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Molecular risk stratification in advanced heart failure patients

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Abstract

Risk stratification in advanced heart failure (HF) is crucial for the individualization of therapeutic strategy, in particular for heart transplantation and ventricular assist device implantation. We tested the hypothesis that cardiac gene expression profiling can distinguish between HF patients with different disease severity. We obtained tissue samples from both left (LV) and right (RV) ventricle of explanted hearts of 44 patients undergoing cardiac transplantation or ventricular assist device placement. Gene expression profiles were obtained using an in-house microarray containing 4217 muscular organ-relevant genes. Based on their clinical status, patients were classified into three HF-severity groups: deteriorating ($n = 12$), intermediate ($n = 19$) and stable ($n = 13$). Two-class statistical analysis of gene expression profiles of deteriorating and stable patients identified a 170-gene and a 129-gene predictor for LV and RV samples, respectively. The LV molecular predictor identified patients with stable and deteriorating status with a sensitivity of 88% and 92%, and a specificity of 100% and 96%, respectively. The RV molecular predictor identified patients with stable and deteriorating status with a sensitivity of 100% and 96%, and a specificity of 100% and 100%, respectively. The molecular prediction was reproducible across biological replicates in LV and RV samples. Gene expression profiling has the potential to reproducibly detect HF patients with highest HF severity with high sensitivity and specificity. In addition, not only LV but also RV samples could be used for molecular risk stratification with similar predictive power.

Keywords: genes • heart failure • remodelling • ventricles • microarrays

Introduction

Risk stratification in advanced heart failure (HF) aims at identifying patients who will rapidly progress to refractory myocardial dysfunction or who are at high risk of sudden cardiac death. This stratification is crucial for the individualization of therapeutic strategy, in particular for the listing and prioritization of patients for heart transplantation, and identification of patients for left ventricular assist devices (LVAD). Prediction models have been mainly developed for moderate HF [1, 2], some of them being applicable to end-stage HF patients [3, 4]. However, because these models have modest predictive capacity, outcome prediction still remains to be improved in advanced HF.

It has already been shown that HF severity correlates with the intensity of the cardiac remodelling process occurring during HF progression [5]. This remodelling process is related to transcriptomal alterations affecting numerous molecular pathways and biological functions, modifying tissue and morphological characteristics of the myocardium [6].

One tool that might lead to better outcome prediction is gene expression profiling. We and others recently showed that gene expression profiling could distinguish, even in advanced HF, subgroups of patients with specific cardiac molecular portraits [7–11].

Here we demonstrate that cardiac gene expression profiling can distinguish among HF patients with different HF severity.

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Methods

Cardiac samples

Cardiac tissue was obtained from explanted hearts of 44 patients with advanced HF who underwent a cardiac transplantation or a total artificial heart placement. Pre-transplant evaluation – including coronary artery angiography and macroscopic and histological examination of the explanted hearts – confirmed the diagnosis, aetiology and severity of the disease for all patients. Extensive individual clinical information can be found in Table S1.

Patients were classified into three severity groups based on their clinical status at the time of transplantation. The individual clinical status was defined based on the United Network for Organ Sharing (UNOS) medical urgency status [12] and occurrence of hospitalizations for acute decompensated heart failure (ADHF) during the 3 months prior to the surgical procedure ('recent ADHF'). UNOS medical urgency status determination is based on several patient characteristics including necessity of mechanical circulatory support, inotrope IV infusion or hospitalization in an intensive care unit. Deteriorating patients were characterized by UNOS-1A status. Stable patients were defined as UNOS-2 patients with no recent ADHF. The remaining patients were classified as intermediate (UNOS-1B status or UNOS-2 status with recent ADHF).

For each of the 44 explanted hearts, two spatially distinct transmural samples were obtained from non-infracted zones of both left ventricle (LV) and right ventricle (RV) immediately after cardiac explantation, leading to a total of 176 distinct tissue samples.

Microarrays

Microarray preparation and hybridization, and expression data acquisition and processing are described in the Supporting Information.

Data analysis

Unsupervised hierarchical clustering was applied to the entire data set median-centred on genes, using the Pearson correlation as a similarity metric and average linkage clustering. Results were displayed using TreeView [13]. Gene clusters were selected using 10 and 0.6 as minimal gene number and minimal correlation, respectively. GoMiner was used to identify functional categories that were over- or underrepresented in specific clusters compared to the list of all analysed genes [14].

'Predictors' and 'molecular severity score'

LV- and RV-specific data were separated into distinct data sets and analysed separately using an identical strategy:

The 'Predictor' was defined as a list of genes differentially expressed between stable and deteriorating patient groups. These genes were identified using 'significance analysis of microarrays' [15] with a maximum false discovery rate of 1% .

LV and RV predictors were used to calculate a transcriptome-based 'molecular severity score' (MSS) for each sample. First, expression profiles were mean-centred and standard deviation scaled on genes. The mean profile was calculated for stable (Ms) and for deteriorating (Md) samples. The MSS of a specific sample was defined as the normalized Euclidean squared distance (ranging from 0 to 1) between the sample and the stable mean profile and was calculated as described below:

$$MSS = \frac{Es}{Es + Ed}$$

where $Es = \sum_{i=1}^{n} [X_i - Ms_i]^2$ and $Ed = \sum_{i=1}^{n} [X_i - Md_i]^2$ and $X =$ the expression profile of the specific analysed sample and $i =$ an index of the n genes included in the 'predictors'. To define the significance level of the obtained MSS, an unpredictable interval in between the stable and deteriorating profiles was calculated. The cut-offs for the unpredictable interval were defined as the 2.5th and 97.5th percentiles of a random-MSS distribution based on 10^4 random permutations of the expression profiles.

Leave-one-out cross-validation was performed on stable and deteriorating samples. For both LV and RV, 50 distinct data sets were produced. Each data set was partitioned into a test set consisting of one sample and a learning set consisting of the 49 other samples. The learning set was used to calculate a MSS using the strategy described. The obtained MSS was employed to predict the MSS value of the test sample. This process was repeated so that the MSS value of each sample was predicted using an MSS estimated from all 49 other samples in the data set.

To test the diagnostic power of our classification, we calculated the sensitivity, the specificity and the positive and negative predictive values of the molecular prediction of stable and deteriorating status using the cross-validation results. We also analysed MSS values obtained from all samples using receiver operating characteristic curves using the jrocfit procedure available at www.jrocfit.org.

To test whether the obtained classification was independent of the method used, we also classified stable and deteriorating samples using the prediction analysis of microarrays method [16] using a previously published strategy [10, 17] (see Supporting Information for detailed methods).

Reproducibility

We tested between-sample reproducibility of the MSS values of all biological duplicates. Expression data from biological duplicates were separated to generate two comparable data sets. MSS from the duplicate sets were compared using the correlation coefficient. Analyses were performed separately on the LV- and RV-specific data sets.

Potential biases

We tested whether between-group variations in drug treatment could have biased the predictor discovery. To avoid confounding factors, subgroups of samples from the same chamber and the same severity group were analysed separately. For each predictor, expression profiles of samples positive and negative for a specific drug treatment were gene-by-gene compared using a Student's t-test with $P < 0.01$. We also tested the predictive power of our predictor in aetiology- and age-based subgroups of patients using the same strategy.

Results

We profiled cardiac gene expression in a cohort of 44 advanced HF patients using a 4217-oligonucleotide microarray containing

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genes selected for their involvement in muscular organ (patho)physiology. Based on the analysis of clinical information the 44 patients were classified into three HF-severity groups: deteriorating ($n = 12$), intermediate ($n = 19$) and stable ($n = 13$). After raw data extraction and consolidation, 4035 genes were validated for further analysis.

Hierarchical clustering and functional annotation

The 176 cardiac samples and the 4035 selected genes were clustered according to their expression profiles using a hierarchical clustering procedure (Fig. 1). Samples were grouped in two major clusters mainly based on the expression profile of a 387-gene cluster (white bar). This patient molecular clustering was not correlated with the clinical severity classification. However, within each of the two major clusters, stable and deteriorating samples were preferentially classified into distinct sub-clusters ($P < 0.001$ within each major cluster, χ^2 test).

Gene clusters were selected by automated analysis of the gene classification. Functional annotation revealed enrichment of genes involved in a specific biological process or tissue type for most of the clusters. Clusters that were too small to obtain a statistically significant annotation using GoMiner software (annotations 'natriuretic peptides' and 'cell metabolism') were functionally annotated based on literature analysis.

Several of the clusters showed marked differential expression between stable and deteriorating samples for LV and/or RV samples. 'Cell metabolism', 'natriuretic peptides' and 'extracellular matrix' gene clusters displayed higher expression for deteriorating samples than for stable samples in both LV and RV. 'Cytoskeleton' and 'cell death' gene clusters displayed higher expression for stable samples than for deteriorating samples in both LV and RV. Interestingly, the 'mitochondrion' gene cluster displayed higher expression for stable samples than for deteriorating samples in RV but not LV.

Prediction of clinical status

Two-class statistical analysis of gene expression profiles of the 24 deteriorating and 26 stable samples resulted in the identification of a 170-gene and 129-gene predictor for LV and RV, respectively (Table S2). Sixty-six genes were present in both LV and RV predictors.

Figure 2 shows individual MSS values calculated for the stable and deteriorating groups. The overall good classification rate was 95/100, whereas one stable LV sample was predicted as deteriorating and four LV samples were in the unpredictable interval. All RV samples were correctly predicted.

We also used data from all samples to generate receiver operating characteristic curves for each predictor (Fig. S1). The LV and RV molecular predictor could accurately identify patients with stable and deteriorating statuses (all area under curve >0.95).

A cross-validation strategy was also employed to account for data over-fitting due to reclassification of the samples used to define the predictors. The overall good classification rate was 94/100, whereas one stable sample was predicted as deteriorating and five samples were in the unpredictable interval. The LV molecular predictor identified patients with stable and deteriorating status with a sensitivity of 88% and 92%, and a specificity of 100% and 96%, respectively. The RV molecular predictor identified patients with stable and deteriorating status with a sensitivity of 100% and 96%, and a specificity of 100% and 100%, respectively. The difference in proportion of samples correctly classified for LV (45/50) and RV (49/50) samples was not statistically significant ($P = 0.20$, Fisher exact test). Equivalent prediction power results were obtained when using the prediction analysis of microarrays prediction method. (Table S3).

We also tested the predictive power of a single-gene predictor based on NPPB gene expression levels (Fig. S2). Using this predictor, a high misclassification rate was observed for deteriorating patients in both LV and RV samples.

We investigated whether a significant correlation exists between the MSS and several clinical parameters. Only heart rate correlated significantly with the MSS for both LV and RV data. Left ventricle end-diastolic diameter and brain natriuretic peptide blood level significantly correlated with LV data only, whereas Systolic Arterial Pressure correlated significantly only with RV data. Interestingly, left ventricle ejection fraction did not correlate with the MSS.

Intermediate group analysis

In agreement with the clinical classification, intermediate samples – which were not used for the construction of the predictors – were on average classified in-between the two other groups (Fig. 3). Progression of clinical severity for the LV samples was associated with a gradual increase of the MSS mean values ($P < 0.001$ for overall and all pairwise comparisons, one-way analysis of variance on ranks followed by Dunn test). Similar results were observed for RV samples. In addition, we observed that 54 of the 76 intermediate samples (66%) exhibited MSS values outside the unpredictable interval and could have been predicted as either stable or deteriorating.

Effects of potential biases

The three patient groups were comparable regarding sex, age, HF aetiology and LV ejection fraction (Table 1). As expected, differences in severity levels were associated with significant inter-group variations regarding treatment with adrenergic agonists, phospho-diesterase inhibitors, β-blockers and angiotensin converting enzyme inhibitors/angiotensin receptor blockers. Significant differences in expression related to these medications were found for only 0–7.0% of the genes included in the LV and RV predictors. Furthermore, significant differences in expression related to age and HF aetiology were found for 1.2% to 2.9% and for 0.6% to 2.3% of the genes, respectively. Removing these

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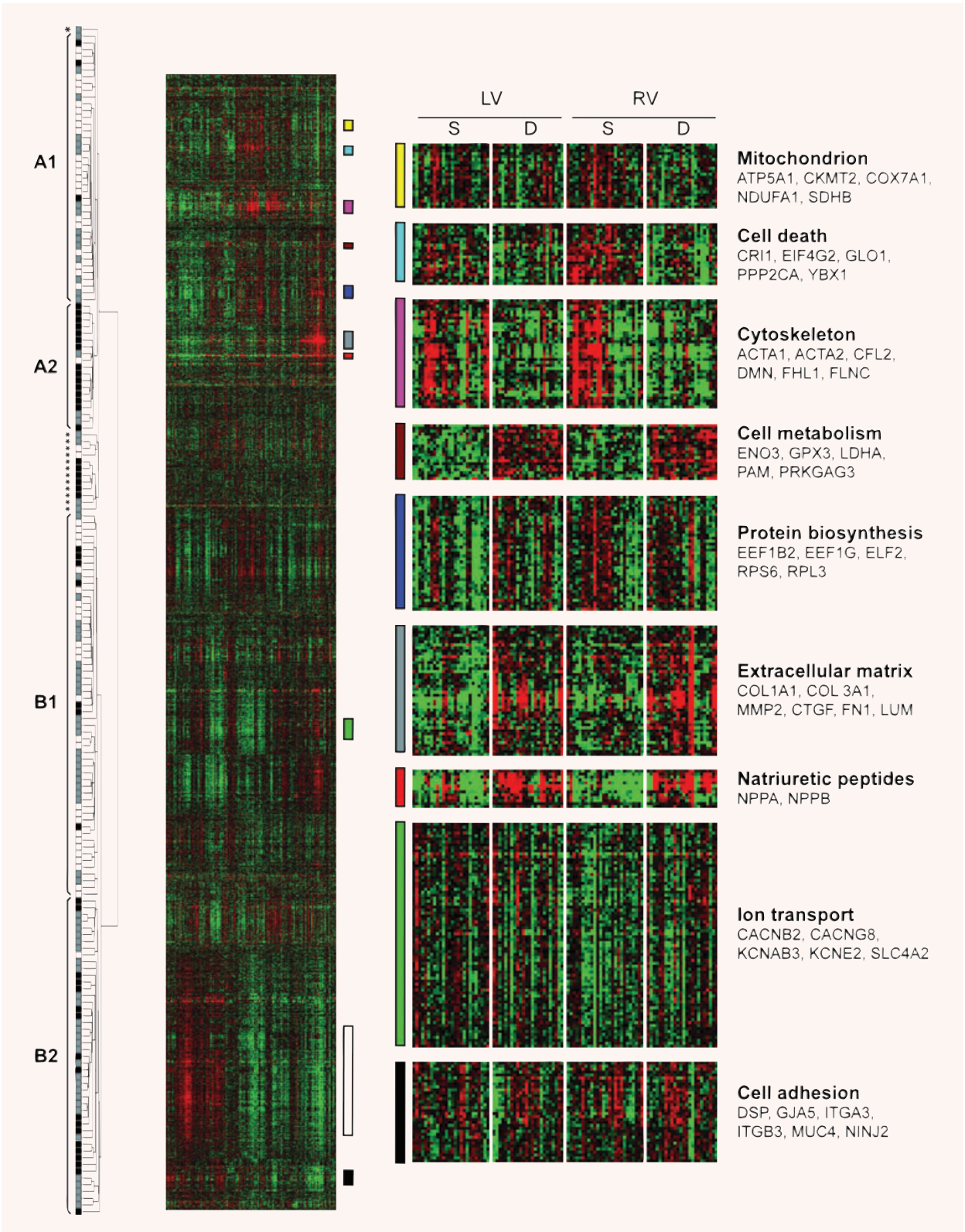

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Fig. 1 Two-way hierarchical clustering of gene expression data. Left: Classification tree of the samples. The dendrogram is based on similarity of the gene expression profiles of the 176 analysed samples. Samples were separated into four main clusters (A1, A2 and B1, B2). Only clusters containing at least 15 samples were considered as significant. Some samples (indicated *) were not included in any cluster. White, grey and black boxes on the left side of the dendrogram denote stable, intermediate and deteriorating clinical status, respectively. Middle: Heat map of expression values for 176 samples and 4035 genes after hierarchical clustering of both genes and samples. Each column represents the 4035-gene expression profile for one sample. Each row represents the 176-sample expression profile for one gene. Results are presented using a colour code. Green and red represent lower and higher expression levels relative to the median expression level of the gene, respectively. Right: Selected gene clusters indicated by coloured bars in the middle part of the figure. Intermediate samples were removed and remaining samples were ordered based on their origin (LV: left ventricle, RV: right ventricle) and the clinical status of the patient (S: stable, D: deteriorating). On the right side, functional annotation of the clusters is shown. Some genes representative of the functional annotation of the cluster are indicated using their HUGO gene nomenclature committee symbol.

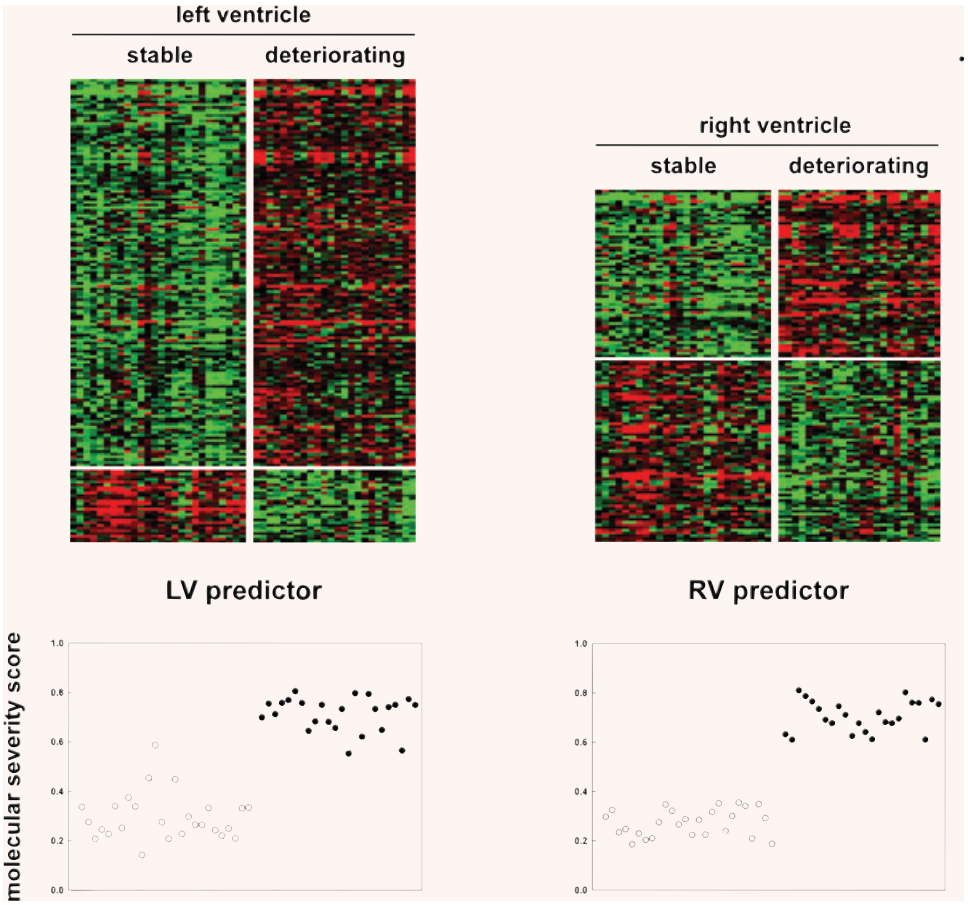

Fig. 2 Prediction of HF severity based on gene expression profiles. Top: Gene expression profiles of stable and deteriorating samples for the LV and RV severity predictors. Each column represents the gene expression profile for one sample. Each row represents the relative expression level for one gene. Colour code as in Fig. 1. Bottom: patient classifications for the LV and RV severity predictors. Open and filled circles correspond to stable and deteriorating LV samples, respectively. Open and filled triangles correspond to stable and deteriorating RV samples, respectively. Dashed lines denote upper and lower limits of the unpredictable interval.

genes from the predictors did not modify the good classification rates of the samples (data not shown). We also performed distinct prediction analysis for ischemic and non-ischemic patients. The results show that our classification can be accurately applied to both ischemic and non-ischemic patients (Fig. S3).

Biological reproducibility

We aimed to test whether our classification was reproducible across biological replicates. A significant correlation between MSS values obtained for the duplicate sets was observed (Fig. 4), with a better correlation for RV samples than for LV samples.

Discussion

We produced and analysed the largest set to date of transcriptomal profiles of LV and RV samples from a cohort of 44 HF patients. Ventricular samples were analysed using a dedicated

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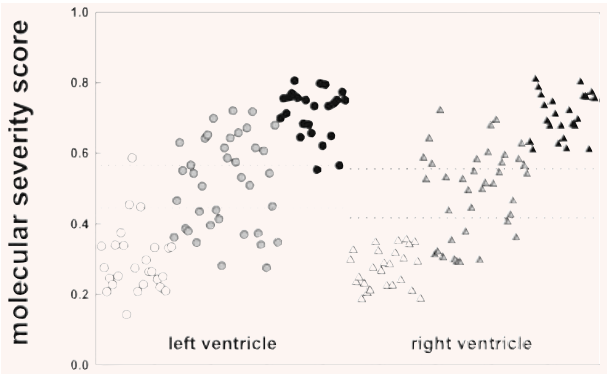

Fig. 3 Prediction of HF severity in all samples. Individual MSS values obtained for the LV and RV predictors are presented for all 176 analysed samples. Open and black-filled circles correspond to stable and deteriorating LV samples, respectively. Open and black-filled triangles correspond to stable and deteriorating RV samples, respectively. Intermediate samples are shown in grey. Dashed lines denote upper and lower limits of the unpredictable interval.

microarray representing genes selected for their contribution to muscular organ (patho)physiology. Replication at both the biological and the technical level, and control of experimental variations at the different steps of the study allowed detection of even subtle expression changes. We identified a set of genes of which expression changes discriminated between patients with different clinical severity levels and established that clinical deterioration of HF patients was associated with a molecular deterioration expression profile in both LV and RV. Therefore, our study confirms the potential of cardiac gene expression profiling to identify outcome predictors in patients with advanced HF.

Related findings in previous studies

It has previously been shown that gene expression profiling can discriminate between cardiac patients with different clinical characteristics [7–11, 18]. Aetiology-related gene expression profiles have been identified in Chagas disease, and hypertrophic, dilated, viral and ischemic cardiomyopathies [9, 10, 19]. In a recent study, Heidecker *et al.* identified a transcriptomic signature that could predict clinical outcome of new-onset idiopathic dilated cardiomyopathy patients [17]. Taken together, these findings offer valuable information regarding the molecular basis of HF related to distinct aetiologies and they could lead to individualized therapeutic strategies in HF.

Other clinical characteristics such as age and sex have also been shown to have an effect on the transcriptomal profile of HF patients [20]. In our study, the molecular severity markers correctly classified HF patients independent of aetiology or age. Because most of our patients were male, we could not validate our classification in female HF patients. We also showed that our results were unchanged when another prediction method was used [10, 17].

Potential clinical significance of findings

Prognosis evaluation for advanced HF patients

The results of our study suggest that gene expression profiling has the potential to detect HF patients with highest HF severity with high sensitivity and specificity. Prognosis evaluation is fundamental for the indication of LVAD implantation and heart transplantation in advanced HF patients. Patients depending on intravenous inotropic therapy have the worst prognosis and should benefit from urgent or elective LVAD implantation or urgent transplantation whenever possible [21]. However, risk stratification remains particularly difficult for ambulatory advanced HF patients not depending on intravenous inotropic therapy or prolonged hospitalization, with major impairment of their functional capacities and poor survival [22, 23]. Specific risk scores are not yet available for advanced HF patients but become mandatory in the context of this growing cohort of patients [24, 25].

Our results showed that the LV and RV predictors lead to a better prediction of clinical status than the NPPB predictor, in particular regarding the prediction of the deteriorating status. It has previously been shown that the NPPB mRNA level in the LV and the BNP peripheral blood level are correlated [26]. The B-type natriuretic peptide (BNP) blood level is widely used as a clinical predictor for HF patients. However the BNP blood level predictive value is still controversial in the specific condition of end-stage HF [27, 28]. A previous report showed that a lower natriuretic peptide blood level, that usually implies a better outcome, may also imply poor outcome in severe HF patients [28]. Similarly, our results show a low NPPB mRNA level for patient in the deteriorating status group.

Our results provide a rational to develop prospective clinical research studies using gene expression measurement techniques in advanced HF. Although microarrays are a unique tool to screen the largest number possible of potential biomarkers, which was the aim of this study, other techniques such as quantitative RT-PCR will be of greater interest to develop a clinically relevant outcome predictor based on a set of selected biomarkers.

Transcriptomal remodelling of the right ventricle

Our results suggest that molecular prediction using samples taken from RV may be as powerful as molecular prediction using samples taken from LV. Most of the patients with advanced HF have severe LV dysfunction, whereas RV dysfunction intensity is variable among these patients. In addition, transcriptome remodelling of the RV in HF has been evaluated to a lesser extent than for the LV. Our data show that most of the molecular processes disturbed in the LV are also disturbed in the RV. In addition, sensitivity and specificity of prediction of both stable and deteriorating statuses using RV samples were at least equivalent to those obtained using LV samples. These results are in agreement with a previous study showing accurate prediction of clinical outcome of new-onset HF

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Table 1 Clinical characteristics of HF severity patient groups

	Stable $n = 13$	Deteriorating $n = 12$	Intermediate $n = 19$	*P*-value
Male / female	12/1	10/2	16/3	0.747
Age, years	50 (15)	49 (9)	48 (12)	0.559
Initial cardiac disease, CAD / DCM / other	5 / 6 / 2	4 / 7 / 1	8 / 7 / 4	0.840
HF duration, months	32 (29)	24 (33)	29 (32)	0.459
Heart rate, per min.	69 (13)	100 (16)	76 (16)	$< 0.001^{*,\ddagger}$
Systolic arterial pressure, mmHg	102 (17)	97 (9)	103 (12)	0.509
LVEF,%	24 (11)	22 (7)	24 (7)	0.829
LVEDD, mm	73 (13)	66 (7)	65 (10)	0.254
MPAP, mmHg	24 (12)	33 (10)	32 (10)	0.095
Blood urea nitrogen, mmol/l	9.1 (5.6)	9.8 (4.2)	9.0 (4.1)	0.884
Serum creatinine, μmol/l	107 (26)	107 (22)	101 (36)	0.642
Medications,% of patients				
ACEI / ARB	100	58	84	0.024*
β-Blockers	69	0	26	$< 0.001^{*,\dagger}$
Adrenergic agonists	0	100	42	$< 0.001^{*,\dagger,\ddagger}$
Phosphodiesterase inhibitors	0	67	0	$< 0.001^{*,\ddagger}$
Aldosterone blockers	77	58	53	0.420
Statin	46	33	32	0.724
Digoxin / digitoxin	46	25	26	0.502
UNOS medical urgency status	2	1A	1B or 2	
Number of recent ADHF episodes	0	1.8 (0.4)	1.7 (0.8)	

CAD: coronary artery disease; DCM: dilated cardiomyopathy; LVEF: left ventricle ejection fraction; LVEDD: left ventricle end diastolic diameter; MPAP: mean pulmonary artery pressure, ACEI: angiotensin converting enzyme inhibitors; ARB: angiotensin receptor blockers; ADHF: acute decompensated heart failure.

Data are presented as 'mean (S.D.)' when appropriate. *P* -value indicates the result of a comparison among the three patient groups using Fisher's exact test or Kruskal–Wallis rank sum test. If $P < 0.05$, groups were compared two-by-two. $^{*}P < 0.05$ between deteriorating and stable; $^{\dagger}P < 0.05$ between intermediate and stable; $^{\ddagger}P < 0.05$ between deteriorating and intermediate.

An ADHF episode was defined as recent if it occurred during the 3 months before the heart transplantation/total artificial heart placement. HF duration was defined as the delay between onset of HF symptoms and heart transplantation/total artificial heart placement. Values for LVEF, LVEDD, blood urea nitrogen and serum creatinine corresponded to pre-operative measurements. All patients were treated with loop diuretics (furosemide and/or bumetanide). Only medications related to HF therapy are presented. The clinical profile was determined based on the patients' medical urgency status in the UNOS classification and the occurrence of recent ADHF episodes.

patients using a transcriptomic signature obtained from RV endomyocardial biopsies [17].

For some specific clinical situations, such as arrythmogenic RV dysplasia or severe LV infarction with unaffected RV, RV and LV function/morphology may clearly differ. In our study, we could not obtain samples for these very specific groups of patients. Therefore, our results cannot be extended to these patients. Although these clinical profiles represent a relatively moderate percentage of advanced HF patients, our results can be applied to a majority of patients in advanced HF.

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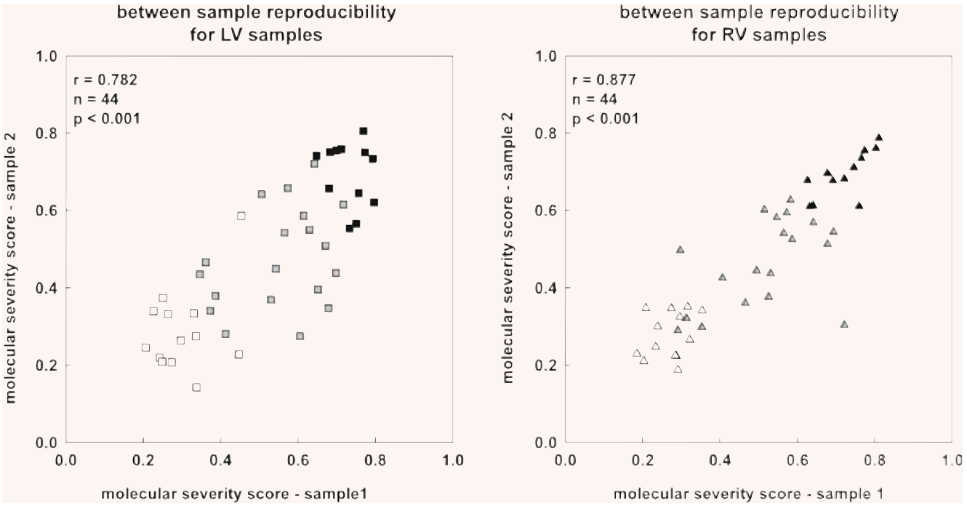

Fig. 4 Between-sample reproducibility. Between-sample reproducibility was assessed using MSS values calculated from biological replicates. Subgroup analysis based on the origin of the sample (LV or RV) is shown. The correlation coefficient was used as a between-sample reproducibility index. Squares: LV samples; Triangles: RV samples. Open symbols: stable samples; grey-filled symbols: intermediate samples; black-filled symbols: deteriorating samples.

Prediction reproducibility

Measurement reproducibility is another crucial point when developing a predictor of HF severity. Relatively high variability of widely used biomarkers like BNP or N-terminal proBNP blood levels may be a problem for patient management [29]. Our results show that gene expression profiling is reproducible among biological replicates. Reproducibility was higher for RV samples, reinforcing the interest of RV sample utilization to develop a molecular predictor in advanced HF. A hypothesis is that regional tissue heterogeneity may be higher in LV than in RV. One cause may be the presence of infarct scars that preferentially affect the LV. However, ventricular samples analysed in this study were obtained after careful dissection of the ventricles excluding infarct scars. We also did not observe a higher variability of MSS values obtained for LV samples in patients affected by coronary artery disease compared to other patients.

Potential limitations

Complexity of myocardial remodelling

Although transcriptional remodelling is an important mechanism of cardiac remodelling occurring in HF, post-translational modifications are also of crucial importance. Therefore, additional techniques such as Western blot and possibly additional experiments would be necessary to verify a mechanistic role for a single gene/protein, which was not the scope of this study. This study was designed to identify transcriptomic biomarkers that would reveal to be useful for patient classification. We also showed that, at the functional level, most of the identified biomarkers are involved in molecular functions that are important for myocardial remodelling associated with HF.

Effect of medication

Therapeutic interventions, in particular medications, may induce modifications of the cardiac transcriptome [30]. We tested the hypothesis that the patient classification may be modified by angiotensin converting enzyme inhibitors/angiotensin receptor blockers, β-blockers and inotropic drugs. A very low number of genes included in the distinct predictors displayed differential expression associated with different drug intake. Removing these genes from the predictors did not modify the patients' classification. Therefore, medications do not strongly modify the expression level of our predictors.

Clinical classification

We compared our molecular predictors to a two-parameter clinical classification that has not been previously evaluated in advanced HF. Because we used samples taken at the time of cardiac transplantation, it was not possible to compare our predictors to a relevant clinical end-point like mortality or hospitalization for ADHF. We suggested that the use of parameters measured at the time of transplantation would better reflect the clinical phenotype at this time and decided to combine two established predictors of HF severity to classify patients. The UNOS medical urgency status has been specifically developed for advanced HF patients listed for cardiac transplantation. The UNOS-1A status at the time of listing is associated with a 1-month mortality $>30\%$ whereas UNOS-2 patients have a 1-month mortality $<10\%$ [4]. The mortality rate on the UNOS waiting list is more than 4-fold higher for UNOS-1A than for UNOS-2 patients [23]. To better define our group of stable patients we combined the UNOS medical urgency status with the occurrence of ADHF episodes. Frequent rehospitalizations have been recognized as a strong predictor of HF patient mortality [24]. Other HF severity prediction scores have been developed in advanced HF [3, 4]. Comparison of one of these HF severity predictors to the UNOS medical urgency status did not reveal a higher predictive power [4]. Other predictors included the measurement of peak oxygen consumption that cannot be recorded in the most severely affected patients [3].

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We analysed expression profiles of patients with advanced HF at the time of cardiac transplantation. Further clinical studies are needed to determine whether gene expression profiling of cardiac tissue provides sensitive prognostic information for advanced ambulatory HF patients using clinical end-points like mortality or hospitalization for HF.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 ROC curves for the prediction of stable and deteriorating statuses in LV and RV samples.

Fig. S2 Predciction of HF severity based on the Natriuretic Peptide Precursor B (NPPB) gene expression level.

Fig. S3 Separate prediction of HF severity for coronary disease (top) and non-coronary artery disease (bottom) related samples.

Table S1. Clinical characteristics of advanced HF patients.

Table S2. Two-class statistical analysis of gene expression profiles.

Table S3. Sample classification using Prediction Analysis for Microarrays.

Data and Methods

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