Circulation: Genomic and Precision Medicine

ORIGINAL ARTICLE

Peripheral Blood RNA Levels of *QSOX1* and *PLBD1* Are New Independent Predictors of Left Ventricular Dysfunction After Acute Myocardial Infarction

BACKGROUND: The identification of patients with acute myocardial infarction (MI) at risk of subsequent left ventricular (LV) dysfunction remains challenging, but it is important to optimize therapies. The aim of this study was to determine the unbiased RNA profile in peripheral blood of patients with acute MI and to identify and validate new prognostic markers of LV dysfunction.

METHODS: We prospectively enrolled a discovery cohort with acute MI (n=143) and performed whole-blood RNA profiling at different time points. We then selected transcripts on admission that related to LV dysfunction at follow-up and validated them by quantitative polymerase chain reaction in the discovery cohort, in an external validation cohort (n=449), and in a representative porcine MI model with cardiac magnetic resonance–based measurements of infarct size and postmortem myocardial pathology (n=33).

RESULTS: RNA profiling in the discovery cohort showed upregulation of genes involved in chemotaxis, IL (interleukin)-6, and NF-κB (nuclear factor-κB) signaling in the acute phase of MI. Expression levels of the majority of these transcripts paralleled the rise in cardiac troponin T and decayed at 30 days. RNA levels of *QSOX1*, *PLBD1*, and *S100A8* on admission with MI correlated with LV dysfunction at follow-up. Using quantitative polymerase chain reaction, we confirmed that *QSOX1* and *PLBD1* predicted LV dysfunction (odds ratio, 2.6 [95% CI, 1.1–6.1] and 3.2 [95% CI, 1.4–7.4]), whereas *S100A8* did not. In the external validation cohort, we confirmed *QSOX1* and *PLBD1* as new independent markers of LV dysfunction (odds ratio, 1.41 [95% CI, 1.06–1.88] and 1.43 [95% CI, 1.08–1.89]). *QSOX1* had an incremental predictive value in a model consisting of clinical variables and cardiac biomarkers (including NT-proBNP [N-terminal pro-B-type natriuretic peptide]). In the porcine MI model, whole-blood levels of *QSOX1* and *PLBD1* related to neutrophil infiltration in the ischemic myocardium in an infarct size–independent manner.

CONCLUSIONS: Peripheral blood *QSOX1* and *PLBD1* in acute MI are new independent markers of LV dysfunction post-MI.

Maarten Vanhaverbeke, MD, PhD
Mélanie Vausort, MSc
Denise Veltman, MSc
Lu Zhang, MSc
Ming Wu, MD, PhD
Griet Laenen, MSc
Hilde Gillijns, BSc
Yves Moreau, PhD
Jozef Bartunek, MD, PhD
Frans Van De Werf, MD, PhD
Yvan Devaux, PhD
Stefan Janssens, MD, PhD
Peter R. Sinnaeve, MD, PhD
on behalf of EU-CardioRNA COST Action CA17129

Key Words: biomarkers ■ gene expression ■ inflammation ■ myocardial infarction ■ ventricular dysfunction, left

© 2019 The Authors. *Circulation: Genomic and Precision Medicine* is published on behalf of the American Heart Association, Inc., by Wolters Kluwer Health, Inc. This is an open access article under the terms of the Creative Commons Attribution Non-Commercial-NoDerivs License, which permits use, distribution, and reproduction in any medium, provided that the original work is properly cited, the use is noncommercial, and no modifications or adaptations are made.

https://www.ahajournals.org/journal/circgen

Downloaded from http://ahajournals.org by on May 6, 2020

Despite marked improvements in the treatment of acute myocardial infarction (MI), the long-term outcome after MI remains poor. The rate of rehospitalization for heart failure at 1 year post-MI ranges from 5% ≤20%, despite state-of-the-art therapy.[1] Up to 15% of patients with ST-segment–elevation MI have a left ventricular (LV) ejection fraction <40% at follow-up.[2] Heart failure post-MI encompasses high morbidity and a high cost for rehospitalizations, drugs, and device therapies. Therefore, position papers have recently emphasized the need to identify new predictors and identify new targets for intervention.[3–5]

Since inflammation plays a pivotal role in the acute injury during MI and subsequent repair, a better characterization of the inflammatory drivers might improve the identification of patients at increased risk for adverse outcome.[6] Conventional markers such as white blood cell count and C-reactive protein are indeed associated with outcome.[7–9] However, a more comprehensive assessment of the inflammatory response upstream of these markers is lacking, but it is indispensable to identify new targets for intervention and improve the identification of high-risk patients.

Detailed assessment of the inflammatory response using whole-blood RNA in dedicated RNA tubes is a promising approach, providing detailed information on leukocyte function without the need for expedite sample handling or complex cell isolation techniques. Both coding and selected long noncoding RNAs were reported to be regulated in peripheral blood of patients with acute MI and to correlate with outcome.[10–12]

Therefore, the aim of this study was to characterize the unbiased circulating RNA signature during and after MI and to identify and validate new prognostic markers for LV dysfunction at follow-up.

METHODS

We prospectively enrolled a discovery cohort of patients with acute MI (n=143) and performed whole-blood RNA profiling at different time points. Selected transcripts related to LV dysfunction were validated in a validation cohort (n=449) using quantitative polymerase chain reaction and in a porcine model of MI with myocardial histology and cardiac magnetic resonance (CMR) imaging. Detailed methods and data supporting the findings are available in the Data Supplement. RNA profiling data are publicly available in the Gene Expression Omnibus (GSE123342). The study protocol complies with the Declaration of Helsinki, was approved by the institution's ethical committee, and all patients signed informed consent. Animal experiments were approved by the institution's ethical committee and performed according to the institutional guidelines.

RESULTS

Characteristics of the Study Cohorts

Demographic and clinical variables of patients in the discovery and validation cohorts are shown in Table 1. In the discovery cohort, the samples of 143 of 180 prospectively enrolled patients were eligible for measurement of RNA expression (study flow chart; Figure I in the Data Supplement). Forty-three percent of patients had ST-segment–elevation MI (n=61), and 13% (n=18) developed LV dysfunction at follow-up. Patients in the validation cohort had a higher risk profile: 78% of patients (n=352) had ST-segment–elevation MI, and 18% of patients (n=79) had LV dysfunction at follow-up. There were no baseline differences in the use of ACE (angiotensin-converting enzyme) inhibitors or β-blockers in patients with versus without LV dysfunction at follow-up.

Unbiased RNA Profiling Identifies Pathways Related to Inflammation in the Acute Phase of MI

In the discovery cohort, we first compared the unbiased RNA expression profile of 65 patients on admission with MI to 22 stable controls matched in 3:1 ratio (Figure 1A). This resulted in 1084 differentially expressed transcripts (false discovery rate–adjusted $P<0.05$; Figure 1B), including 469 annotated protein-coding genes and 25 long noncoding transcripts (8 intergenic RNAs, 4 antisense RNAs, and 13 pseudogenes; Figure 1C). Unbiased network analysis shows clustering related to Toll-like receptor and NF-κB (nuclear factor-κB) signaling, chemotaxis, and ubiquitination (Figure 1D). Gene ontology analysis confirms significant enrichment of biological processes related to immune response and chemotaxis in acute MI (Figure 1E, blue). The underlying pathways related to the observed immune response were identified using pathway analysis: 9 canonical pathways were significantly enriched in the acute phase of MI (Fisher exact false discovery rate–adjusted $P<0.0005$), shown in Figure 1E (green). The identified transcripts were predominantly expressed on the plasma membrane (Figure 1F, blue), and tissue expression profiles confirmed that they were predominantly originating from circulating leukocytes (Figure 1F, green). Taken together, the global transcriptome response highlights upregulation of different pattern recognition receptors (Toll-like receptors such as *TLR4* and *TLR2*, C-type lectin receptors such as *CLEC4E* and *CLEC4D*, and Nod-like receptors such as *NLRC4* and *NLRP12*), resulting in downstream NF-κB (*NFKBIA* and *MYD88*) signaling. Additional markers of leukocyte activation (*IRAK3*, *IL1R2*, and *IL18R1*) and mean arterial pressure kinase signaling were also upregulated. A full list of all differentially expressed transcripts, biological processes, and pathways is available in the Data Supplement.

RNA Expression in the Acute Phase Clusters With Acute Cardiac Injury and Conventional Markers of Inflammation

A large number of transcripts significantly correlated with ejection fraction in the acute phase of MI (374

Downloaded from http://ahajournals.org by on May 6, 2020

Table 1. Baseline Characteristics of the Discovery Cohort, Validation Cohort, and Stable Controls

	Discovery Cohort (n=143)	Validation Cohort (n=449)	Controls (n=22)	*P* Value*
Age, y; median (range)	63 (30–88)	61 (30–91)	60 (47–90)	0.118
Sex, male; n (%)	113 (79.0)	339 (75.5)	17 (77.2)	0.430
Body mass index, median (range)	27 (18–46)	27 (18–51)	27 (20–38)	0.952
Medical history, n (%)				
Current smoker	55 (38.5)	205 (45.9)	4 (18.2)	0.147
Hypertension	66 (46.2)	217 (48.8)	15 (68.2)	0.701
Hypercholesterolemia	112 (78.3)	199 (45.3)	21 (95.5)	<0.001
Diabetes mellitus	18 (12.6)	98 (22.0)	0 (0)	0.015
Prior MI	14 (9.8)	51 (11.4)	5 (22.7)	0.649
Medication on admission, n (%)				
Aspirin	42 (29.4)	85 (19.2)	18 (81.8)	0.010
β-Blocker	40 (28.0)	94 (21.3)	13 (59.1)	0.086
ACE inhibitor	25 (17.5)	56 (12.8)	11 (50.0)	0.162
Angiotensin receptor antagonist	11 (7.7)	51 (11.6)	2 (4.5)	0.272
Statin	45 (31.5)	89 (20.1)	18 (81.8)	0.006
MI presentation, n (%)				
ST-segment elevation	61 (42.7)	352 (78.4)		<0.001
Anterior infarction	59 (41.3)	348 (77.5)		<0.001
CABG	13 (9.1)	3 (0.7)		<0.001
PCI	141 (98.6)	433 (96.4)		0.266
White blood cells on admission†				
Total white blood cells, 10^9/L	9.5 (4.3–21.1)	10.8 (3.1–29.0)	7.0 (4.8–11.0)	<0.001
Neutrophils, %	66 (34–89)	74 (24–94)	62 (36–81)	<0.001
Lymphocytes, %	23 (6–55)	16 (3–70)	27 (10–51)	<0.001
Monocytes, %	8 (4–15)	6 (1–22)	8 (4–11)	<0.001
Biomarkers, peak values†				
CRP, mg/L	10.30 (0.60–251.00)	8.80 (0.30–333.40)		0.227
cTnT, ng/L	994 (20–18 680)	3820 (10–26 930)		<0.001
LV EF ≤40% at follow-up, n (%)	18 (12.6)	79 (17.6)	0 (0)	0.194

ACE indicates angiotensin-converting enzyme; CABG, coronary artery bypass grafting; CRP, C-reactive protein; cTnT, cardiac high-sensitivity troponin T; EF, ejection fraction; LV, left ventricle; MI, myocardial infarction; and PCI, percutaneous coronary intervention.

**P* value for discovery vs validation cohort.

†Median values (range).

Downloaded from http://ahajournals.org by on May 6, 2020

transcripts with adjusted Spearman $P<0.05$), peak cTnT (684 transcripts), and white blood cell count (688 transcripts). RNA expression also clustered with type of presentation, with a gradient from patients with ST-segment–elevation MI over non–ST-segment–elevation MI to stable coronary artery disease (Figure II in the Data Supplement). At 30-day and 1-year follow-up, RNA expression largely normalized: only 5.8% and 3.4% of the initial 1084 transcripts remained differentially expressed compared with stable controls, with low fold changes. Further analyses, therefore, focused on RNA expression patterns in the acute phase of MI.

Identification of QSOX1, PLBD1, and S100A8 on Admission for the Prediction of LV Dysfunction at Follow-Up in the Discovery Cohort

In light of the high correlation between RNA expression and cardiac injury and inflammation, we next investigated whether RNA expression at the time of admission is related to LV function at follow-up. We selected *QSOX1*, *PLBD1*, and *S100A8* as 3 top RNAs that were significantly upregulated in the acute phase of MI and related to LV function at follow-up, for further evaluation using quantitative polymerase chain reaction. We

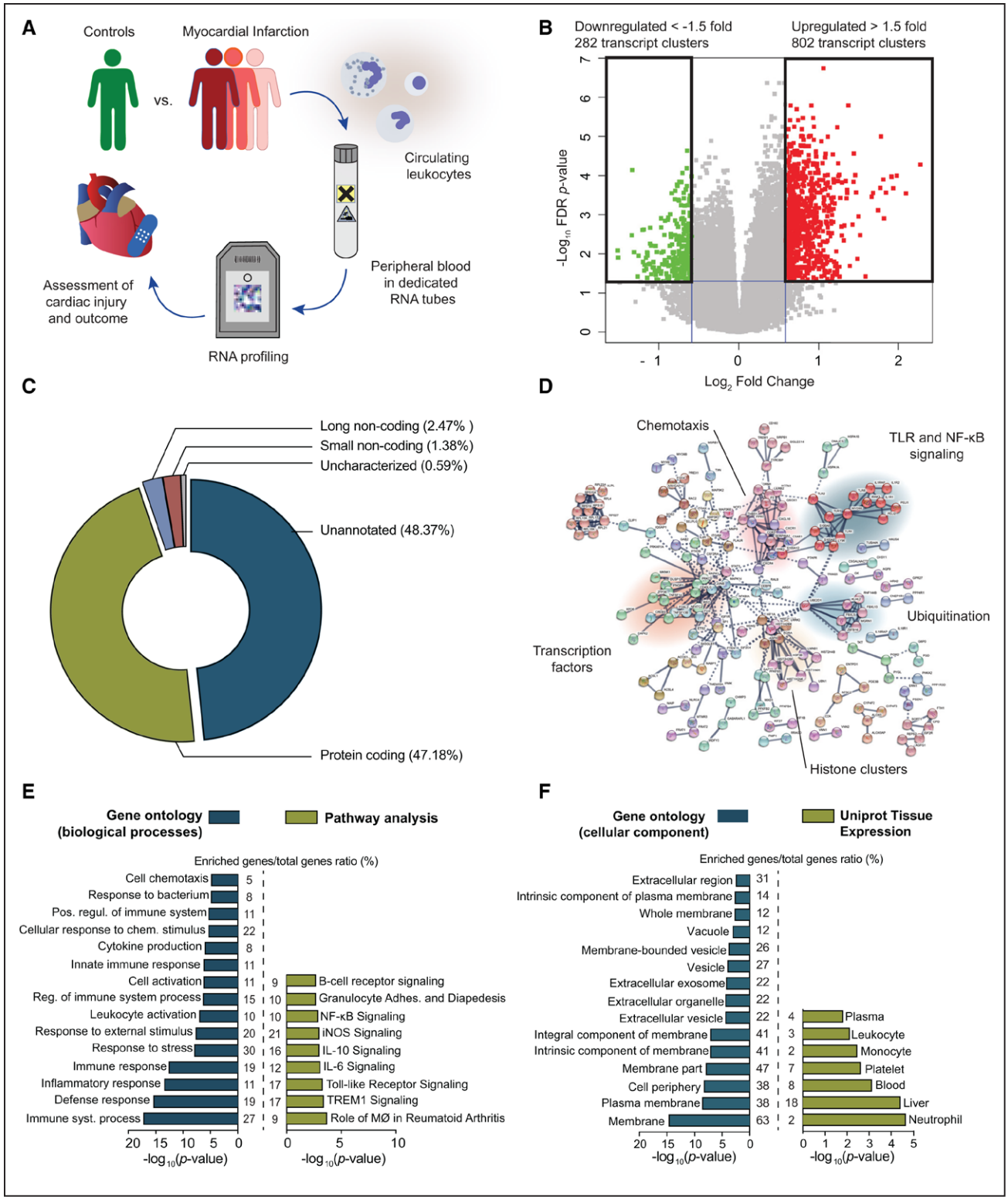

Figure 1. Coding and noncoding RNAs in whole blood reflect inflammatory pathways involved in the acute phase of myocardial infarction (MI). **A**, We performed unbiased RNA profiling in peripheral blood of patients presenting with acute MI, with the specific aim of identifying patterns that relate to cardiac injury and outcome. **B**, This approach revealed 802 upregulated and 282 downregulated transcript clusters in the acute phase of MI (downregulated to the left in green, upregulated to the right in red), with a fold change compared with stable controls >1.5 or <1.5 and adjusted $P<0.05$. **C**, These transcripts were predominantly protein coding (47%) but also included long noncoding transcripts (1.7%), although many remained unannotated (48.5%). **D**, Network analysis shows that many of these transcripts cluster together in networks associated with immune response, chemotaxis, and Toll-like receptor/NF-κB (nuclear factor-κB) signaling. **E**, These biological processes are confirmed by gene ontology analysis (**left**, blue), and additional pathways in IL (interleukin) signaling, MAPK signaling, and neutrophil activation were activated (**right**, green). **F**, The identified transcripts are predominantly expressed on the cell membrane (**left**, blue), and were enriched in circulating leukocytes (**right**, green). All *P* values are false discovery rate (FDR) adjusted for multiple testing. iNOS indicates inducible NO synthase; MØ, macrophages; MAPK, mitogen-activated protein kinase; TLR, toll-like receptor; and TREM1, triggering receptor expressed on myeloid cells 1.

Downloaded from http://ahajournals.org by on May 6, 2020

first confirmed that these 3 transcripts were significantly upregulated in MI on admission (n=138) compared with stable controls (n=20) using quantitative polymerase chain reaction (Figure 2A): 1.77-fold change for *QSOX1* (*P*=0.013), 1.58 for *PLBD1* (*P*<0.001), and 2.40 for *S100A8* (*P*<0.001). Second, *QSOX1* and *PLBD1*, but not *S100A8*, were significantly higher on admission in patients who developed LV dysfunction at 1-year follow-up (n=18), compared with those who did not (n=100; Figure 2B): 1.50-fold change for *QSOX1* (*P*=0.021), 1.39-fold change for *PLBD1* (*P*<0.001), and 1.06-fold for *S100A8* (*P*=0.45; Figure 2B). In binary logistic regression, *QSOX1* and *PLBD1* were univariate predictors of LV dysfunction at follow-up (odds ratio [OR], 2.6 [95% CI, 1.1–6.1] and OR, 3.2 [95% CI, 1.4–7.4], respectively), whereas S100A8 was not (OR, 1.2 [95% CI, 0.8–1.7]).

External Validation Confirms QSOX1 and PLBD1 as Independent Predictors of LV Dysfunction at Follow-Up

In the independent validation cohort, *QSOX1* and *PLBD1* were confirmed to be significantly higher expressed in the group of patients with LV dysfunction at 4 months of follow-up (n=79) compared with patients without LV dysfunction (n=370): 1.31-fold (*P*<0.001) and 1.32-fold (*P*<0.001), respectively (Figure 3A). Consistent with the discovery cohort, no difference was observed for *S100A8* (1.14-fold; *P*=0.487). *QSOX1* and *PLBD1* showed statistically significant positive correlation with leukocyte and neutrophil count, peak levels of cTnT, NT-proBNP (N-terminal pro-B-type natriuretic peptide), and negative correlation with ejection fraction at 4 months of follow-up, with moderate correlation coefficients (Table 2; Table II in the Data Supplement).

In logistic regression, *QSOX1* and *PLBD1* were univariate predictors of LV dysfunction at 4 months with ORs of 1.74 (95% CI, 1.37–2.22) and 1.71 (95% CI, 1.33–2.19), respectively (Figure 3B). In multivariable analysis, *QSOX1* and *PLBD1* were independent predictors of LV dysfunction (ORs, 1.43 [95% CI, 1.08–1.89] and 1.41 [95% CI, 1.06–1.88], respectively), as well as admission levels of NT-proBNP and peak levels of CPK (creatine phosphokinase; Figure 3C through 3E). *S100A8* was not a significant predictor of LV dysfunction in this cohort.

The incremental predictive value of these 3 new markers on top of conventional clinical and biochemical variables was calculated using the Akaike information criteria (AIC) of prediction models. The use of the AIC instead of the area under the curve allows for adjusting for the multiplication of variables entered into the model, therefore, avoiding model overfitting. A lower AIC indicates a better model fit. The AIC was calculated for the clinical model alone and after adding each combination of 1 to 3 genes (Table 3). All models were able to significantly predict LV dysfunction (Wald χ^2 test *P*<0.001). While the addition of *S100A8* to the clinical model did not provide a significant incremental predictive value, adding *QSOX1* or *PLBD1* to the clinical model improved prediction. The best improvement of prediction (lowest AIC) was observed for the clinical model with *QSOX1*. Adding *PLBD1* did not provide a further incremental predictive value. In reclassification analysis, the addition of *QSOX1* to the clinical model was able to reclassify a moderate but significant proportion of patients (1.6%) misclassified by the clinical model alone with a net reclassification index of 0.397 (95% CI, 0.161–0.634) and an integrated discrimination index of 0.017 (95% CI, 0.003–0.031; Table 3). Adding *QSOX1* improved the sensitivity of the model from 69.6% to 73.4% and specificity from 69.5% to 70.5% (Figure III in the Data Supplement). Since the 3 identified transcripts correlated with leukocyte counts, we next adjusted the expression of *QSOX1*, *PLBD1*, and *S100A8* for white blood cell count. The predictive value

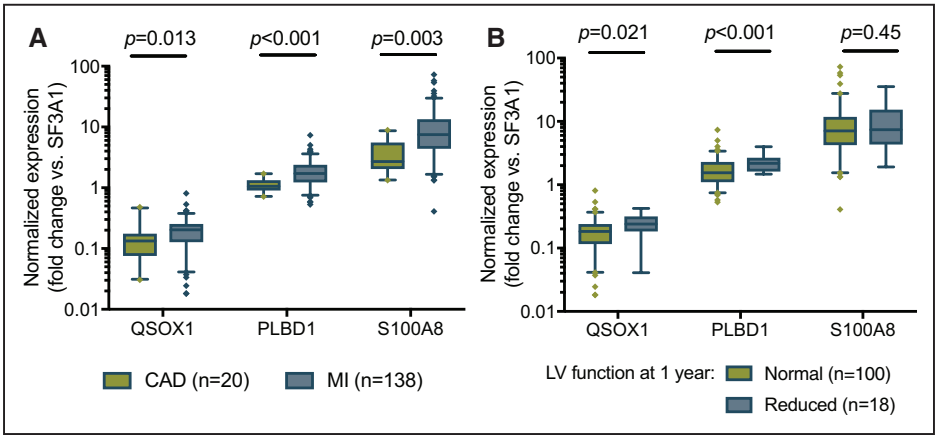

Figure 2. Expression levels of QSOX1, PLBD1, and S100A8 in the discovery cohort.
A, In the discovery cohort, *QSOX1*, *PLBD1*, and *S100A8* are upregulated in acute myocardial infarction (MI; n=138) compared with stable controls (n=20). **B**, *QSOX1* and *PLBD1* on admission were significantly higher in patients who developed left ventricular (LV) dysfunction (n=18) compared with those who did not (n=100). CAD indicates coronary artery disease; PLBD1, Phospholipase B Domain Containing 1; QSOX1, Quiescin sulfhydryl oxidase 1; S100A8, S100 calcium-binding protein A8; and SF3A1, Splicing factor 3 subunit 1.

Downloaded from http://ahajournals.org by on May 6, 2020

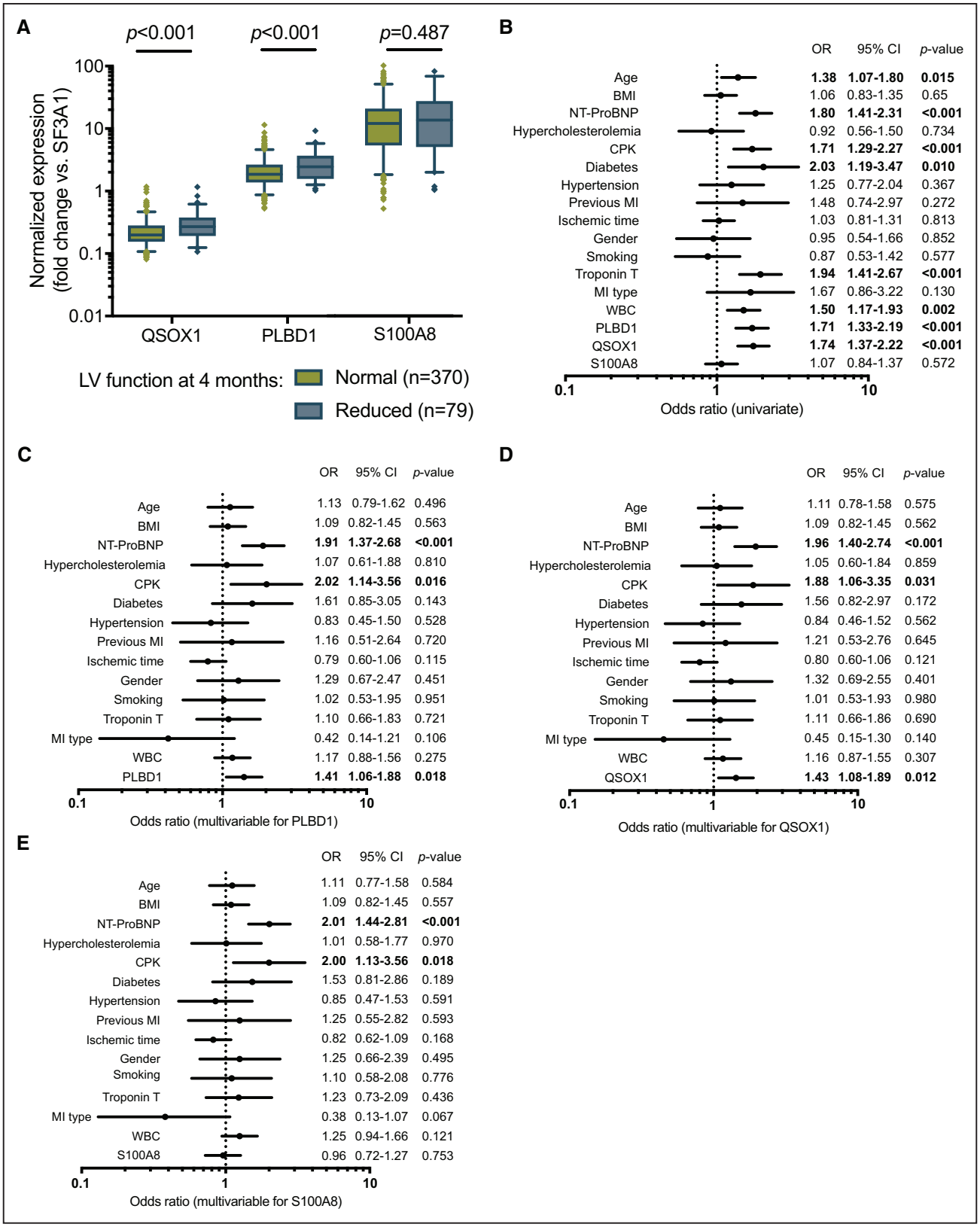

Figure 3. QSOX1 and PLBD1 are independent predictors of left ventricular (LV) dysfunction in the validation cohort.
A, In the validation cohort (n=449), *QSOX1* and *PLBD1* expression at reperfusion was upregulated in patients developing LV dysfunction (n=79) compared with those who did not (n=370). **B**–**E**, The ability of *QSOX1*, *PLBD1*, and *S100A8* to predict LV dysfunction was determined using univariate (**B**) and multivariable analyses (**C**–**E**). The parameters included in the clinical model were age, body mass index (BMI), admission level of NT-proBNP (N-terminal pro-B-type natriuretic peptide), hypercholesterolemia, peak levels of CPK (creatine phosphokinase), diabetes mellitus, hypertension, previous myocardial infarction (MI), ischemic time (ie, delay between chest pain onset and reperfusion), sex, smoking, peak levels of cardiac troponin T, MI type (ST-segment–elevation MI vs non–ST-segment–elevation MI), and white blood cell count (WBC). Odds ratios (ORs) with 95% CIs are shown, and significant associations are in bold. PLBD1 indicates Phospholipase B Domain Containing 1; QSOX1, Quiescin sulfhydryl oxidase 1; S100A8, S100 calcium-binding protein A8; and SF3A1, Splicing factor 3 subunit 1.

Downloaded from http://ahajournals.org by on May 6, 2020

Table 2. Spearman Correlation in Patients Between Expression Levels of *QSOX1*, *PLBD1*, and *S100A8*, Blood Biomarkers on Admission, Peak Levels of Cardiac Biomarkers, and EF at 4-mo Follow-Up in the Validation Cohort

	QSOX1		*PLBD1*		*S100A8*	
	Coeff	*P* Value	Coeff	*P* Value	Coeff	*P* Value
Blood biomarkers* (admission levels)						
White blood cell count	0.254	<0.001	0.262	<0.001	0.184	<0.001
Neutrophils						
%	0.272	<0.001	0.26	<0.001	0.117	0.017
10^9/L	0.296	<0.001	0.302	<0.001	0.174	<0.001
Lymphocytes						
%	−0.262	<0.001	−0.268	<0.001	−0.078	0.111
10^9/L	−0.096	0.051	−0.115	0.018	0.038	0.442
Monocytes						
%	−0.073	0.135	−0.016	0.74	−0.143	0.003
10^9/L	0.128	<0.001	0.181	<0.001	0.019	0.702
Platelet count	0.097	0.045	0.080	0.099	0.005	0.912
CRP	0.247	<0.001	0.285	<0.001	0.175	<0.001
Cardiac biomarkers (peak levels)						
cTnT	0.3	<0.001	0.247	<0.001	0.105	0.030
NT-proBNP	0.055	0.263	0.116	0.017	−0.030	0.535
EF at 4 mo	−0.2	<0.001	−0.163	<0.001	−0.043	0.366

Coeff indicates correlation coefficient; CRP, C-reactive protein; cTnT, cardiac high-sensitivity troponin T; EF, ejection fraction; and NT-proBNP, N-terminal pro-B-type natriuretic peptide.

*White blood cell counts are expressed in absolute values (billion cells per liter of blood). Neutrophils, lymphocytes, and monocytes are expressed both as a percentage of white blood cell count and as absolute values.

Downloaded from http://ahajournals.org by on May 6, 2020

of the 3 genes was conserved, and we observed similar predictive values after adjustment (Figure IV in the Data Supplement).

Circulating QSOX1 and PLBD1 Relate to Neutrophil Count and Neutrophil Infiltration in Porcine Ischemic Myocardium

To provide direct evidence that whole-blood RNA expression as surrogate tissue is associated with myocardial injury, we correlated RNA levels from porcine blood with serial cTnT measurements, CMR-based infarct size, and myocardial tissue analysis in a representative experimental MI model. Baseline characteristics of the animals are shown in Table 4. Expression levels of *QSOX1*, *PLBD1*, and *S100A8* in whole blood were significantly upregulated at 120 minutes after transient left anterior descending artery occlusion (1.6-, 2.2-, and 4.0-fold; Figure 4A).

Consistent with the human data, *QSOX1*, *PLBD1*, and *S100A8* at 120 minutes after reperfusion correlat-

Table 3. Prediction and Reclassification Analyses in the Validation Cohort

Gene Added to the Clinical Model	Wald χ^2 Test *P* Value	AIC	LRT (vs Clinical Model)	NRI			IDI		
				Value	95% CI	*P* Value	Value	95% CI	*P* Value
None	<0.001	390.56							
PLBD1	<0.001	386.90	0.017	0.244	0.002 to 0.485	0.048	0.012	−0.001 to 0.026	0.073
QSOX1	<0.001	386.14	0.011	0.397	0.161 to 0.634	0.001	0.017	0.003 to 0.031	0.019
S100A8	<0.001	392.46	0.753	0.005	−0.238 to 0.248	0.966	6×10^{-4}	−0.001 to 0.003	0.533
PLBD1+QSOX1	<0.001	387.78	<0.001	0.336	0.097 to 0.575	0.006	0.017	0.002 to 0.031	0.024

The parameters included in the clinical model were age, BMI, admission level of NT-proBNP, hypercholesterolemia, peak levels of CPK, diabetes mellitus, hypertension, previous MI, ischemic time (ie, delay between chest pain onset and reperfusion), sex, smoking, peak levels of cardiac troponin T, MI type (ST-segment–elevation vs non–ST-segment–elevation MI), and WBC. The Wald χ^2 test indicates the overall significance of the model. A lower AIC indicates a better model fit. The LRT compares the fit of a model with genes to the clinical model alone. The NRI and IDI were used to quantify the ability of genes to reclassify patients misclassified by the clinical model. AIC indicates Akaike information criteria; BMI, body mass index; CPK, creatine phosphokinase; IDI, integrated discrimination index; LRT, likelihood ratio test; MI, myocardial infarction; NRI, net reclassification index; NT-proBNP, N-terminal pro-B-type natriuretic peptide; and WBC, white blood cell.

Table 4. Baseline Characteristics, Pathology, and CMR Imaging in Pigs Undergoing Myocardial Ischemia-Reperfusion Injury (n=33)

	Baseline (n=33)	120 min Reperfusion (n=31)	*P* Value
Death (refractory VF)	n=2		
VF with successful defibrillation	n=10		
Hemodynamic and biochemical measurements			
Systolic blood pressure, mm Hg	95±12	89±5	0.059
Diastolic blood pressure	63±10	54±8	0.002
MAP, mm Hg	78±11	70±5	<0.001
Heart rate (per min)	99±19	103±25	0.57
WBC count, 10^9/L	16.68±5.00	16.67±3.90	0.98
Neutrophil count, %	37±12	40±11	0.13
Lymphocyte count, %	54±14	51±12	0.13
Monocyte count, %	5±2	6±3	0.45
cTnT, ng/L	13±6	13 521±12 221	<0.001
Pathology	Ischemic zone	Remote zone	
Neutrophils (per mm^2)	261±78	160±65	<0.001
Macrophages (per mm^2)	229±111	175±100	<0.001
MPO activity (mU/mg protein)	535±263	78±70	<0.001
CMR measurements			
Ejection fraction, %		34±9	
LVEDVi, mL/m^2		88±16	
LVESVi, mL/m^2		57±11	
Infarct size (%LV mass)		27±9	
Microvascular obstruction (%LV mass)		7±10	
Edema (%LV mass)		37±7	

Data are shown as mean±SD. CMR indicates cardiac magnetic resonance; cTnT, cardiac high-sensitivity troponin T; LV, left ventricle; LVEDVi, left ventricular end-diastolic volume index; LVESVi, left ventricular end-systolic volume index; MAP, mean arterial pressure; MPO, myeloperoxidase; VF, ventricular fibrillation; and WBC, white blood cell.

ed significantly with neutrophil count (Table 5). *S100A8* and *PLBD1*, but not *QSOX1*, moderately correlated with the area under the curve of cTnT release (*P*=0.020 and *P*=0.142; n=31). However, none of the 3 transcripts correlated to CMR-based infarct size (n=18).

We next assessed the correlation between these transcripts and neutrophil and macrophage infiltration in the ischemic myocardium. A representative histological section, stained with a neutrophil-specific MPO (myeloperoxidase) antiserum, is shown in Figure 4B. Circulating *QSOX1* and *PLBD1* significantly correlated with neutrophil infiltration (r=0.47, *P*=0.024 and r=0.48, *P*=0.0007; Table 5). *PLBD1* and to a lesser extent *QSOX1* also correlated with neutrophil (MPO) activity in tissue (r=0.40, *P*=0.030 and r=0.37, *P*=0.087). In contrast, *S100A8* correlated significantly with macrophage infiltration (r=0.42, *P*=0.018).

Origin of Circulating QSOX1, PLBD1, and S100A8 in Isolated Leukocytes and in Ischemic Myocardium

To identify the origin of the identified expression changes, we measured *QSOX1*, *PLBD1*, and *S100A8* in leukocyte subtypes and in the ischemic myocardium. *QSOX1* in whole blood only weakly correlated with expression levels in circulating neutrophils (r=0.43, *P*=0.04), and it did not correlate with expression levels in isolated lymphocytes or monocytes (Table 5). Moreover, *QSOX1* showed only minor differential expression in these cells (Figure 4C). However, circulating *QSOX1* highly correlated with expression in the ischemic zone of the myocardium (r=0.61, *P*=0.002). In contrast, *PLBD1* levels in whole blood highly correlated with expression in circulating lymphocytes (r=0.584, *P*<0.001), and the latter paralleled the expression observed in whole blood after ischemic injury (Figure 4D). However, *PLBD1* levels in peripheral blood did not correlate with expression levels in the myocardium. Also peripheral blood *S100A8* appeared to parallel expression in neutrophils and lymphocytes, in which it was also differentially expressed (Figure 4E). In contrast to *QSOX1*, *S100A8* was not related to the levels in the myocardium.

DISCUSSION

New strategies to identify patients at risk for developing LV dysfunction after MI are needed to improve outcome. We here report that whole-blood transcriptome profiling at the time of hospital admission for acute MI reveals significant upregulation of pattern recognition receptors and transcripts involved in NF-κB, IL (interleukin)-6, and TLR signaling. Moreover, we identified *QSOX1* and *PLBD1* as new markers for the prediction of LV dysfunction at follow-up, independent of conventional risk factors, and validated these findings in a large independent MI cohort. In a large animal MI model representative of human disease, circulating *QSOX1* and *PLBD1* were related to neutrophil count and neutrophil infiltration in the ischemic myocardium. Circulating *QSOX1* levels highly related to those measured in the ischemic myocardium, while *PLBD1* levels correlated with levels in lymphocytes. Taken together, these findings indicate the diagnostic potential of whole-blood RNA to reveal disease-specific regulatory pathways in MI and to identify patients with increased risk for LV systolic dysfunction at follow-up.

The first key observation in our unbiased analysis is the identification of over 1084 coding and noncoding transcripts that are differentially expressed in whole blood of patients with ST-segment–elevation or non–ST-segment–elevation MI. These findings are in line with previous studies, which reported smaller numbers of differentially expressed transcripts in acute MI by includ-

Downloaded from http://ahajournals.org by on May 6, 2020

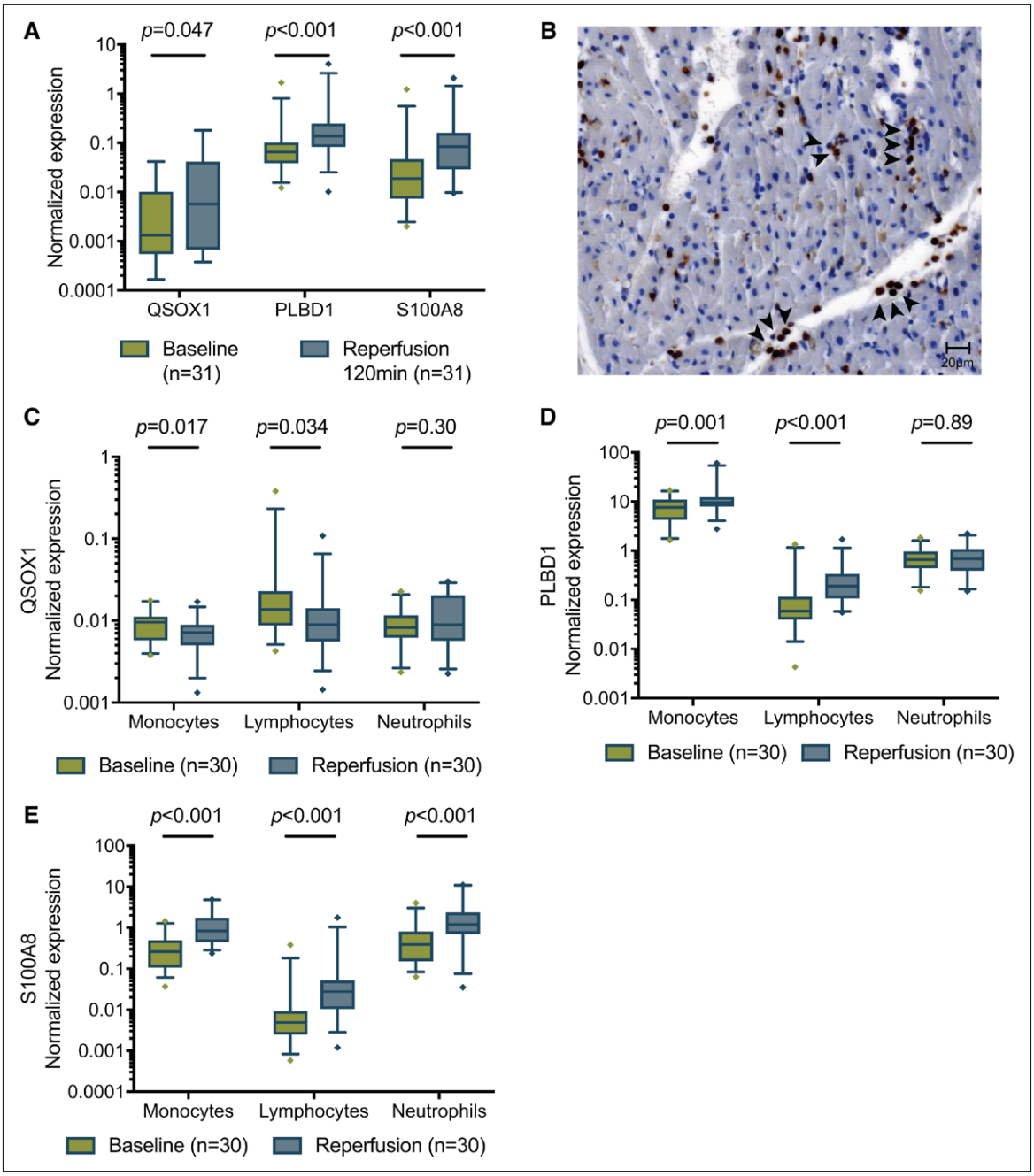

Figure 4. RNA expression in whole-blood and leukocyte subpopulations following acute myocardial infarction (MI) in pigs 120 min after reperfusion. The identified transcripts were validated in 33 pigs undergoing acute myocardial infarction. **A**, All 3 transcripts that were upregulated in acute MI in patients were also upregulated in whole blood of pigs undergoing MI. **B**, Representative light microscopy image of MPO (myeloperoxidase)-stained porcine ischemic myocardium, showing neutrophils (black arrowheads), cardiomyocytes, and capillaries. **C**–**E**, Expression levels of *QSOX1*, *PLBD1*, and *S100A8* in isolated circulating monocytes, lymphocytes, and neutrophils. PLBD1 indicates Phospholipase B Domain Containing 1; QSOX1, Quiescin sulfhydryl oxidase 1; and S100A8, S100 calcium-binding protein A8.

ing only selected patients with ST-segment–elevation MI or by using a targeted approach for coding RNA or selected transcripts.[10,12–15] Moreover, we show that 63% of the identified transcripts relate to the extent of myocardial injury in the acute phase of MI. Many of the identified transcripts in our study relate to sterile inflammation and code for pattern recognition surface receptors such as *TLR2*, *TLR4*, and *CLEC4E*. These receptors respond to damage-associated molecular patterns, including *HMGB1* and necrotic cell content, which are released during myocardial injury.[16–18] Pathway analysis also showed activation of IL-1 signaling (*IL1R2*, *IL18R1*, *IRAK3*, *NFKBIA*, and *MYD88*). Several clinical trials have targeted some of these pathways in the setting of acute MI, including IL-1, CD11, and P-selectin, but failed to show marked clinical benefit.[19–23] The identified transcripts in our study may point to alternative pathogenic pathways in acute MI and warrant further exploration.[3,4]

Downloaded from http://ahajournals.org by on May 6, 2020

Table 5. Spearman Correlation Between Porcine Peripheral Blood RNA Expression and RNA Expression in Isolated Leukocyte Subsets and Ischemic Myocardium

	Porcine Blood RNA Expression					
	QSOX1		*PLBD1*		*S100A8*	
	Coeff	*P* Value	Coeff	*P* Value	Coeff	*P* Value
Blood biomarkers*						
White blood cell count	0.267	0.208	0.375	0.038	0.314	0.085
Neutrophils, %	0.522	0.009	0.565	<0.001	0.592	<0.001
Lymphocytes, %	–0.526	0.010	–0.573	<0.001	–0.599	<0.001
Monocytes, %	0.368	0.084	0.367	0.046	0.368	0.046
Cardiac biomarkers						
cTnT*	0.231	0.277	0.318	0.081	0.349	0.054
cTnT (AUC)	0.279	0.187	0.270	0.142	0.417	0.020
CMR measurements						
Ejection fraction, %	0.121	0.681	0.059	0.824	0.152	0.559
Infarct size (%LV mass)	–0.114	0.683	–0.111	0.663	0.150	0.553
Microvascular obstruction (%LV mass)	–0.021	0.944	0.314	0.205	0.201	0.424
Edema (%LV mass)	–0.021	0.944	–0.015	0.951	0.150	0.553
Pathology†						
Neutrophils (per mm^2)	0.470	0.024	0.483	0.007	–0.049	0.798
Macrophages (per mm^2)	0.416	0.043	0.321	0.079	0.422	0.018
MPO activity	0.373	0.087	0.404	0.030	0.157	0.407
RNA expression‡						
Ischemic myocardium	0.610	0.002	–0.222	0.231	0.267	0.147
Neutrophils	0.431	0.040	0.135	0.477	0.553	0.002
Lymphocytes	0.0791	0.720	0.584	<0.001	0.718	<0.001
Monocytes	0.0346	0.876	–0.103	0.588	0.096	0.614

AUC indicates area under the curve; CMR, cardiac magnetic resonance; Coeff, correlation coefficient; cTnT, cardiac high-sensitivity troponin T; LV, left ventricle; and MPO, myeloperoxidase.

*Measured at 120 min after reperfusion.

†Stainings in the ischemic myocardium.

‡Comparison of gene expression in tissue or isolated cells with expression of the respective gene in whole blood.

Downloaded from http://ahajournals.org by on May 6, 2020

The second main finding is that, besides conventional markers of cardiomyocyte death such as cTnT, we identified a number of inflammation-related transcripts that also relate to LV dysfunction at follow-up. We validated *QSOX1* and *PLBD1*. Although expression of *QSOX1* and *PLBD1* shows some intragroup variability and the improvement of the risk prediction by adding *QSOX1* to a full model including NT-proBNP is significant but moderate, the unbiased observation that the inflammatory process measured at RNA level relates to LV function at follow-up, independent from conventional markers of cardiomyocyte death, is a key finding.

QSOX1 (quiescin sulfhydryl oxidase 1) is a sulfhydryl oxidase involved in cellular growth and extracellular matrix remodeling. Our mechanistic studies in a representative large animal model suggest that the circulating *QSOX1* levels may relate to those observed in the ischemic myocardium. Previously, *QSOX1* was induced in the LV of pressure-overloaded rat hearts and has been described as a marker of acute heart failure.[24] QSOX1 has been shown to exhibit a cardioprotective response upon acute stress by orchestrating adequate protein folding in the endoplasmatic reticulum. Indeed, QSOX1$^{-/-}$ knockout mice showed a phenotype of dilated cardiomyopathy and enhanced inflammation.[25] Alternatively, circulating QSOX1 also alters the redox status of soluble proteins in plasma.[26] PLBD1 (Phospholipase B domain containing 1) is a phospholipase, which can generate lipid mediators of inflammation and was first identified in neutrophils.[27] While our data confirm the expression of *PLBD1* in monocytes and neutrophils, they also show that increased *PLBD1* levels in the setting of acute MI are attributable to significant upregulation in circulating lymphocytes. Finally, we could not validate *S100A8* expression as a marker of LV dysfunc-

tion. S100A8 (S100 calcium-binding protein A8) is a S100 calcium-binding protein and can act as a damage-associated molecular pattern to initiate neutrophil chemotaxis.[16] Although S100A8 signaling is related to vascular inflammation and has been shown to aggravate post-MI heart failure, its expression may represent a more generic inflammatory signal detectable in many leukocyte subpopulations.[28,29]

Other complementary RNA-based strategies to improve risk stratification in patients with MI are currently being explored, including circular RNA in whole blood but also long noncoding RNA or microRNA in plasma.[11,30] Nevertheless, whole blood seems to be a valuable source for risk stratification in MI, since neutrophils and lymphocytes contribute substantially to the expression pattern in whole blood.[31,32]

We recognize the limitations of our study. First, as baseline medication use was different between the acute MI patients and the control group with stable coronary artery disease, we cannot completely exclude that the identified RNA profile and pathways in the profiling phase of the study are to some extent driven by imbalances in baseline medication use. However, medication use in patients with MI who did or did not develop LV dysfunction was well balanced. Second, we lack CMR-based assessments of infarct size in our patients, and additional CMR-derived prognostic information including microvascular obstruction. However, in the subset of pigs with available CMR imaging (n=18 of 33), we did not observe associations between RNA expression and infarct size. Third, blood samples from patients (and pigs) were obtained in the acute phase of MI, at the time of reperfusion. Because the inflammatory response is time dependent and RNA profiling was performed on admission, we cannot exclude different RNA profiles at later time points. Fourth, in our bioinformatics analysis, we did not assess the possible contributions of the platelet transcriptome to the whole-blood RNA expression profile. Finally, extended follow-up preclinical studies are required to identify the exact role of *QSOX1* and *PLBD1* as specific markers of cardiac inflammation in acute MI and predictors of LV dysfunction.

In conclusion, whole blood as surrogate tissue provides a valuable noninvasive platform to differentiate dysregulated immune-related pathways in patients with MI at risk for LV dysfunction. Increased expression levels of *QSOX1* and *PLBD1* represent promising new predictors of functional impairment and targets for personalized treatment. Future studies will need to investigate whether optimizing interventions at discharge in acute MI patients, based on RNA levels measured on admission, may improve patient outcome.

ARTICLE INFORMATION

Received June 16, 2019; accepted November 13, 2019.

The Data Supplement is available at https://www.ahajournals.org/doi/suppl/10.1161/CIRCGEN.119.002656.

Correspondence

Maarten Vanhaverbeke, MD, PhD, KU Leuven Campus Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium. Email vanhaverbeke.maarten@gmail.com

Affiliations

Department of Cardiovascular Sciences (M. Vanhaverbeke, D.V., M.W., H.G., J.B., F.V.D.W., S.J., P.R.S.). Department of Electrical Engineering ESAT, STADIUS Center for Dynamical Systems, Signal Processing and Data Analytics, KU Leuven (G.L., Y.M.). Department of Cardiovascular Medicine, University Hospitals Leuven, Belgium (M. Vanhaverbeke, F.V.D.W., S.J., P.R.S.). Cardiovascular Research Unit, Luxembourg Institute of Health, Strassen (M. Vausort, L.Z., Y.D.). Cardiovascular Center, OLV Hospital, Aalst, Belgium (J.B.).

Acknowledgments

The authors acknowledge all personnel of the cardiac catheterization laboratory and cardiac intensive care unit of the University Hospitals Leuven. We gratefully acknowledge the staff of the microarray facility VIB Nucleomics Core, in particular, Ruth Maes and Wout Van Delm. We furthermore thank Margaretha Van Kerrebroeck and Sofie Van Soest for the assistance during the animal experiments. We thank Christelle Nicolas, Bernadette Leners, Torkia Lalem, and Mara Luchetti for their contribution in the validation study. This article is based on collaboration supported by the European Union Cooperation in Science and Technology Action CardioRNA CA17129. Dr Vanhaverbeke, Dr Bartunek, Dr Van De Werf, Dr Janssens, and Dr Sinnaeve contributed to the study design; Dr Vanhaverbeke, Dr Wu, G. Laenen, H. Gillijns, Dr Moreau, Dr Janssens, and Dr Sinnaeve contributed to methodology; Dr Vanhaverbeke, D. Veltman, H. Gillijns, Dr Janssens, and Dr Sinnaeve enrolled patients and performed follow-up; porcine experiments were performed by Dr Vanhaverbeke, D. Veltman, Dr Wu, and H. Gillijns; data from the validation cohort was provided by M. Vausort, L. Zhang and Dr Devaux; data analysis was done by Dr Vanhaverbeke, M. Vausort, D. Veltman, L. Zhang, Dr Wu, G. Laenen, Dr Moreau, Dr Sinnaeve, and Dr Janssens; Dr Vanhaverbeke, M. Vausort, Dr Devaux, Dr Janssens, and Dr Sinnaeve contributed to the writing of the manuscript; all authors performed the revision and editing of the manuscript; and Dr Vanhaverbeke, Dr Van De Werf, Dr Devaux, Dr Janssens, and Dr Sinnaeve contributed to resources and funding acquisition.

Sources of Funding

Research was funded by Research Foundation Flanders—a score grant from the University of Leuven (PF10/014) and the Frans Van de Werf Fund for Clinical Cardiovascular Research. Dr Sinnaeve is a clinical investigator for the Research Foundation Flanders. Dr Janssens is holder of a named chair at KU Leuven, financed by AstraZeneca. The independent validation cohort was supported by the Ministry of Higher Education and Research and the Society for Research on Cardiovascular Diseases of Luxembourg.

Disclosures

None.

REFERENCES

1. Cung TT, et al. Cyclosporine before PCI in patients with acute myocardial infarction. *N Engl J Med*. 2015;373:1021–1031. doi: 10.1056/NEJMoa1505489
2. Janssens SP, et al; NOMI Investigators. Nitric oxide for inhalation in ST-elevation myocardial infarction (NOMI): a multicentre, double-blind, randomized controlled trial. *Eur Heart J*. 2018;39:2717–2725. doi: 10.1093/eurheartj/ehy232
3. Hausenloy DJ, et al. Novel targets and future strategies for acute cardioprotection: position paper of the European Society of cardiology working Group on cellular biology of the heart. *Cardiovasc Res*. 2017;113:564–585. doi: 10.1093/cvr/cvx049
4. Perrino C, et al. Epigenomic and transcriptomic approaches in the post-genomic era: path to novel targets for diagnosis and therapy of the ischaemic heart? Position paper of the European Society of cardiology working Group on cellular biology of the heart. *Cardiovasc Res*. 2017;113:725–736. doi: 10.1093/cvr/cvx070
5. Houser SR. The American Heart Association's new institute for precision cardiovascular medicine. *Circulation*. 2016;134:1913–1914. doi: 10.1161/CIRCULATIONAHA.116.022138

Downloaded from http://ahajournals.org by on May 6, 2020

6. Frangogiannis NG. Regulation of the inflammatory response in cardiac repair. *Circ Res*. 2012;110:159–173. doi: 10.1161/CIRCRESAHA.111.243162
7. Ørn S, et alet al. C-reactive protein, infarct size, microvascular obstruction, and left-ventricular remodelling following acute myocardial infarction. *Eur Heart J*. 2009;30:1180–1186. doi: 10.1093/eurheartj/ehp070
8. Barron HV, et al. Association between white blood cell count, epicardial blood flow, myocardial perfusion, and clinical outcomes in the setting of acute myocardial infarction: a thrombolysis in myocardial infarction 10 substudy. *Circulation*. 2000;102:2329–2334. doi: 10.1161/01.cir.102.19.2329
9. Arruda-Olson AM, et al. Neutrophilia predicts death and heart failure after myocardial infarction: a community-based study. *Circ Cardiovasc Qual Outcomes*. 2009;2:656–662. doi: 10.1161/CIRCOUTCOMES.108.831024
10. Zhang Y, et al. Reciprocal changes of circulating long non-coding RNAs ZFAS1 and CDR1AS predict acute myocardial infarction. *Sci Rep*. 2016;6:22384. doi: 10.1038/srep22384
11. Vausort M, et al. Myocardial infarction-associated circular RNA predicting left ventricular dysfunction. *J Am Coll Cardiol*. 2016;68:1247–1248. doi: 10.1016/j.jacc.2016.06.040
12. Kiliszek M, et al. Altered gene expression pattern in peripheral blood mononuclear cells in patients with acute myocardial infarction. *PLoS One*. 2012;7:e50054. doi: 10.1371/journal.pone.0050054
13. Vausort M, et al. Long noncoding RNAs in patients with acute myocardial infarction. *Circ Res*. 2014;115:668–677. doi: 10.1161/CIRCRESAHA.115.303836
14. van der Pouw Kraan TC, et al. Systemic toll-like receptor and interleukin-18 pathway activation in patients with acute ST elevation myocardial infarction. *J Mol Cell Cardiol*. 2014;67:94–102. doi: 10.1016/j.yjmcc.2013.12.021
15. Suresh R, et al. Transcriptome from circulating cells suggests dysregulated pathways associated with long-term recurrent events following first-time myocardial infarction. *J Mol Cell Cardiol*. 2014;74:13–21. doi: 10.1016/j.yjmcc.2014.04.017
16. Chen GY, et al. Sterile inflammation: sensing and reacting to damage. *Nat Rev Immunol*. 2010;10:826–837. doi: 10.1038/nri2873
17. Ma Y, et al. Temporal neutrophil polarization following myocardial infarction. *Cardiovasc Res*. 2016;110:51–61. doi: 10.1093/cvr/cvw024
18. Arslan F, et al. Innate immune signaling in cardiac ischemia. *Nat Rev Cardiol*. 2011;8:292–300. doi: 10.1038/nrcardio.2011.38
19. Abbate A, et al; VCU-ART Investigators. Interleukin-1 blockade with anakinra to prevent adverse cardiac remodeling after acute myocardial infarction (Virginia Commonwealth University Anakinra Remodeling Trial [VCU-ART] Pilot study). *Am J Cardiol*. 2010;105:1371–1377.e1. doi: 10.1016/j.amjcard.2009.12.059
20. Abbate A, et al. Effects of interleukin-1 blockade with anakinra on adverse cardiac remodeling and heart failure after acute myocardial infarction [from the Virginia Commonwealth University-Anakinra Remodeling Trial (2) (VCU-ART2) pilot study]. *Am J Cardiol*. 2013;111:1394–1400. doi: 10.1016/j.amjcard.2013.01.287
21. Tardif JC, et al. Effects of the P-selectin antagonist inclacumab on myocardial damage after percutaneous coronary intervention for non-ST-segment elevation myocardial infarction: results of the SELECT-ACS trial. *J Am Coll Cardiol*. 2013;61:2048–2055. doi: 10.1016/j.jacc.2013.03.003
22. Faxon DP, et al; HALT-MI Investigators. The effect of blockade of the CD11/CD18 integrin receptor on infarct size in patients with acute myocardial infarction treated with direct angioplasty: the results of the HALT-MI study. *J Am Coll Cardiol*. 2002;40:1199–1204. doi: 10.1016/s0735-1097(02)02136-8
23. Oyama J, et al. Reduced myocardial ischemia-reperfusion injury in toll-like receptor 4-deficient mice. *Circulation*. 2004;109:784–789. doi: 10.1161/01.CIR.0000112575.66565.84
24. Mebazaa A, et alet al. Unbiased plasma proteomics for novel diagnostic biomarkers in cardiovascular disease: identification of quiescin Q6 as a candidate biomarker of acutely decompensated heart failure. *Eur Heart J*. 2012;33:2317–2324. doi: 10.1093/eurheartj/ehs162
25. Caillard A, et al. QSOX1, a novel actor of cardiac protection upon acute stress in mice. *J Mol Cell Cardiol*. 2018;119:75–86. doi: 10.1016/j.yjmcc.2018.04.014
26. Israel BA, et al. Disulfide bond generation in mammalian blood serum: detection and purification of quiescin-sulfhydryl oxidase. *Free Radic Biol Med*. 2014;69:129–135. doi: 10.1016/j.freeradbiomed.2014.01.020
27. Xu S, et al. The identification of a phospholipase B precursor in human neutrophils. *FEBS J*. 2009;276:175–186. doi: 10.1111/j.1742-4658.2008.06771.x
28. Du CQ, et al. The elevated serum S100A8/A9 during acute myocardial infarction is not of cardiac myocyte origin. *Inflammation*. 2012;35:787–796. doi: 10.1007/s10753-011-9375-8
29. Morrow DA, et al. Myeloid-related protein 8/14 and the risk of cardiovascular death or myocardial infarction after an acute coronary syndrome in the Pravastatin or Atorvastatin Evaluation and Infection Therapy: Thrombolysis in Myocardial Infarction (PROVE IT-TIMI 22) trial. *Am Heart J*. 2008;155:49–55. doi: 10.1016/j.ahj.2007.08.018
30. Kumarswamy R, et al. Circulating long noncoding RNA, LIPCAR, predicts survival in patients with heart failure. *Circ Res*. 2014;114:1569–1575. doi: 10.1161/CIRCRESAHA.114.303915
31. Wang L, et al. Disease-specific classification using deconvoluted whole blood gene expression. *Sci Rep*. 2016;6:32976. doi: 10.1038/srep32976
32. Palmer C, et al. Cell-type specific gene expression profiles of leukocytes in human peripheral blood. *BMC Genomics*. 2006;7:115. doi: 10.1186/1471-2164-7-115

Downloaded from http://ahajournals.org by on May 6, 2020