority of this population it is a consequence of myocardial infarction) [5].

Genome-wide gene expression profiling is a valuable strategy for discovering new potential biomarkers for diagnosis/prediction of disease severity [6] and in the identification of novel drug targets [7]. Transcriptional analysis has been successfully applied to numerous complex diseases, including coronary artery diseases and heart failure.

In this review we focus on recent studies on the identification of novel biomarkers for cardiovascular diseases and briefly discuss recent transcriptomic research conducted on cardiac tissues as well as blood samples.

CURRENTLY USED CARDIAC BIOMARKERS

Biomarkers are measurable and quantifiable biological parameters that can serve as indices for health and physiology assessments [8]. They should be easily measured with a potential to serve as a marker for the occurrence of a disease incident and its severity; some biomarkers also provide prognostic information. In acute coronary syndromes (ACS) several clinical factors have been identified, grouped with clinical scores, that help to stratify the risk of the disease (e.g., the GRACE score [9]). Translating into the clinic – they help to make decisions about a particular patient and inform the clinicians how aggressively the patient should be treated.

One of the most important markers of myocardial infarction is cardiac troponin (cTn). Elevated cTn (I or T) is essential to the diagnosis of myocardial infarction [2]. In acute

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coronary syndromes elevated cTn has also a prognostic value [10] and is a crucial biomarker in the GRACE score. The release of cTn into the blood stream can be caused by any type of myocardial injury, not only ischemic injury, therefore in clinical settings other than MI, the detection of elevated cTn values should prompt a careful search for other possible etiologies of cardiac damage [11]. cTn levels may remain elevated for up to two weeks after MI.

Another group of biomarkers of myocardial infarction commonly used in the clinical practice are natriuretic peptides: B-type natriuretic peptide (BNP) or N-terminal proB-type natriuretic peptide (NT-proBNP) [12]. These peptides are secreted from the heart in response to cardiac hemodynamic stress. In patients with MI the level of natriuretic peptides are correlated with the infarct size and left ventricle (LV) dysfunction, therefore they are powerful prognostic biomarkers. They augment the predictive value of the GRACE score in patients with MI [13].

The next important biomarker currently in use in the clinical setting is C-reactive protein (CRP). This is an acute phase protein, the most extensively studied systemic marker of inflammation [14]. Several prospective studies have reported that an increased CRP level is an independent predictor of cardiovascular events among apparently healthy individuals [15, 16] as well as in acute coronary syndrome [17, 18]. The major problem with CRP is a lack of specificity, and the true prognostic usefulness of CRP is still under debate [19].

There are numerous other biomarkers reportedly related to cardiovascular performance, like Mid-Regional pro-Atrial Natriuretic Peptide (MRproANP), Endothelin-1 and C-Terminal portion of pro-Endothelin-1 (CTproET1 Myeloperoxidase (matrix metalloproteinases (MMP) and many others. All those markers are not routinely used in the clinical practice but they are currently under investigation [20].

METHODS OF GLOBAL GENE EXPRESSION ANALYSIS

Two major types of high-throughput techniques are used for evaluating transcriptomes: microarray analysis and RNA sequencing. Microarrays are the older and still more often used method which allows simultaneous analysis of thousands of transcripts. Microarrays are considered as a 'closed system' because they are restricted to the known transcript sequences [21], albeit so-called genomic microarrays representing the entire DNA sequence of a given species are free of that limitation.

The second technique for transcriptomic estimation developed recently is next generation sequencing (NGS). The power of NGS platforms lies in their ability to provide a higher depth of sample coverage, lower background, better sensitivity and quantitative data. Their main disadvantages comprise complex sample preparation, short read-lengths, and the necessity to use sophisticated computer systems and advanced bioinformatics tools to process the huge volumes of sequence data [22].

Because the microarray technology is useful in determination of gene sets whose altered expression correlates with the pathogenesis of a disease, an increasing number of studies have used microarrays in clinical cardiovascular research.

GENE EXPRESSION PROFILING IN ANIMAL MODELS OF MYOCARDIAL INFARCTION AND HEART FAILURE

Animal models of myocardial infarction and heart failure have been invaluable as tools for elucidation of pathophysiological and molecular changes involved in HF, and in the development of novel HF therapies. Human myocardial tissues are hard to obtain and usually come from heterogeneous patient cohorts, with different disease duration, age, sex, and receiving various medications. All these factors may cause significant heterogeneity of transcriptional patterns. Animal models provide homogenous populations and allow harvesting myocardial tissues at will at defined stages of the disease. However, animal models which mimic distinct features of human heart failure cannot fully capture the complexity of the disease [23]. In contrast to the uniform transcriptional response observed in animal models, human HF microarray studies displayed a greater variability. The reasons for this discrepancy include (1) the effects of medications and (2) processes of fibrosis, inflammation and apoptosis developing in patients over a period of several years [24].

The majority of microarray studies on myocardial infarction have been performed in rodents. They have revealed that the development of cardiac hypertrophy leads to substantial alterations in expression of genes involved in the metabolism, cell growth and maintenance, apoptosis, cytoskeletal architecture, extracellular matrix remodeling, calcium regulation, signal transduction and inflammatory response [25-31]. Mitochondrial respiratory genes and lipid metabolism genes were downregulated, suggesting metabolic reprogramming and a shift away from the use of fatty acids as an energy source in the infarcted myocardium [26, 31]. Interestingly, products of some of the differentially expressed genes identified in those studies had already been used as molecular markers in cardiology, including those encoding ANP and BNP, troponin I, collagen propeptides, matrix metalloproteinases, or proteins involved in inflammation.

Studies involving meta-analyses of diverse microarray datasets and systematic network modeling have recently been used to identify new markers of myocardial infarction and heart failure, with prospective clinical applicability.

For example, an important meta-analysis of 28 experimental (mouse, rat, dog) and human HF microarray studies was done by Barth *et al.* [32]. Although the animal models differed in the etiology of HF (ischemic and nonischemic), they consistently showed a common gene expression pattern: downregulation of major metabolic pathways and upregulation of cell signaling pathways, showing similarity to a fetal gene program. In contrast, the human HF samples displayed a greater heterogeneity, even showing the opposite pattern. Therefore while well-controlled animal models could help in elucidating functional and molecular changes associated with cardiovascular disease and heart failure, the conclusions from such studies require validation in humans.

GENE EXPRESSION PROFILING IN THE HUMAN HEART

The greatest limitation of microarray analyses of the human myocardium is the general unavailability of patients' cardiac tissues. The tissues are collected mainly at the time

of heart transplantation, left ventricular assist device (LVAD) placement, or endomyocardial biopsies. Because of these limitations the majority of global gene expression data are those obtained from patients in the end-stage of cardiomyopathies of different etiologies leading to heart failure. In this review we focus on ischemic cardiomyopathy (ICM), in which the most prevalent initiating factor is coronary artery disease. However, some findings in nonischemic cardiomyopathy (NICM) and dilated cardiomyopathy (DCM) are also mentioned, since these etiologies often exhibit shared gene expression patterns of heart failure irrespective of the initiating factor. A summary of the results of selected microarray analyses of patients' myocardial samples is shown (Table **1**).

Early microarray studies involved few patients and usually compared gene expression between failing (DCM) and non-failing hearts [reviewed in 33]. They have identified a number of differentially expressed genes related to various pathways and functions, only partially overlapping between studies. Asakura and Kitakaze [33] analyzed seven independent microarray studies and identified 107 HF-related genes present in at least two of the seven different gene sets. Among them were genes involved in cell structure regulation, cell growth, oxidative phosphorylation and mitochondrial functioning. Of particular interest were four genes encoding extracellular molecules, among them *NPPA* encoding atrial natriuretic peptide (ANP), a peptide that had already been used as a biomarker of heart failure. Three others (*POSTN* encoding periostin, *PTN* encoding pleiotrophin, and *SERPINA3* encoding serine protease inhibitor alpha 1-antichymotrypsin) were likely to have a diagnostic potential and are possibly promising targets of HF therapy.

Another approach was to correlate gene expression levels with clinical parameters such as pulmonary artery pressure, left ventricular ejection fraction, and brain natriuretic peptide (BNP) mRNA level [34, 35]. Twelve patients with end-stage HF mainly due to DCM or myocardial infarction were included in those studies. MYLK3 (myosin light chain kinase 3), GPR37L1 (G-protein-coupled receptor 37 like 1), and GPR35 (G-protein-coupled receptor 35) were the newly identified targets and their role in HF pathogenesis was confirmed in the zebrafish and transgenic mouse models.

Several gene expression studies have been conducted to generate classifiers based on transcriptional profiles to differentiate between ischemic and nonischemic cases, since such correct classification is clinically highly relevant. Beisvag *et al.* [36] obtained gene expression classifiers allowing good prediction of CAD and DCM etiology among randomized samples of heart tissues. Four genes appeared in all selected classifiers: matrix metalloproteinase 3 (*MMP3*), fibulin 1 (*FBLN1*), ATP-binding cassette, sub-family B, member 1 (*ABCB1*) and iroquois homeobox protein 5 (*IRX5*). Most of the biological processes represented by the differentially expressed genes were common to both etiologies, however, more genes involved in catabolism were upregulated in the CAD samples. In contrast, Steenman *et al.* [37] failed to clearly differentiate between ischemic and idiopathic dilated cardiomyopathy employing dedicated cDNA microarrays. In the both studies rather small number of myocardial samples was used (24 and 17 patients, respectively).

A larger study (48 myocardial samples) was performed to discriminate between ischemic and nonischemic cardiomyopathy using a specially developed 90-gene etiology prediction profile [38]. The majority of genes in that profile were involved in signal transduction, metabolism, and cell growth/maintenance (most were upregulated in ICM). In their later study Kittleson *et al.* [39] compared differential gene expression in ICM and NICM relative to non-failing hearts and showed a number of common and unique genes involved in the development of HF. Forty-one genes were shared between the two types of cardiomyopathy, mainly those involved in cell growth/maintenance and signal transduction. There were also genes encoding components of the cytoskeleton, sarcomere and extracellular matrix, and genes implicated in the fetal gene program induction. Genes unique to ICM were predominantly from the functional classes of catalytic activity, whereas those unique to NICM were related to metabolism.

More recently, Kuner *et al.* [40] performed a class prediction analysis on three independent microarray data sets comprising a total of 279 human myocardial samples. They applied potential classifiers identified in a single study to the publicly available data sets and noted poor separation between ICM and NICM samples (misclassification rates above 25%). Worse still, as many as 40% of the 279 myocardial samples were misclassified when the 90-gene prediction profile for ICM and NICM published earlier by Kittleson and colleagues [38] was used. Although there were substantial similarities in the transcriptomic response in ICM and NICM, clear-cut differences could also be found between these two etiologies, with an overrepresentation of cytokine signaling pathways and immediate-early response genes in the ICM samples. Similarly, a transcriptional analysis of 92 explanted hearts (32 ICM, 27 DCM, and 33 non-failing) revealed no significant differences between the ICM and DCM groups [41]. In both groups a considerable number of downregulated metabolic pathways was found, with significantly decreased expression of genes regulated by peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) and estrogen-related receptor α (ERRα), central to the mitochondrial energy metabolism. The alterations in the PGC-1α and ERRα target gene sets were associated with an impaired left ventricular systolic function and were predictive for the heart failure phenotype. Taken together, the findings discussed above highlight the critical importance of using large datasets and validating the obtained results in multiple independent populations when looking for robust gene expression classifiers.

In an attempt to identify outcome predictors of HF severity, left and right ventricular samples from 44 patients (17 CAD, 20 DCM, 7 other) were analyzed using dedicated microarrays representing genes related to muscular organ pathophysiology [42]. A 170-gene predictor for left ventricle samples and a 129-gene predictor for right ventricle samples identified patients with stable and deteriorating status with high sensitivity and specificity. Thus, a reliable assay based on selected biomarkers to perform risk stratification and individualization of therapy in HF patients seems achievable.

A separate group of microarray studies concerned gene expression profiling after left ventricular assist device

Table 1. Selected Microarray Studies Performed on Human Cardiac Tissues

Disease description	No. of patients	Main pathways/functions indicated by authors	No. of selected genes	Potential biomarkers	Platform	References
Heart failure	In total 59 DCM/NICM/ICM *vs* 33 controls (reexamination of 7 studies)	Cell structure regulation Cell growth Oxidative phosphorylation Mitochondrial dysfunction	107	POSTN, PTN, SERPINA3	Affymetrix GeneChip Hu6800 (A-D), Affymetrix GeneChip HuFl 6800, Affymetrix GeneChip HG-U133A, CardioChip, Uni-Gene RZPD cDNA, ABI high-density oligonucleotide	[33] (review)
End-stage heart failure	12 CHF *vs* 2 controls	Cardiovascular system development and function Skeletal and muscular system development and function Cellular assembly and organization	313 correlated to clinical parameters	MYLK3, GPR37L1, GPR35	Affymetrix GeneChip HG-U95	[34, 35]
End-stage heart failure	11 CAD and 9 DCM *vs* 4 controls	Catabolism Regulation of protein kinase activity	153 in CAD and 147 in DCM	MMP3, FBLN1, ABCB1, IRX5	custom-made cDNA arrays (Norwegian Microarray Consortium)	[36]
End-stage cardiomyopathy, post-LVAD cardiomyopathy, newly diagnosed cardiomyopathy	16 ICM and 32 NICM	Signal transduction Metabolism Cell growth/ maintenance	90	90-gene etiology prediction profile	Affymetrix GeneChip U133A	[38]
End-stage cardiomyopathy	10 ICM and 21 NICM *vs* 6 controls	Catalytic activity (ICM) Metabolim (NICM), Cell growth/ maintenance and signal transduction (shared genes)	72 in ICM and 257 in NICM	ACE2 (upregulated only in NICM), TNFRSF11B (downregulated in both NICM and ICM)	Affymetrix GeneChip U133A	[39]
End-stage heart failure	150 ICM and 129 NICM (3 independent microarray studies)	Cytokine signaling pathways, inflammatory response and chemotaxis (ICM) Cytoskeletal torganization, antigen processing and presentation (NICM)	458 classifier genes	FOS and JUNB in ICM	Unigene 3.1 cDNA (37.5 K), Affymetrix GeneChip U133 2.0 plus, Affymetrix GeneChip U133A	[40]

(Table 1) contd….

Disease description	No. of patients	Main pathways/functions indicated by authors	No. of selected genes	Potential biomarkers	Platform	References
End-stage heart failure	31 ICM and 27 DCM vs 33 controls	Mitochondrial energy metabolism Cell signaling/growth Inflammatory response Cytoskeletal and membrane organization	340 upregulated and 144 downregulated	Downregulation of *PGC-1α* and *ERRα* target genes	Affymetrix GeneChip U133 plus 2.0	[41]
Stable, intermediate and deteriorating heart failure	17 CAD, 20 DCM, 7 other	NI	NI	170-gene predictor (left ventricle) and 129-gene predictor (right ventricle) for HF severity	Custom-made multi-dedicated microarrays (MyoChips)	[42]
Heart failure (LVAD implantation)	4 ICM, 5 IDCM, 2 other	NI	41 upregulated and 10 downregulated	APJ and its ligand APLN	Agilent Human 1 Catalog Array	[43]

NI- not indicated.

(LVAD) placement, which could improve cardiac function resulting in "reverse remodeling". Chen *et al.* [43] compared samples of the left ventricle harvested at the time of LVAD implantation and later at the time of cardiac transplantation from eleven HF patients (4 ICM, 5 DCM, 2 other). There were no significantly differentially regulated genes between those diagnostic groups. The APJ receptor (angiotensin receptor-like 1) was identified as the most significantly upregulated in cardiac tissue after LVAD therapy. The secreted ligand for this receptor is an inotropic neurohormone named apelin, which was later found markedly reduced in the plasma from patients with severe chronic heart failure [44]. These findings have led recently to the development of a novel potential therapeutic approach for patients with heart failure [45]. However, the majority of genes altered in HF did not show "reverse" expression changes in the LVAD-supported tissues as tested in 199 human myocardial samples from failing, LVAD-supported, and non-failing hearts [46].

CURRENT STATUS OF BLOOD MICROARRAY STUDIES

Peripheral blood mononuclear cells (PBMCs) are easier to get then a tissue biopsy, so their use could greatly facilitate large-scale clinical studies. Numerous studies have shown that circulating blood cells are involved in the pathogenesis of many diseases and may serve as biomarkers of pathological changes occurring in other tissues [47]. To date, gene expression studies related to chronic heart failure (CHF) have mainly involved microarray analysis of myocardial tissues. Only few studies used PBMCs as the source of mRNA for transcriptonal analysis. One of the earliest reports of blood transcriptomic analysis, published in 2002 used RNA isolated from blood from chronic heart failure patients [48]. The authors focused on a panel of cytokine genes and their role in CHF. Of the 375 genes analyzed, 34 were upregulated and two downregulated in CHF. The authors underscored the enhanced expression of several ligands from the TNF superfamily which could play a role in the pathophysiology of CHF.

VanBuren *et al.* [49] analyzed blood gene expression profiles from 71 subjects with heart failure and 15 controls and identified 197 "mortality genes" that were significantly associated with a patient's outcome. Functional analysis showed that genes associated with T cell receptor signaling were most significantly overrepresented. In a study presented by Vo and colleagues [50], transcription profiles from PBMCs of healthy aged donors and aged patients in the acute phase of heart failure and at recovery were compared. That investigation identified 22 transcripts differentially abundant between the acute phase of heart failure and healthy controls. Transcripts involved in inflammation and oxidative stress were more abundant while those associated with T-cell functions were less abundant in the failing heart. Those results were compared with the transcriptomic signatures of two other major acute geriatric disorders, infectious diseases and hip fracture. Remarkably, transcript levels of *BCL2, CASP, CCL5, DDIT3, ERG3, IL10RB, IL1R2, SERPINB2* and *TIMP1* were affected in a similar manner in all three disease states. The authors concluded that the selected genes should be considered as potential therapeutic targets in those geriatric pathologies. In an interesting study Lin *et al.* [51] performed transcriptomic microarray, iTRAQ proteomic, and nuclear magnetic resonance metabolomic analyses of blood samples from 29 end-stage CHF patients (16 ischemic heart disease – IHD, 13 nonischemic cardiomyopathy – NINC) and 20 normal cardiac function controls (NCF). Seventy-five genes were found to be differentially expressed between the CHF and NCF groups, mainly genes associated with

Table 2. Selected Microarray Studies Performed on Human Blood Samples

Disease description	No. of patients	Main pathways/functions indicated by authors	No. of selected genes	Potential biomarkers	Clinical assessment/clasification	Platform	References
Heart failure	71 HF *vs* 15 controls	T cell receptor signaling, FC Epsilon RI Signaling Signaling Natural killer Cell Signaling	197	CD3E, LAT, TK, CD3G, CD3D, CD28, TRA@, TXK, CALM1, BTK	NYHA Classification	Affymetrix GeneChip U133Plus2.0	[49]
Chronic heart failure	12 ICM / 12 NIDCM *vs* controls	Apoptosis Cell cycle Proliferation Cell surface receptor Cell metabolism chemotaxis Immune/stress response Signal transduction Transcription.	65	CCR2, CX3, CR1, EGR1, EGR2 EGR3	NYHA and LVEF Classification	Affymetrix GeneChip U133	[52]
Geriatric heart failure	33 aged patients with HF *vs* 28 healthy aged	Stress response, Immunosenescence and/or inflammation.	22	CD28, CD69, LCK, HMOX1, TNFRSF1 PRDX6	Age and clinical parameters	Custom made arrays (135 selected transcript),Eppendorf	[50]
Myocardial infarction	16 low EF patients (< 40%) *vs* 16 high EF patients (> 40%)	Cell surface transduction Intracellular signalling Proliferation Transcription	35	VEGFB, THBS1 PGF	LVEF classification	Oligonucleotide microarrays	[53]
Myocardial infarction	21 MI *vs* 14 stable CAD (3 time points)	Cellular Growth Proliferation Cell-To-Cell Signaling Cell Movement	24	SOCS3, FAM20,Il-10 Il-6	Clinical characterization	Affymetrix Human Gene 1.0 ST	[55]
Coronary artery disease	27 CAD *vs* 14 controlsl	Pro- and anti-oxidant molecules, Extracellular matrix, Cell motility proteins, and signaling Receptors and transcription factors	50	CAPG, MGST1, CSPG2, ALOX5, VIG4,NS5ATP13T, CD4, IL1RN, HP, CSF3R, CSF2RA, HK3, RNASE2, CREB5	Invasive angiography	41K Human Whole Genome Arrays (Agilent)	[56]
Coronary artery disease	120 (CADi 23) *vs* 121 controls	Cell growth apoptosis, Iinflammation	160	FTL, FKBP8, TUBA3, PNPLA2, UBXD1, MARCH2, ITPK1, PINK1,	CAD-index (Duke Coronary Artery Disease Index)	Affymetrix GeneChip U133A	[57]
Coronary artery disease	12 CAD *vs* 12 controls 10 CAD rehabilitation patients	Mitochondrial dysfunction and oxidative phosphorylation pathways	365	NDUFB3 UQCRQ COX7C ATP5I	Clinical classification	Illumina Sentrix humanref-6 beadchips	[58]

response to cardiac damage, inflammation response, blood coagulation/cell adhesion and apoptosis. Interestingly, the IHD and NINC sub-groups showed similar patterns. Notably, all three "-omics" strategies approaches gave convergent results, which supports their robustness.

In another study, Cappuzzello *et al.* [52] investigated gene expression profiles in PBMCs of chronic heart failure patients with ischemic (ICM) or nonischemic dilated (NIDCM) cardiomyopathy. Statistical analyses identified 65 genes comprising a molecular signature of CHF patients *versus* control subjects. Those genes were involved in apoptosis, cell cycle, proliferation, cell surface receptor functioning, cell metabolism, immune response, stress response, signal transduction, and transcription. The authors eventually selected *CCR2, CX3CR1, EGR1, EGR2,* and *EGR3* genes as biomarkers that could be regarded with high confidence as associated with the disease.

In order to identify prognostic biomarkers in MI patients, Devaux *et al.* [53] combined microarray analysis of blood cells with protein interaction networks. They constructed and analyzed a protein-protein interaction network of angiogenesis and identified 53 proteins highly specialized in regulation of cell growth. Among those, 38 proteins were found differentially expressed at the transcription level between low (≤40%) and high (>40%) LV ejection fraction (EF) patients (n=32). Three genes (*VEGFB. THBS1, PGF*) were finally selected as able to predict significant LV dysfunction (EF≤40%) with a stronger prognostic value than BNP and troponin T. The same group [54] showed that elevated level of TGFBR1 in blood cells of acute MI patients is associated with LV remodeling.

Our own investigations concentrated on leukocyte gene expression signatures of the acute phase of MI [55]. From 28 patients with ST-segment elevation myocardial infarction (STEMI), blood was collected on the 1st day of myocardial infarction, after 4–6 days, and after 6 months. On admission, genes linked with lipid/glucose metabolism, platelet function and atherosclerotic plaque stability were affected (signaling of PPAR, IL-10, IL-6). Analysis at discharge highlighted specific immune response (upregulation of immunoglobulins). The main finding of that work was a significant upregulation of *SOCS3* and *FAM20* genes in the first 4–6 days of myocardial infarction in all patients.

A number of microarray analyses have been conducted in order to identify genes potentially related to the range of coronary artery disease. Wingrove *et al.* [56] performed a transcriptomic screen to detect genes responsible for manifestation and extent of CAD. They found 106 genes (50 from microarray experiments and 53 from literature data) associated with the severity of CAD, of which 14 were confirmed in an independent cohort. Analysis of biological pathways for those genes showed that pro- and antioxidant molecules, extracellular matrix, cell motility proteins, and signaling receptors and transcription factors were enriched. Different results were obtained by Sinnaeve *et al.* [57] who also used microarray analysis to investigate correlations between the gene expression pattern and the severity of coronary artery disease. They found 160 differentially expressed transcripts, mainly involved in bone marrow cell differentiation, cell growth or growth arrest, apoptosis, cell adhesion and matrix modulation, and inflammatory and immune response. Another study [58], on gene expression in whole blood identified 365 differentially expressed genes in patients with CAD *versus* healthy controls. Additionally, a group of ten patients with CAD were compared before and after completion of a cardiac rehabilitation program following surgical coronary revascularization. Expression of *NDUFB3, UQCRQ, COX7C* and *ATP5I* were greater in the CAD patients relative to control subjects. Completion of the rehabilitation period was associated with a downregulation of two of those genes *COX7C* and *ATP5I*, three related ones, engaged in the same pathways *NDUFA1*, *CASP3* and *ATP5L*.

Results of selected microarray studies conducted on blood samples from different cardiopathological cases are summarized (Table **2**). The results are clearly discordant owing to the different designs of the studies, diverse experimental techniques and methods of data analysis. In addition, blood is composed of multiple cell subsets, therefore variations in the number of particular blood cell types might hinder the identification of disease-related changes of gene expression. For these reasons all newly proposed biomarkers have to be tested carefully in a different cohort and, preferably, also using a different technique to verify their diagnostic and/or stratification value. Despite of these concerns, the use of human peripheral blood cells as surrogate biopsy material represents a powerful tool to explore disease pathogenesis and to choose drug targets.

CONCLUSIONS

Microarray analysis of the myocardial transcriptome can identify differentially expressed genes encoding extracellular secreted molecules which have the potential of becoming circulating biomarkers for diagnostic and prognostic purposes. Furthermore, transcriptional profiling of diseased myocardium and blood cells enables the developing of multigene expression classifiers to identify disease etiology or outcome predictors of heart failure severity. One should bear in mind, however, that large-scale studies in multiple cohorts are critical for validation of the transcriptomic results and introducing the proposed biomarkers to the clinical practice.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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