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A 3-gene panel improves the prediction of left ventricular dysfunction after acute myocardial infarction☆

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ABSTRACT

Background: Identification of patients at risk of poor outcome after acute myocardial infarction (MI) would allow tailoring healthcare to each individual. However, lack of prognostication tools renders this task challenging. Previous investigations suggested that blood transcriptome analysis may inform about prognosis after MI. We aim to independently confirm the value of gene expression profiles in the blood to predict left ventricular (LV) dysfunction after MI.

Methods and results: Five genes (LMNB1, MMP9, TGFBR1, LTBP4 and TNXB) selected from previous studies were measured in peripheral blood samples obtained at reperfusion in 449 MI patients. 79 patients had LV dysfunction as attested by an ejection fraction (EF) ≤40% at 4-month follow-up and 370 patients had a preserved LV function (EF > 40%). LMNB1, MMP9 and TGFBR1 were up-regulated in patients with LV dysfunction and LTBP4 was down-regulated, as compared with patients with preserved LV function. The 5 genes were significant univariate predictors of LV dysfunction. In multivariable analyses adjusted with traditional risk factors and corrected for model overfitting, a panel of 3 genes — TNXB, TGFBR1 and LTBP4 – improved the prediction of a clinical model ($p = 0.00008$) and provided a net reclassification index of 0.45 [0.23–0.69], $p = 0.0002$ and an integrated discrimination improvement of 0.05 [0.02–0.09], $p = 0.001$. Bootstrap internal validation confirmed the incremental predictive value of the 3-gene panel.

Conclusion: A 3-gene panel can aid to predict LV dysfunction after MI. Further independent validation is required before considering these findings for molecular diagnostic assay development.

1. Introduction

Left ventricular (LV) remodeling after acute myocardial infarction (MI) is a complex process which, when adequately regulated, restores the functional capacity of the infarcted heart. However, LV remodeling can become maladaptive, leading to LV dysfunction and ultimately heart failure (HF). Heart failure is a grave condition with an ever increasing prevalence [1]. Prediction of the development of maladaptive LV remodeling after MI is challenging and would be a major breakthrough. A plethora of cardiovascular disease biomarkers have been identified [2], but there is still a need for novel biomarkers to identify patients at risk of developing LV remodeling and dysfunction after MI. While current guidelines recommend the use of brain natriuretic peptides (BNP)-monitoring to guide diagnosis and treatment of patients with acute or chronic HF [3], such biomarker-guided practice is not recommended in the post-MI setting.

In past studies [4–9], we examined whether gene expression profiles of blood cells may be used to predict LV remodeling and dysfunction after MI. The hypothesis of an association between blood cells transcriptome and outcome after MI was supported by the capacity of immune circulating cells to inform about some features of the inflammatory and healing processes that occur after MI [10]. It is now well established that immune cells play a major role in ischemic heart disease [11]. They link systemic to cardiac inflammation [12] and regulate LV remodeling [13]. Interestingly, gene expression profiles of blood cells are associated with LV remodeling in rats with aldosteronism and hypertensive heart disease [14]. Furthermore, gene expression profiles

Abbreviations: MI, myocardial infarction; LV, left ventricle; EF, ejection fraction; HF, heart failure; STEMI, ST-segment–elevation myocardial infarction; NSTEMI, non-ST-segment–elevation myocardial infarction; PCI, percutaneous coronary intervention; BMI, body mass index; TIMI, thrombolysis in MI; BNP, brain natriuretic peptides; Nt-proBNP, N-terminal proBNP; cTnT, cardiac troponin T; CPK, creatine phospho kinase; WBC, white blood cells; AIC, Akaike Information Criteria; IDI, integrated discrimination improvement; NRI, net reclassification index; CI, confidence intervals; OR, Odds ratios; ΔΔCt, relative quantification method; TGFBR1, transforming growth factor beta receptor I; TNXB, tenascin XB; LTBP4, latent transforming growth factor beta binding protein 4; LMNB1, lamin B1; MMP9, matrix metalloproteinase 9.

☆ The authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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of blood cells correlate with the presence and extent of coronary artery disease in patients undergoing angiography [15] and are used for the diagnosis of obstructive coronary artery disease in symptomatic non-diabetic patients [16].

In past investigations, microarray- and system-based approaches for biomarker discovery allowed the identification of several genes whose expression levels in peripheral blood cells may inform about prognosis after MI [4–9]. However, these studies were limited by small sample size. Here, we aimed to confirm in a large cohort of MI patients the association between the expression levels in the blood of 5 genes and LV dysfunction after MI. The rationale beyond the selection of these 5 genes is three-fold. Firstly, the 5 genes predicted LV dysfunction with areas under the receiver operating characteristic curve above 0.70 in discovery studies [5,6,8]. Secondly, their expression levels in blood cells were moderate to high and could reliably be detected using quantitative PCR. Lastly, they were expressed in biopsies from failing hearts (unpublished data), consistently with the recruitment of peripheral blood cells to the injured heart [11]. The selected 5 genes are: transforming growth factor beta receptor I (TGFBR1), tenascin XB (TNXB), latent transforming growth factor beta binding protein 4 (LTBP4), lamin B1 (LMNB1), and matrix metalloproteinase 9 (MMP9).

2. Methods

2.1. Patients and blood samples

From a total of 960 consecutive patients of the Luxembourg Acute Myocardial Infarction Registry completed at the Institut National de Chirurgie Cardiaque et de Cardiologie Interventionnelle and the Department of Cardiology of the Centre Hospitalier de Luxembourg [17], we enrolled 449 patients for which blood samples and follow-up data were available. All patients had acute MI and were treated with primary percutaneous coronary intervention (PCI). Acute ST-segment–elevation MI (STEMI) was the final diagnosis for 352 patients and 97 patients had non-ST-segment–elevation MI (NSTEMI). STEMI was defined by (1) clinically significant ST elevation (>1 mm); (2) occluded major coronary artery: thrombolysis in MI (TIMI) 0 flow in the left anterior descending, circumflex, or right coronary artery; and (3) peak creatine phospho kinase (CPK) activity >600 U/L (3 times above the upper limit of the reference interval). NSTEMI was defined by (1) no significant ST-elevation but significant ST depression (>1 mm); (2) significant lesion in a major coronary artery requiring PCI; and (3) positive cardiac troponin T (cTnT) concentration after 24 h (>0.03 μg/L). Most NSTEMI patients had a severe or sub-occlusive lesion in the left anterior descending, circumflex, or right coronary artery. Blood samples were withdrawn at the time of reperfusion via an arterial catheter into PAXgene™ RNA tubes (BD Biosciences, Erembodegem, Belgium). Left ventricular (LV) ejection fraction (EF) was determined after 4 months using echocardiography. This time-point was chosen to ensure the completion of LV remodeling which dictates whether the heart will recover properly or show signs of dysfunction. The protocol has been approved by the ethics committee and the national committee for data protection of Luxembourg. All patients signed an informed consent. Consecutive patients were enrolled in the present study.

2.2. Measurement of gene expression

2.2.1. RNA extraction

Total RNA was extracted from PAXgene™ tubes with the PAXgene™ blood RNA kit (Qiagen, Venlo, Netherlands) as described by the manufacturer. Extracted RNA was purified and concentrated using the RNeasy® MinElute™ kit (Qiagen). On-column DNase I treatment (Qiagen) was undertaken to digest potential contaminating genomic DNA. After extraction, RNA was quantified with the ND-1000 spectrophotometer (NanoDrop® Technologies, Wilmington, USA). RNA quality was evaluated with the 2100 Bioanalyzer® apparatus (Agilent Technologies, Massy, France) and the RNA 6000 Nano chips.

2.2.2. Reverse-transcription and quantitative PCR

1 μg of total RNA was reverse-transcribed using the Superscript II RT kit (Life technologies, Belgium). Absence of contaminating genomic DNA was checked using controls with RNA but lacking reverse transcriptase. PCR was performed in a CFX96 thermocycler with the IQ™ SYBR® Green Supermix (BioRad, Nazareth, Belgium). PCR primers were designed with the Beacon Designer software (Premier Biosoft, USA). The housekeeping gene SF3A1 was used for normalization purposes. The choice of SF3A1 has been previously rationalized [18]. For each gene measurement, a melting curve analysis and the sequencing of PCR products allowed attesting for the specificity of the amplification. An inter-run calibrator was used to normalize the variations between PCR plates. Gene expression levels were calculated by the relative quantification method (ΔΔCt) using the CFX Manager 2.1 software which takes into account primer efficacy (Bio-Rad). Primer sequences, hybridization temperatures and PCR efficiencies are provided in Table 3 (Supplementary data).

2.3. Statistical analyses

All statistical analyses started with a Shapiro-Wilk test to assess data normality. *t*-Test and Mann–Whitney test were used to compare two groups of continuous variables following a Gaussian or non-Gaussian distribution, respectively. The Chi-square test was used for qualitative data. The SigmaPlot v12.0 software was used for statistical analyses. All tests were two-tailed and a P value <0.05 was considered significant.

For prediction analyses, missing values were replaced using 100-fold multiple imputation. Continuous variables were scaled to mean = 0 and standard deviation = 1. Expression of genes was log10-transformed. Univariate and multivariable analyses with logistic regression were conducted to evaluate the ability of genes to predict LV dysfunction, either alone or in combination with various demographic and clinical parameters. Odds ratios (OR) with 95% confidence intervals (CI) were calculated. The Wald chi-square test was used to measure the statistical significance of the models. The Likelihood ratio test was used to compare 2 models. Bootstrap internal validation was used to test the robustness of the models. For each bootstrap sample, the whole model selection was performed to select the best model according to the Akaike Information Criteria (AIC). Computation of the net reclassification index (NRI) and the integrated discrimination improvement (IDI) was used for reclassification analyses. All prediction analyses were performed on the R version 2.14.2 statistical platform using the packages Hmisc, PredictABEL, lmtest and bootStepAIC.

3. Results

3.1. Study population

Characteristics of the 449 MI patients enrolled in this study are presented in Table 1 (Supplementary data). Median age was 61 and 75% were males. All patients had a successful revascularization. LV function was evaluated 4 months after MI and patients with an EF ≤40% were considered as having LV dysfunction. Of the 449 MI patients enrolled in the study, 79 (18%) had LV dysfunction and 370 (82%) had a preserved LV function at 4-month follow-up. As compared to patients without LV dysfunction, patients with LV dysfunction were older, had higher white blood cell counts at admission, higher peak levels of CPK and cTnT, higher admission levels of NT-proBNP, and had more often type 2 diabetes.

3.2. Gene expression levels according to LV function

Expression levels of LMNB1, MMP9, TGFBR1, TNXB and LTBP4 were assessed using quantitative PCR in whole blood samples obtained at reperfusion in 449 MI patients. Levels of LMNB1, MMP9 and TGFBR1 were up-regulated in patients with LV dysfunction (4-month EF ≤40%) as compared to patients with preserved LV function (4-month EF >40%). LTBP4 showed the opposite trend, being down-regulated in patients with LV dysfunction, and TNXB displayed comparable expression levels between patients with LV dysfunction and patients with preserved LV function (Fig. 1).

3.3. Gene expression in leukocyte subsets

To investigate the cellular origin of the 5 genes, neutrophils, monocytes and lymphocytes were isolated from the peripheral blood of healthy donors. LMNB1 and MMP9 were mostly expressed in neutrophils, TNXB and LTBP4 were predominant in monocytes and lymphocytes, and TGFBR1 was expressed at a similar level in all cell types (Fig. 1 in the Online Supplement).

3.4. Univariate analyses

We first determined the ability of the 5 genes individually to predict LV dysfunction (4-month EF ≤40%) using univariate logistic regression. The 5 genes were significant univariate predictors of LV dysfunction with odds ratios (OR) [95% confidence intervals (CI)] of 1.40 [1.12–1.74], 0.75 [0.57–0.98], 1.35 [1.07–1.69], 1.43 [1.15–1.79] and 1.26 [1.02–1.57] for LMNB1, LTBP4, MMP9, TGFBR1 and TNXB, respectively (Fig. 2).