Disease Markers

Emerging Biomarkers of Adverse Cardiac Remodeling: from Acute Myocardial Infarction to Advance Heart Failure

Lead Guest Editor: Alexander Berezin
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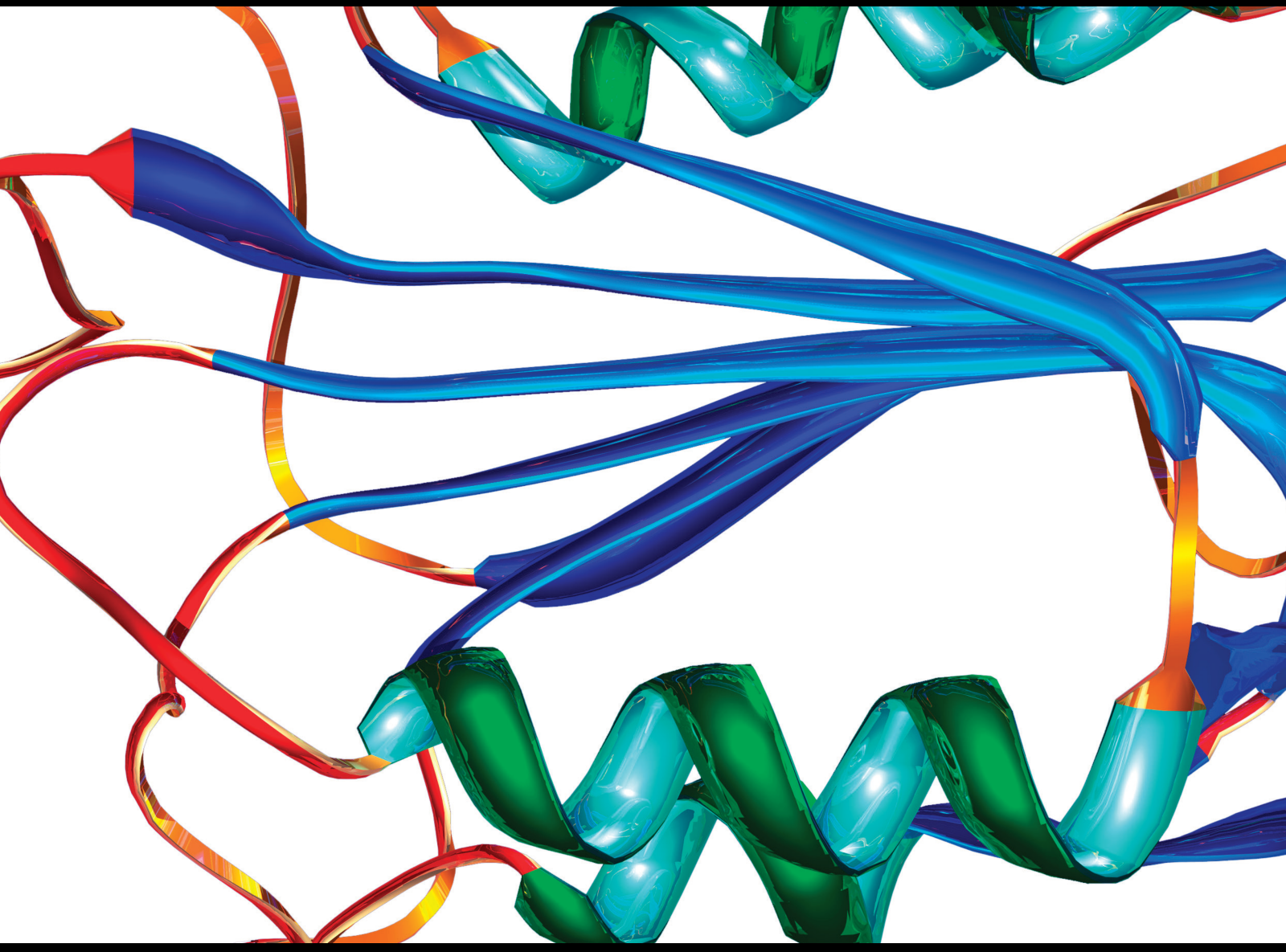

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Corrigendum

Corrigendum to "Circulating microRNAs as Novel Potential Biomarkers for Left Ventricular Remodeling in Postinfarction Heart Failure"

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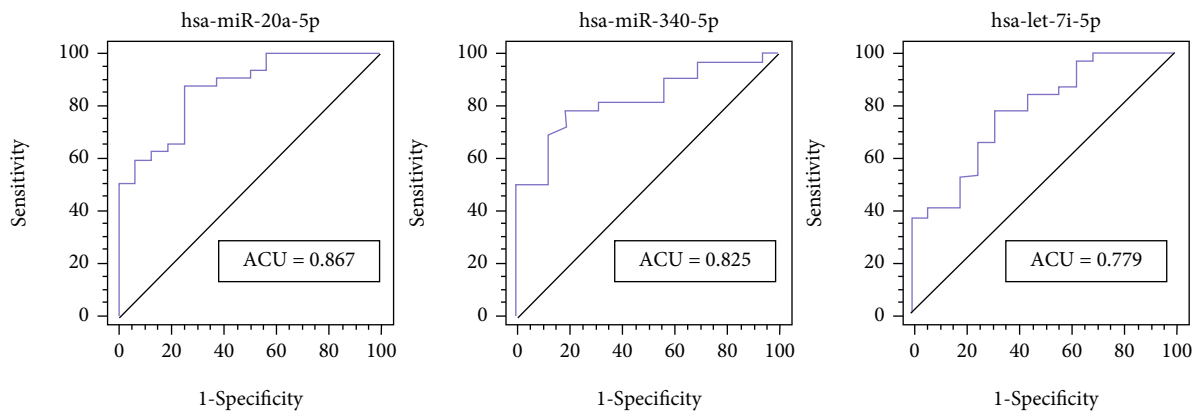
In the article titled "Circulating MicroRNAs as Novel Potential Biomarkers for Left Ventricular Remodeling in Postinfarction Heart Failure" [1], a duplication of the panels in Figure 7(b) was noted on PubPeer [2].

The authors have re-examined the data and explained that the error occurred due to the incorrect use of the ROC curve drawing software (MedCale). The authors provided the corrected ROC curve graphs and the underlying data. The corrected Figure 7(b) is shown below:



(b)

Figure 7: Expression of candidate plasma miRNAs in the patient population. (a) The expression levels of plasma miR-20a-5p, miR-340-5p, and let-7i-5p in patients with postinfarction HF ($n = 32$) and control patients with stable angina and without significant coronary lesions and HF ($n = 16$). (b) ROC curves and the AUCs of miR-20a-5p, miR-340-5p, and let-7i-5p. $^{*}P < 0:05$ versus patients with stable angina and without significant coronary lesions and HF. HF: heart failure; ROC: receiver operator characteristic; AUC: area under the curve.

References

[1] G. Gao, W. Chen, M. Liu, Y. Xu, and P. Yang, "Circulating microRNAs as novel potential biomarkers for left ventricular remodeling in postinfarction heart failure," *Disease Markers*, vol. 2019, Article ID 5093803, 13 pages, 2019.

[2] R. Breviscapa, "Circulating microRNAs as novel potential biomarkers for left ventricular remodeling in postinfarction heart failure," 2020, https://pubpeer.com/publications/BB480A0CC0D11318B74932DB57FE7E#1/.

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Research Article

Radixin Relocalization and Nonmuscle α-Actinin Expression Are Features of Remodeling Cardiomyocytes in Adult Patients with Dilated Cardiomyopathy

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Background. Pediatric patients show an impressive capacity of cardiac regeneration. In contrast, severely deteriorated adult hearts do usually not recover. Since cardiac remodeling—involving the expression of fetal genes—is regarded as an adaptation to stress, we compared hearts of adult patients suffering from dilated cardiomyopathy (DCM) with remodeling of cultured neonatal (NRC) as well as adult (ARC) rat cardiomyocytes and the developing postnatal myocardium. *Methods*. NRC and ARC were stimulated with serum and cardiac morphogens derived from DCM hearts. Protein synthesis (PS) as well as protein accumulation (PA) was measured, and cell survival was determined under ischemic conditions. Fetal markers were investigated by Western blot. Biomarkers of remodeling were analyzed in controls, DCM, and 2- to 6-month-old children with tetralogy of Fallot as well as in neonatal and adult rats by immunofluorescence. *Results*. In NRC, serum and morphogens strongly stimulated PS and PA and the reestablishment of cell-cell contacts (CCC). In ARC, both stimulants increased PS and CCC, but PA was only elevated after serum stimulation. In contrast to serum, morphogen treatment resulted in the expression of fetal genes in ARC as determined by nonmuscle α-actinin-1 and α-actinin-4 expression (NM-actinins) and was associated with increased survival under ischemia. NM-actinins were present in cardiomyocytes of DCM in a cross-striated pattern reminiscent of sarcomeres as well as in extensions of the area of the intercalated disc (ID). NM-actinins are expressed in NRC and in the developing heart. Radixin staining revealed remodeling of the area of the ID in DCM almost identical to stimulated cultured ARC. *Conclusions*. Remodeling was similar in ARC and in cardiomyocytes of DCM suggesting evolutionary conserved mechanisms of regeneration. Despite activation of fetal genes, the atrophy of ARC indicates differences in their regenerative capacity from NRC. Cardiac-derived factors induced NM-actinin expression and increased survival of ischemic ARC while circulating molecules were less effective. Identification of these cardiac-derived factors and determination of their individual capacity to heal or damage are of particular importance for a biomarker-guided therapy in adult patients.

1. Introduction

The function of the heart is not only the continuous supply of oxygen and nutrients but also the removal of degradation products such as CO_2 by pumping blood through the circulatory system. When the healthy heart is challenged by increased physiological workload, for example, during pregnancy or in high-performance athletes, the myocardium reacts with hypertrophy but sustains cardiac architecture as well as the differentiation status of the ventricle. However, under chronic conditions such as dilated cardiomyopathy, the "status quo" is disturbed and ventricular remodeling occurs which affects the amount and the composition of proteins in cardiac cells and the extracellular matrix. Often, inflammatory processes even at a low grade might continuously contribute to cardiac remodeling by changing the level of cytokines and leukocyte subsets in the tissue as well as in the circulation [1–4].

Cardiac remodeling has been defined in a consensus paper from an international forum in 2000 as "genome expression, molecular, cellular and interstitial changes that are manifested clinically as changes in size, shape and function of the heart after cardiac injury" [5]. Importantly, the etiology of adult cardiac remodeling might significantly differ between various heart diseases, but the transition from compensatory processes to heart failure shares many common pathways [5–10]. By keeping this in mind, it becomes clear that strategies to determine the mechanism behind the transition to heart failure, its therapy, and risk stratification are still poorly established and require more basic knowledge [11].

One common pathway in the development of heart failure is the reactivation of fetal genes. Activation of fetal genes involves a switch in the protein expression from adult to fetal/neonatal isoforms such as smooth muscle α-actin and myosin heavy chains, but also fetal nonisoforms like the ERM protein moesin are reexpressed in cardiomyocytes [8, 12, 13]. However, despite the activation of several evolutionary conserved survival pathways under pathological conditions, the regenerative capacity of the adult myocardium still remains poor. When the heart fails to meet metabolic demands, patients develop chronic heart failure from which adult people usually do not recover. In contrast, there are a number of clinical examples showing that young children have an astonishing regenerative capacity. Children with anomalous origin of the left coronary artery from the pulmonary artery with severely dilated hearts regenerate after surgical correction [14]. Pediatric patients with univentricular hearts recover after volume unloading [15], and children with dilated cardiomyopathy are able to regenerate spontaneously from severely impaired heart function [16]. Thus, activation of fetal genes might be an incomplete attempt of adult cardiomyocytes to obtain a certain level of regenerative capacity similar to that of young hearts in order to adapt to increased physiological as well as pathological workloads. It is quite obvious that a more complete list of fetal genes activated in adult patients as well as the understanding of their regenerative capacity in pediatric hearts will give a wealth of information about healing processes and help to identify disease-relevant cardiac biomarkers and therapeutic targets.

A key approach of our study was the utilization of isolated cardiomyocytes because primary cell cultures reduce the enormous complexity of a direct *in vivo* analysis. This system offers a first-class toolbox to analyze regeneration mechanisms and accelerates the screen for novel heart failure as well as circulating biomarkers by "omics" technologies [4, 12, 17]. Here, we will compare our *in vitro* results with data obtained in the developing and failing heart of patients with dilated cardiomyopathy.

2. Methods

2.1. Study Population. Patients' characteristics are shown in Table 1. Myocardial samples from four patients with aortic stenosis and preserved ejection fraction (EF > 50%) served as controls (CON) [4]. Cardiac tissue samples from six patients with end-stage heart failure due to dilated cardiomyopathy (DCM) were obtained during transplantation. Small tissue samples from 2-, 3-, and 6-month-old patients with tetralogy of Fallot were received during surgery. Moreover, hearts were collected from 3-, 8-, and 15-day-old and (adult) 12-week-old Sprague-Dawley rats after decapitation. Tissue samples were immediately flash-frozen in liquid nitrogen and kept at -80°C until use. This study complies with the Declaration of Helsinki and is approved by the respective responsible ethical committees.

2.2. Cell Culture, Growth, and Western Blot Analysis. Isolation, culture, and stimulation of Sprague-Dawley neonatal and adult rat cardiomyocytes were performed as previously described [18, 19]. Each experiment was performed with 20-30 hearts of 2-3-day-old neonatal pups or one 12-week-old adult heart. Cultures were performed in duplicate. The number of n is indicated in the figures. Adult hearts were perfused for 5 min with Ca^{2+}-free Krebs-Henseleit bicarbonate buffer (KHB, pH 7.4) containing (in mM) 110 NaCl, 2.6 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 11 glucose, and 10 HEPES and gassed with 95% O_2- plus 5% CO_2 at 37°C. Then, after perfusion for 30 min in the same solution containing 0.04% collagenase (Worthington) and 60 μM calcium, ventricles were minced in the same collagenase solution. After two washing steps at 25 g for 3 min with increasing calcium concentrations of 0.2 and 0.5 mM in KHB, myocytes were layered over a 4% BSA gradient (Sigma, fatty acid free) containing 1 mM calcium and centrifuged for 1 min at 15 g. The cell pellet was suspended then in basic medium consisting of Medium 199 with Earle's balanced salts (Sigma) without L-glutamine including 25 mM HEPES, 25 mM $NaHCO_3$, 100 IU/ml penicillin, and 100 μg/ml streptomycin and supplemented with 2 mM L-carnitine, 5 mM creatine, and 5 mM taurine (Sigma). Myocytes were plated on laminin- (10 μg/ml; Sigma) coated chamber slides (Nunc) for fluorescence microscopy analysis and on six-well culture dishes (Falcon) for Western blots, protein synthesis determination, phase-contrast microscopy, and viability. Two hours after plating, the medium was changed, and experiments started after one day. Media were replaced every other day. Addition of 10 μM 1-(b-D-arabinofuranosyl) cytosine prevented nonmyocyte growth. Similarly, neonatal ventricular

Table 1: Clinical data of the analyzed patients. Six patients (3 females and 3 males) developed the phenotype of dilated cardiomyopathy (DCM) without signs of coronary heart disease (CHD) who had undergone heart transplantation (HTX). Left ventricular ejection fraction (LVEF) was lower than 20%. Four patients have aortic stenosis (AoSt) and preserved ejection fraction (EF > 60% ; 2 males and 2 females). Three pediatric patients with tetralogy of Fallot (ToF) served for comparison. NYHA: New York Heart Association; PCI: percutaneous coronary intervention; CABG: coronary artery bypass grafting; AK-OP: aortic valve surgery; MK-OP: mitral valve surgery; bivICD: biventricular implantable cardioverter-defibrillator; ACE: angiotensin-converting enzyme; AT1: angiotensin II receptor; ASS: acetylsalicylic acid; CRP: C-reactive protein; LDH: lactate dehydrogenase; SGOT: serum oxaloacetic transaminase; SGPT: serum glutamic pyruvic transaminase; CK: creatine kinase; NT-pro-BNP: N-terminal probrain natriuretic peptide; PTT: activated and partial thromboplastin time; INR: international normalized ratio; HIV: human immunodeficiency virus; LVESD: left ventricular end-systolic diameter; LVEDD: left ventricular end-diastolic diameter; PVR: pulmonary vascular resistance; PCWP: pulmonary capillary wedge pressure.

Demographic and risk factors	DCM (mean ± s.e.m.)	AoSt (mean ± s.e.m.)	ToF (mean ± s.e.m.)
Number of patients	6	4	3
DCM	6	0	0
CHD	0	0	0
Etiology of DCM	3xi.p., 2xfam, 1xpp	0	0
Age	54 ± 4 years	65 ± 10 years	2, 3, and 6 months
Gender	3 females, 3 males	2 females, 2 males	1 females, 2 males
NYHA class	3.8 ± 0.2	3	0
Body height (cm)	170 ± 5	170 ± 5	64 ± 2
Weight (kg)	64 ± 4	84 ± 9	6.6 ± 0.4
Prior myocardial infarction	0	0	0
Prior PCI or CABG	0	0	0
Prior smoking	2	1	0
Prior hypertension	1	3	0
Prior COPD	1	0	0
Prior diabetes mellitus	0	1	0
Thrombocytopenia	0	0	0
Prior hypercholesterolemia	1	1	0
Pre-CABG	0	0	0
Pre-HTXLVAD	0	0	0
Pre-HTXAK-OP	1	0	0
Pre-HTXMK-OP	1	0	0
Pre-HTXbivICD	5	0	0
Cardiac arrhythmia	3	1	0
Comedication			
Beta blocker (%)	6	4	3
ACE inhibitor and/or AT1 antagonist (%)	4	4	0
Diuretic (%)	6	2	0
Digitalis (%)	0	1	0
Aldosterone antagonist (%)	4	1	0
Sildenafil 20 mg (%)	2	0	0
ASS (%)	0	2	0
Amiodarone (%)	2	0	0
L-Thyroxin (%)	1	1	0
Statins (%)	1	0	0
Laboratory parameters			
Sodium (mmol/l)	137 ± 1	139 ± 0.6	138 ± 0.7
Potassium (mmol/l)	4.5 ± 0.2	4.6 ± 0.4	4.5 ± 0.3
Creatinine (mg/dl)	1.6 ± 0.2	1 ± 0.2	0.2
Hemoglobin (g/dl)	9.7 ± 0.3	12.8 ± 1.4	12.5 ± 0.1
Hematocrit (%)	29.2 ± 1	39.2 ± 4.2	0.37
CRP (mg/l)	1.2 ± 0.4	2.8 ± 2.7	<0.5

Table 1: Continued.

Demographic and risk factors	DCM (mean ± s.e.m.)	AoSt (mean ± s.e.m.)	ToF (mean ± s.e.m.)
Leukocytes (Ts/μl)	10.9 ± 1.3	10.1 ± 1.4	15.3 ± 4
Total cholesterol (mg/dl)	171 ± 19	217 ± 32.5	n.d.
LDHU (l)	403 ± 153	286 ± 30	306 ± 13.3
SGOT (IU/l)	72 ± 41	57 ± 7.3	33 ± 1.2
SGPT (IU/l)	24.2 ± 8.4	67 ± 24	27 ± 4.7
CKU (l)	361 ± 263	75 ± 19	307 ± 144
NT-pro-BNP (pg/ml)	6363 ± 2783	1663 ± 1260	57 ± 21
Quick (thromboplastin time) (%)	80.7 ± 15.2	75 ± 11.7	96 ± 6.7
PTT (s)	31 ± 2	31 ± 2.2	34 ± 1.8
INR	1.4 ± 0.2	1.3 ± 0.2	1 ± 0.1
Hepatitis B & C, HIV	0	0	0
Echo characteristics			
LVEF (%)	19.2 ± 2.4	64 ± 2.4	n.d.
LVESD (mm)	57 ± 4	31 ± 2.2	n.d.
LVEDD (mm)	65 ± 4	53 ± 2.3	n.d.
Hemodynamic parameters			
Cardiac index (l/min∗m)	23.8 ± 4.1 (1.8 ± 0.2)	n.d.	n.d.
PVR (dynes∗s/cm^5)	190 ± 22	n.d.	n.d.
PCWP (mmHg)	26.3 ± 3	n.d.	n.d.
O_2 saturation (%)	46.7 ± 2.5	n.d.	n.d.

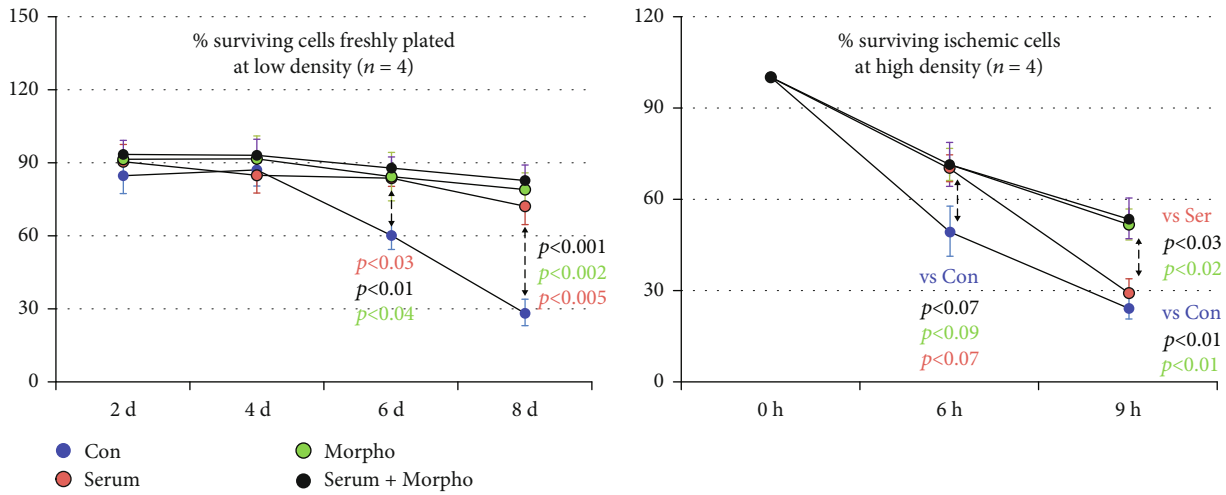
cardiomyocytes were directly minced in the same collagenase solution without calcium supplementation and dissociated under shaking at 37°C for 30–45 min. Dissociated myocytes of digestion cycles 2–4 were collected in basic medium containing 1% newborn calf serum and preincubated for 1.5 h on an uncoated 148 cm^2 dish (Falcon) to remove nonmyocytes. Nonattached neonatal myocytes were collected and then centrifuged at 1000 rpm for 5 min. The cell pellet was suspended in 1% fetal calf serum and plated on fibronectin-coated dishes (10 μg/ml; PromoCell, Heidelberg, Germany) for one day. Before stimulation, cells were washed twice and cultured in basic medium as described above. Neonatal cardiomyocytes were plated at a density of 1×10^4 cells/cm^2 and adult cardiomyocytes at 0.5 (low density) or at 1.5×10^4 cells/cm^2 (high density) as indicated in the figures.

Determination of protein content and synthesis as well as DNA content of neonatal and adult cardiomyocyte cultures was reported previously in detail [18, 19]. Protein synthesis and accumulation were normalized against the DNA content as a relative measure for the cell number. At the end of the experiment, adult cardiomyocyte cultures were washed twice with phosphate-buffered saline (PBS), dissolved in lysis buffer, and processed for Western blot analysis as described previously in detail [20]. The ischemic condition was produced by reducing the O_2 content to 1% (with 5% CO_2) in the incubator, and cardiomyocytes were cultured in glucose-free phosphate-buffered saline (PBS).

2.3. Preparation of Serum, Control Samples, Morphogens, and Microvascular Endothelial Cells. Serum and tissue samples were obtained perioperatively. Cardiac microvascular endothelial cells (MVEC) were isolated from approximately 0.5 g tissue of the explanted hearts (2 patients) with DCM and grown in 20% serum on a 148 cm^2 dish (Falcon). Microvascular endothelial cells were isolated from 6 different regions of one human myocardium. The best two cultures of each patient were used, judged by the initial number of isolated cells, their proliferation rate, and the lowest percentage of apoptotic/dying cells. Cells of the third passage were used for conditioning. Confluent cultures were washed twice, and the cell supernatant was conditioned for two days in stimulant-free pure Medium 199 with Earle's salt as described previously [18]. Proteins of this conditioned medium were concentrated 30-fold by Centricon (Millipore) with a cut-off of 5 kDa (morphogens (Morpho)). For better evaluation of specific effects of stimulants, an appropriate concentration of serum was determined giving a comparable rate of protein synthesis with Morpho at day two. Thus, Morpho was used at a 3-fold concentration and serum at a final concentration of 7.5% for experiments. For comparison, 0.2 mg/ml human serum albumin (Sigma) was added to basic medium in the controls.

2.4. Fluorescence Microscopy. Antibodies are listed in Figure 1(b) used for Western blot analysis, and tissues sections were prepared for confocal microscopy as previously described in detail [2].

2.5. Statistics. For statistical analysis, the unpaired t-test was used. p values less than 0.05 were considered statistically significant.



(a)

CatID	Target	Phospho-sites	Description/function	Host	Abbreviation	Company
ab68194	α-Actinin-1	-	Nonmuscle α-actinin-1	Rabbit	Actn1	Abcam
A7811	α-Actinin-2	-	Sarcomeric α-actinin-2	Mouse	Actn2	Sigma
ab108201	α-Actinin-4	-	Nonmuscle α-actinin-4	Rabbit	Actn4	Abcam
3149	P-Ezrin/radixin/moesin	See below	Cytoskeletal/membranelinker	Rabbit	P-ERM	Cell Signaling
2636	Radixin	Thr564	Cytoskeletal/membranelinker	Rabbit	Rdx	Cell Signaling
3150	Moesin	Thr558	Cytoskeletal/membranelinker	Rabbit	Msn	Cell Signaling
A5228	Smooth muscle α-actin	-	Cytoskeleton	Mouse	α-SMA	Sigma
5046	α-Tubulin	-	Microtubules	Rabbit	α-Tub	Cell Signaling
-	Myomesin	-	Marker of mature sacomeres	Mouse	Myom	Dr. Eppenberger
P5282	F-actin	-	Phalloidin	-	F-actin	Sigma
600-401-103	CollagenI	-	Extracellular matrix	Rabbit	Col I	Rockland
5839	Succinate dehydrogenase	-	Serves as a loading control	Rabbit	SDHA	Cell Signaling

(b)

Figure 1: Endothelial morphogens exert protective effects on ischemic adult cardiomyocytes. Con are albumin-treated cultures. Cardiomyocytes were stimulated with serum, morphogen (Morpho), and serum plus Morpho for the indicated time in days (d) and hours (h). (a) Stimulation increases significantly the number of surviving cardiomyocytes at low density (0.5×10^4 cells/cm^2). However, under ischemic conditions, only morphogen stimulation increased the survival of cardiomyocytes (1.5×10^4 cells/cm^2). (b) List of utilized antibodies. The catalog number (Cat ID), target, phosphorylation site (phospho-site), description/function of the analyzed protein, host, used abbreviations, and distributors are listed.

3. Results

3.1. Serum and Cardiac-Derived Endothelial Morphogens Exert Distinct Types of Adult Cardiomyocyte Remodeling. Serum and morphogens derived from cultured microvascular endothelial cells of two patients with dilated cardiomyopathy were used to analyze patterns of remodeling and dedifferentiation of cultured adult cardiomyocytes (ARC) as well as neonatal myocytes (NRC). Control cultures were treated with human serum albumin. Since we have previously demonstrated that ezrin and the reexpression of moesin monitor dynamic cellular changes, we wanted to analyze radixin (Rdx), the third component of ERM proteins, and its activated form (P-ERM) [12, 21]. Sarcomeric α-actinin (Actn2) identifies cardiomyocytes. The cross-striated pattern of myomesin (Myom) stain marks mature sarcomeres. Dedifferentiation is monitored by smooth muscle α-actin (α-SMA, Acta2) and nonmuscle α-actinin (Actn1 and Actn4) expression. These proteins are hardly expressed in cardiomyocytes of the adult tissue. Antibodies used for confocal fluorescence microscopy and Western blot analysis are listed in Figure 1(b) with corresponding distributors, short descriptions, and abbreviations.

Normal adult cardiomyocytes are rod-shaped with sharp defined intercalated discs. This phenotype is clearly visible in freshly isolated cells (Figure 2(a); Rdx, yellow arrows) as well as in myocytes of the human myocardium (Figure 2(b); Rdx, yellow arrows). In culture, unstimulated cardiomyocytes (Con) round up to a different degree in a 10-day period (Figure 2(c); yellow arrows in the control group), but still some cells maintain their rod-shaped phenotype (Figure 2(c); white arrows in the control group). Cell-cell contacts are random after plating, and no contractile activity is observed.

In contrast, serum-stimulated cardiomyocytes lose their rod-shaped phenotype and increase their surface area by spreading laterally until day 10 (Figure 2(d); long black arrow and magnified image). Cell-cell contacts are partly reestablished to cells in close vicinity by spreading, and some beating cardiomyocytes become visible. Stimulation with

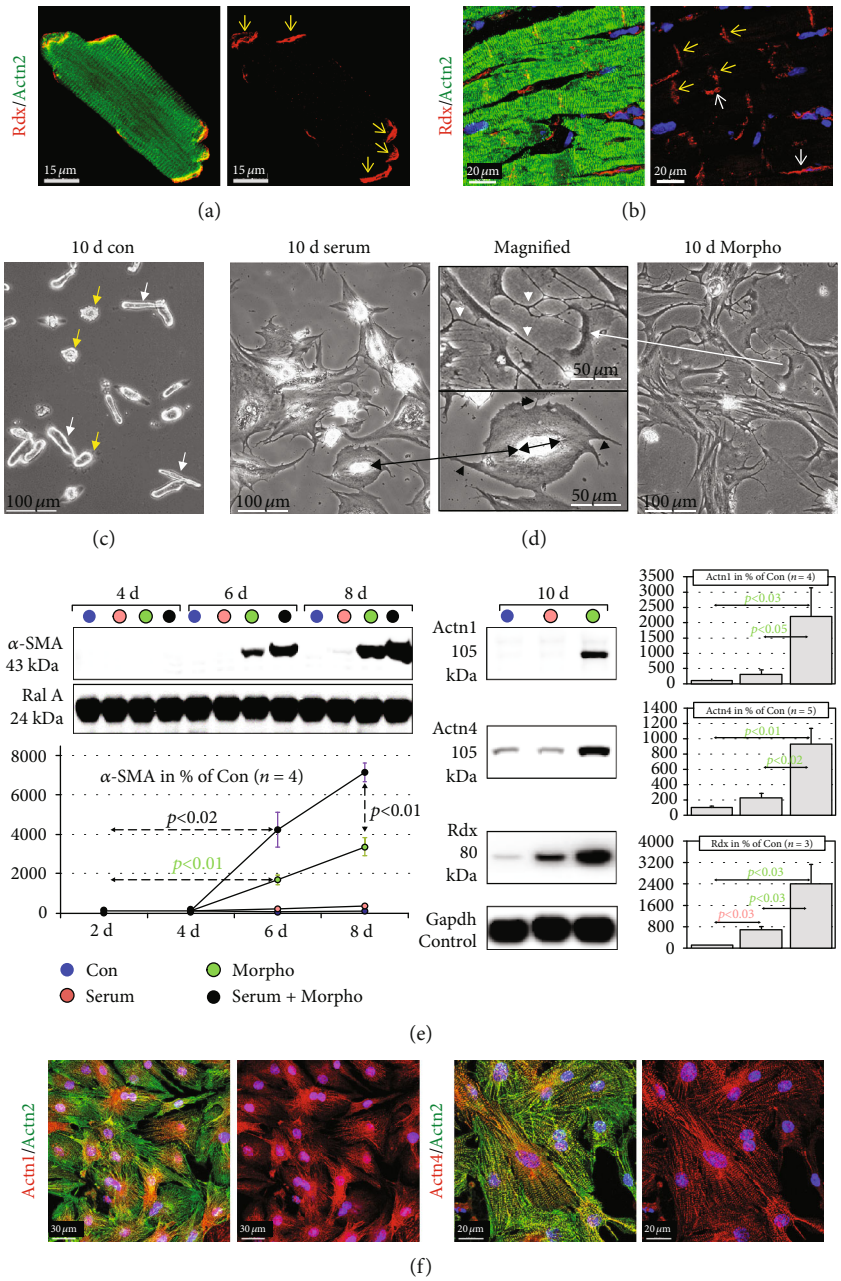


Figure 2: Serum and cardiac endothelial-derived morphogens induce distinct types of cardiomyocyte remodeling. Con are control cultures of adult rat cardiomyocytes treated with 0.2 mg/ml human serum albumin. Serum indicates serum stimulation, and Morpho specifies treatment with cardiac-derived morphogens (Morpho) from microvascular endothelial cells (see Methods) for the indicated time in days (d). Dedifferentiation markers are smooth muscle α-actin (α-SMA) and nonmuscle α-actinin-1 and α-actinin-4 (Actn1 and Actn4). (a) Freshly isolated adult cardiomyocytes show the typical rod-shaped phenotype *in vitro*. Radixin (Rdx, yellow arrows) staining defines the area of the intercalated disc. (b) The same applies to radixin staining of cardiomyocytes in the human myocardium (yellow arrows). Note that also some noncardiomyocytes in the interstitium are positive for radixin (white arrows). The Actn2 antibody stains sarcomeric α-actinin-2 in a cross-striated pattern. (c) A phase-contrast micrograph of the control group shows after 10 days that some cells still maintain a rod-shaped phenotype (white arrows) while other cardiomyocytes appear rounded (yellow arrows). (d) Phase-contrast micrographs show typical differences between the effects of serum and morphogens (Morpho) on cardiomyocytes after 10 days. The magnified image (long black arrow) shows a myocyte with an almost circular spread enlargement and little extensions after serum stimulation (black arrowheads). The double black arrow indicates the length and orientation of the original cell. In contrast, Morpho induces the formation of extremely long extensions with little lateral spreading (white long arrow and white arrowheads in the magnified image). (e) Western blot analysis reveals that Morpho induces dedifferentiation of adult cardiomyocytes. In contrast, serum stimulates very little α-SMA expression but enhances morphogen-induced dedifferentiation. Morphogens induce strongly the expression of Actn1 and Actn4. The expression of radixin (Rdx) reflects cardiomyocyte remodeling. Gapdh serves as a loading control. Statistical analysis is shown. (f) Confocal images show isolated neonatal cardiomyocytes (cell cultures from 3 d-old hearts).

morphogens (Morpho) induces a completely different phenotype (Figure 2(d); long white arrow and magnified image). Myocytes show massive elongation along with the formation of multiple cellular contacts, and only some lateral spreading is recognizable. Furthermore, morphogen-stimulated cultures show multiple long and thin extensions while only few were detected in serum-stimulated myocytes (white arrowheads versus black arrowheads in magnified images). By this, morphogen-stimulated cardiomyocytes establish cell-cell contacts in the immediate and more distant vicinity.

Dedifferentiation of adult cardiomyocytes became apparent after day 4 and was strongly induced by morphogens but only mildly by serum. However, serum enhanced morphogen-induced α-SMA in cardiomyocytes (Figure 2(e)). Both nonmuscle α-actinins 1 and 4 (Actn1 and Actn4) were strongly expressed in morphogen-stimulated adult cardiomyocytes (Figure 2(e)). Since α-SMA as well as Actn1 and Actn4 (Figure 2(f)) is present in neonatal cardiomyocytes, their expression in adult myocytes indicates the activation of fetal pathways.

3.2. Opposing Effects of Serum and Morphogens on Protein Accumulation in Adult and Neonatal Cardiomyocytes. Here, we wanted to know the effect of serum and endothelial morphogens on protein synthesis in cultured adult cardiomyocytes by measuring [^{3}H]-phenylalanine incorporation (pulse labeling) as well as protein accumulation by determination of changes in the total protein content (Figure 3(a)). Protein synthesis of the control group significantly declined steadily until day eight to 44% of the initially measured rate. In contrast, serum- and morphogen-stimulated cardiomyocytes increased continuously their protein synthesis until day eight to 480% and 534%, respectively. Combination of both stimulants showed synergistic effects (1164%). As expected, total protein content decreased in the control group to 58% while serum and serum plus morphogen increased protein content to 124% and 162%, respectively. Surprisingly, in the morphogen-stimulated group, the total amount of protein decreased significantly to 70% indicating strong protein degrading activities thereby causing atrophy of adult cardiomyocytes.

We also wanted to understand the effect of serum and morphogens on the growth of neonatal cardiomyocytes since adult cardiomyocytes develop features of neonatal cells when stimulated with morphogens. Since neonatal cardiomyocytes grow rapidly, we performed a growth analysis at day 2, a time point when adult cultured cardiomyocytes show little differences after treatment. Similar to adult cultures, serum and morphogens increased significantly the level of protein synthesis to 235% and 335%, respectively (Figure 3(b)). In contrast to adult cardiomyocytes, the elevation in protein synthesis correlates in both groups with a significant protein accumulation (145% and 138%, respectively) and indicates hypertrophic growth. In untreated control neonatal cardiomyocytes (Con), the loss of sarcomeric structure is apparent in the diffuse pattern of sarcomeric α-actinin staining (Figure 3(c); Actn2). Cell-cell contacts are rapidly reestablished within 2 days by elongation and spreading of stimulated cardiomyocytes (Figure 3(d)). The formation of long extensions was more pronounced in the morphogen-treated group. Furthermore, mature sarcomeres can be observed in a cross-striated pattern after myomesin staining of serum- (Figure 3(d)) and morphogen-stimulated cardiomyocytes (Figure 3(e); enlarged image).

3.3. Endothelial Morphogens Protect Adult Cardiomyocytes from Stress. Next, we wanted to know whether serum and morphogens exert protective effects on adult cardiomyocytes by using two different cell culture model systems. In the first model, cardiomyocytes were plated at a (low) density of 2×10^3 cells/cm^2 in order to stress cultures by keeping cell-cell contacts at a minimum. Cells were counted at the indicated days. In the damaged myocardium, a reestablishment of cell-cell contacts of surviving cardiomyocytes is necessary for the proper recovery of the contractile function. In the second model, cardiomyocytes were plated at a (high) density of 1.5×10^4 cells/cm^2 under ischemic conditions. Cultures were initially stimulated for 5 d and then kept at 0.2% O_2 plus 5% CO_2 in PBS for the indicated time in hours (h, ischemic model).

In the first model, cardiomyocytes were counted at the indicated time under a phase-contrast microscope and calculated in % of the originally attached number of cells (100%). In the control group, the number of attached cardiomyocytes remained relatively constant until day four and then declined to less than 30% (Figure 1(a)). In contrast, the number of attached cells in serum-, morphogen-, and serum plus morphogen-stimulated groups remained comparatively constant (73%, 79%, and 83%, respectively) indicating that growth itself is good for cells independent of the stimulus [22]. In the ischemic model, the number of attached cells decreased until 9 h to less than 30% in the control as well as in the serum-treated group. Surprisingly, the number of surviving attached cardiomyocytes was significantly higher in morphogen- (52%) and in serum plus morphogen- (54%) treated groups than in serum-stimulated cultures (Figure 1(a); 29%).

3.4. Cardiomyocytes in Patients with DCM Reveal Patterns of Remodeling Similar to Cultured Rat Cardiomyocytes. We wondered whether activation of cultured adult cardiomyocytes reveals similarities with remodeling of myocytes in patients with end-stage heart failure. As mentioned before, cardiomyocytes are rod-shaped and radixin is mainly located at the area of the intercalated disc. Human serum albumin-treated cardiomyocytes round up at the ends within 5 days (1-day recovering time and 4-day treatment), and the typical pattern of radixin staining as well as typical cross-striations is lost but cells still maintain their rod-shaped phenotype (Figure 4(a); Con). Upon growth stimulation, radixin staining reveals various shapes of the area of the intercalated disc (Figure 4(a)). In serum-stimulated cells, the area of IDs appears wider (Figure 4(a); serum, yellow arrows) and radixin accumulates in longitudinal directions paralleling the axis of the cardiomyocyte (Figure 4(a); highlighted by the yellow circle, the long yellow arrow points to the corresponding magnified image). These patterns of radixin stains can also be found in the myocardium of patients with DCM (Figure 4(b), yellow

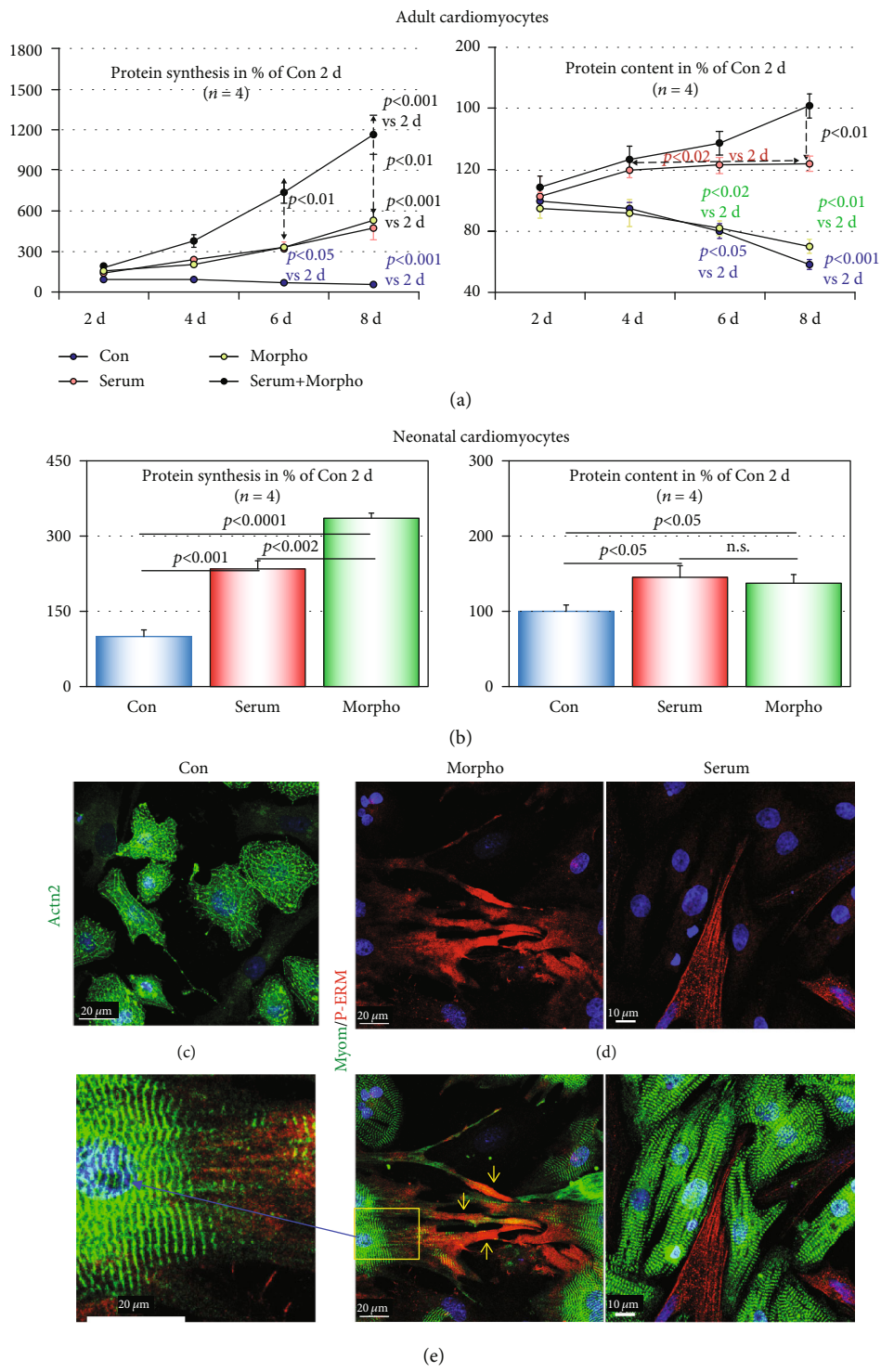


Figure 3: Endothelial morphogens induce atrophy of adult cardiomyocytes but hypertrophic growth of neonatal myocytes. Con are albumin-treated control cultures. Serum indicates serum stimulation, and Morpho specifies treatment with endothelial morphogens for the indicated time in days (d). All values are calculated in % of Con at day 2. (a) The time course of protein synthesis and protein accumulation (total protein content) of adult cardiomyocytes reveals significant increases after serum and serum plus morphogen stimulation. Morphogen-treated cultures show a decrease in protein content despite an elevated protein synthesis. (b) In neonatal cardiomyocytes, protein synthesis and protein content are significantly increased after stimulation for 2 days with serum or morphogen. (c, d) Confocal images of 2-day-old neonatal cardiomyocytes. (c) Unstimulated cardiomyocytes (Con) reveal a diffuse pattern of sarcomeric α-actinin (Actn2) and an absence of cross-striation. (d) Reestablishment of cell-cell contacts as well as the formation of mature sarcomeres (myomesin) is clearly visible after serum and morphogen stimulation. Note the long extensions in morphogen-stimulated cultures (yellow arrows). (e) Cross-striation is visible in the magnified image (blue arrow and yellow rectangle).

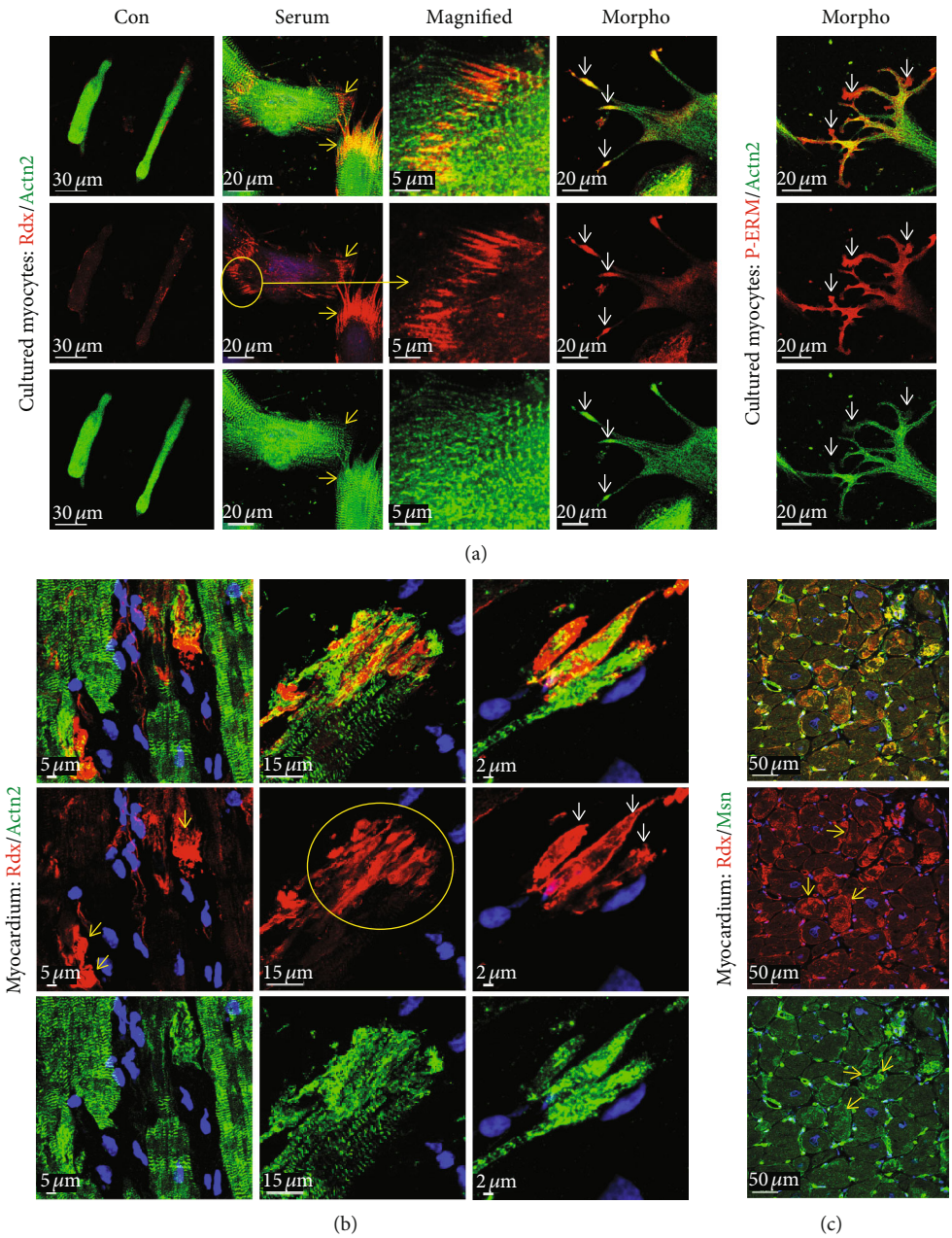


Figure 4: Reorganization of the intercalated disc in cultured adult cardiomyocytes resembles remodeling of ID in patients with DCM. (a) Confocal images of adult cardiomyocytes after different treatments at day 4. In control cultures (Con), radixin (Rdx) is lost at the area of the intercalated disc and cells round up at the ends. Typical cross-striation is lost. After serum stimulation, the shape of IDs appears wider (yellow arrows). Radixin accumulates in a longitudinal configuration paralleling the axis of the cardiomyocyte (yellow circle and long yellow arrow, magnified image). When long thin extensions are formed, knot-like spots at the ends can be observed (Morpho, white arrows). P-ERM indicates activation in these knot-like spots. (b) Similar to cell cultures, cardiomyocytes of the failing heart show various shapes of the ID reflecting probably different stages of remodeling (yellow and white arrows, yellow circle). The area of the ID appears wider (yellow arrows). Radixin accumulates in a longitudinal configuration in cardiomyocytes (yellow circle). Knot-like spots and extensions at the end of cardiomyocytes are clearly visible (white arrows). (c) Radixin (Rdx, red) and moesin (Msn, green) reveal also some diffuse cytoplasmic as well as membranous localization (yellow arrows).

arrows and yellow circle) indicating that these processes are evolutionary conserved. In contrast to serum, treatment with morphogens induces the formation of long thin extensions and knob-like spots at cell ends (Figure 4(a); Morpho, white arrows). These thin extensions and knob-like spots can also be observed in the human failing heart (Figure 4(b); white arrows). An overall cytoplasmic and membranous accumulation of radixin comparable to moesin in the diseased myocardium is also visible (Figure 4(c); yellow arrows) [21].

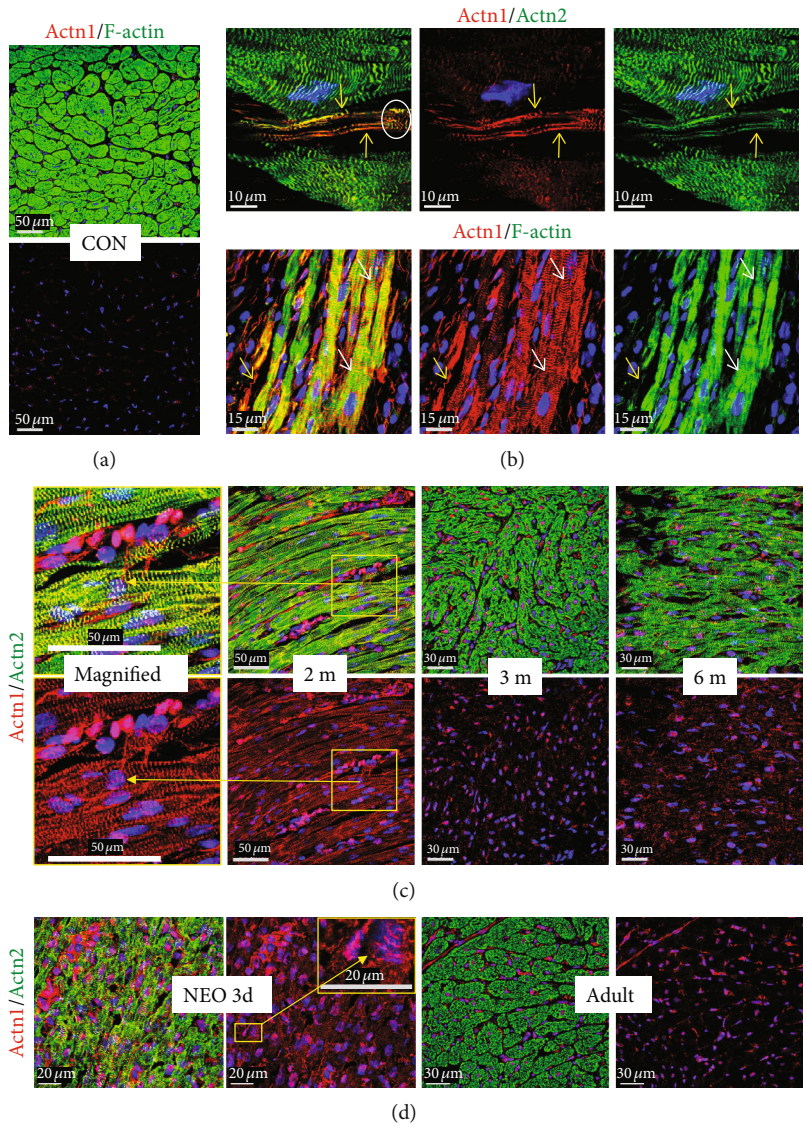


Figure 5: Nonmuscle α-actinin-1 (Actn1) is reexpressed and involved in remodeling of the area of the intercalated disc in patients with dilated cardiomyopathy. (a) Nonmuscle α-actinin-1 (Actn1) is completely absent in normal cardiomyocytes of the adult heart (CON). (b) Actn1 is reexpressed in myocytes of the failing human heart (HF). Note the formation of elongations (yellow arrows) from the area of the intercalated disc and the cross-striated pattern of Actn1-positive cardiomyocytes (white arrows). (c) Actn1 is present in myocytes of the myocardium of a 2-month- (2 m-) old patient with tetralogy of Fallot but absent in older infants (3 (3 m) and 6 (6 m) months). Note the cross-striated pattern of cardiomyocytes in the magnified image (yellow rectangle, long yellow arrows). (d) Actn1 is strongly expressed in cardiomyocytes of 3-day-old rat (NEO 3d) but is absent in the adult heart (Adult). Note that vessels and interstitial cells also express Actn1.

A further marker of cardiomyocyte remodeling is α-actinin-1 (Actn1). It is not expressed in cardiomyocytes of the normal adult myocardium (Figure 5(a); CON), but this nonmuscle α-actinin can be detected in long thin extensions of myocytes in the failing heart (Figure 5(b); yellow arrows). Furthermore, disruption of IDs is especially observed in areas of the myocardium with disturbed cell-cell contacts. The reexpression of the nonmuscle α-actinin-1 as well as the presence of sarcomeric α-actinin might stabilize these thin newly formed extensions. Another nonmuscle α-actinin, Actn4, is expressed and located at the area of the intercalated disc as well as in its extensions (Figure 6(a); small yellow arrows in HF and a long yellow arrow pointing to the magnification in a yellow rectangle).

Another surprising observation was the appearance of both nonmuscle actinins, Actn1 and Actn4, in a cross-striated pattern in cardiomyocytes of patients with heart failure (Actn1 indicated by white arrows in Figure 5(b) and Actn4 displayed by a white circle and a long white arrow pointing from Figure 6(a) to the magnified image in Figure 6(b)). While the functional role is unknown, the cross-striated presence of both nonmuscle actinins in the myocardium of newborn rats suggests a potential role in the early postnatal development of sarcomeres (Actn1

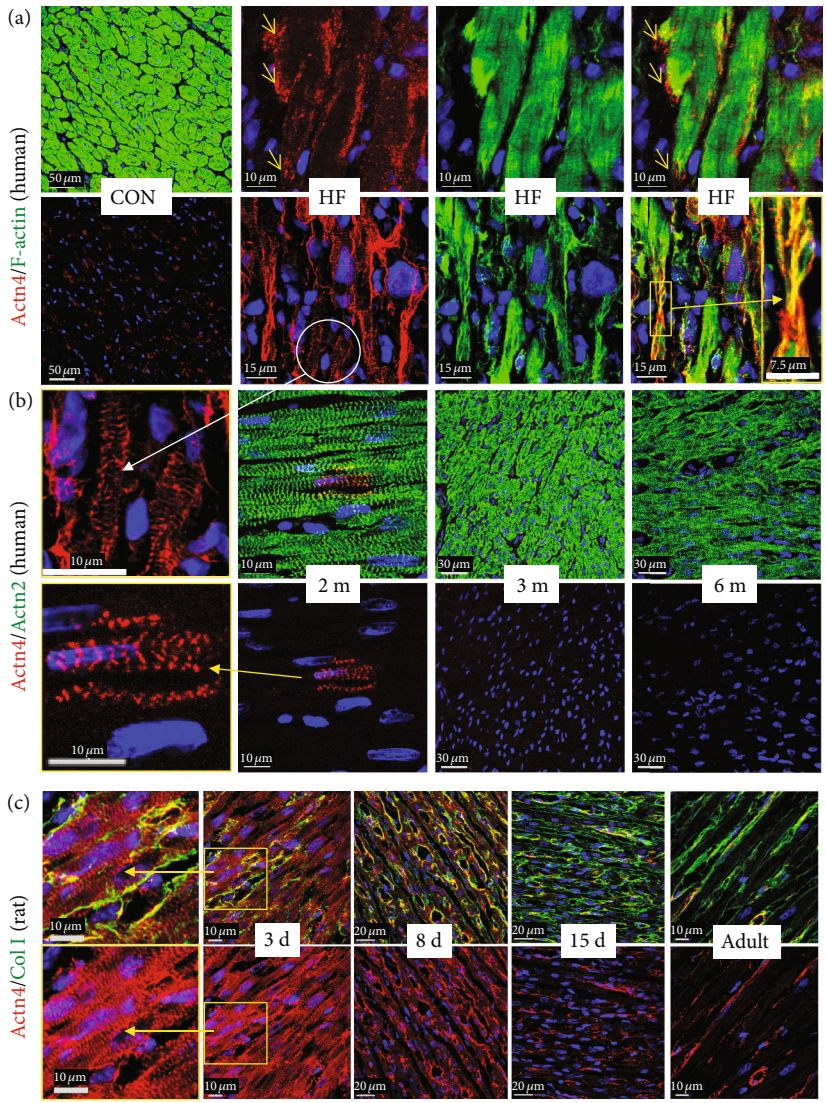


Figure 6: Nonmuscle α-actinin-4 (Actn4) is expressed and involved in remodeling of cardiomyocytes in patients with dilated cardiomyopathy. (a) Actn4 is hardly detectable in cardiomyocytes of the human adult heart (CON). In the failing heart (HF), remodeling of the area of the intercalated disc (yellow arrows) and formation of long extensions are visible (long yellow arrow, enlarged image with a yellow frame). Actn4-positive extensions of cardiomyocytes can be recognized in yellow by the overlay of green sarcomeric actinin and red Actn4. Some cross-striation (white circle and long white arrow, magnification in (b)) can be recognized. (b) Few cardiomyocytes are positive for Actn4 in the myocardium of a 2-month-old child with tetralogy of Fallot (2 m) but absent in older infants (3 (3 m) and 6 (6 m) months). (c) Actn4 is strongly expressed in cardiomyocytes of the early postnatal heart (3 days) but decreases steadily with increasing age and is absent in the adult. Note that interstitial cells and vessels express nonmuscle Actn4. Col I indicates collagen 1.

in an enlarged yellow frame image in Figure 5(d) and enlarged yellow frame images of Actn4 in Figure 6(c)). A cross-striated pattern of both nonmuscle α-actinins was also observed in cardiomyocytes of human infants (enlarged images of Actn1 in Figure 5(c) and Actn4 in Figure 6(b)). Furthermore, the postnatal decrease in both nonmuscle proteins in rats (Actn1 in Figure 5(d) and Actn4 in Figure 6(c)) and humans (Actn1 in Figure 5(c) and Actn4 in Figure 6(b)) during cardiac maturation indicates that these nonmuscle α-actinins are reactivated in the adult failing heart.

4. Discussion

Here, we demonstrate that remodeling of cultured adult cardiomyocytes shows similarities with myocytes of patients suffering from dilated cardiomyopathy. Remodeling of cardiomyocytes is regarded as a mechanism to cope with increased stress and involves the activation of fetal genes which gives cardiomyocytes the plasticity to adapt to environmental challenges. One of these fetal markers is nonmuscle α-actinin-1 (Actn1) which we previously observed in storage clusters of cardiomyocytes in the failing heart [7]. Since autophagic

and ischemic cell death is a frequent event in failing hearts but colocalization of Actn1 with cell death markers was rarely observed [7], we hypothesized that nonmuscle actinins might exert certain functions and are genes expressed in developing cardiomyocytes.

Actinins (Actn1, Actn2, Actn3, and Actn4) are coded by four genes located on different chromosomes and are highly conserved between species with an almost identical length in mouse, rats, and humans. Actn3 is only expressed in the skeletal muscle but not in the heart, while Actn2 occurs in both muscle types. We also did not detect Actn3 in the myocardium of patients with heart failure by confocal microscopy (not shown). Actinins stabilize adjacent sarcomeres and anchor filaments to cell-cell contacts by cross-linking actin. Mutations in Actn1, Actn2, and Actn4 are linked to human heritable diseases [23]. Actn1 and Actn4 belong to the so-called nonmuscle α-actinins and reveal a high degree of sequence homology with similar actin binding properties. Despite functional similarities, diverse cell biological roles have been reported indicating that their functions are only partly overlapping. Actn1-knockout mice are not viable while Actn4-deficient mice develop a severe glomerular disease indicating that Actn1 does not compensate for the loss of α-actinin-1 [24]. Nuclear localization of Actn4 suggests a role in transcriptional regulation which has not been reported for Actn1 [23]. In the normal adult cardiomyocyte, nonmuscle α-actinins are hardly detected, but we observed a reexpression of Actn1 in the remodeling myocardium. Actn1 is reexpressed in cardiomyocytes of patients with myocardial infarction, aortic stenosis, and dilated cardiomyopathy [7, 8, 17, 25]. In mice with myocarditis and acute myocardial infarction, we observed a reexpression of this α-actinin in cardiomyocytes [8, 17]. Here, we detected surprisingly Actn1 in the extensions of cardiomyocytes indicating an important role of this nonmuscle α-actinin in the reestablishment of cellular contacts in the damaged myocardium. In addition to Actn1, we observed also a strong expression of Actn4 in cardiomyocytes of patients with dilated cardiomyopathy suggesting similar functions. However, there appears to be a timely restricted and distinct expression pattern of both nonmuscle α-actinins during development. Actn1 was expressed in a large number of 2-month-old human cardiomyocytes whereas few cells were positive for Actn4 at this stage. Since both nonmuscle α-actinins were identified in cultured neonatal rat cardiomyocytes as well as in the newborn rat heart, their expression in the adult human myocardium indicates that they are reactivated. Surprisingly, despite classification as nonmuscle α-actinins, staining of Actn1 and Actn4 produced a distinct cross-striated pattern reminiscent of sarcomeric α-actinin (Actn2) which is localized at the Z-disc. It is intriguing to speculate that these two nonmuscle α-actinins are involved in myofibrillogenesis and mechanotransduction in cardiomyocytes of the diseased adult myocardium as well as in the developing neonatal heart.

A further feature of remodeling is the obvious change of the architecture around the intercalated discs (IDs). IDs are the contact sites between individual cardiomyocytes and ensure mechanical and electrochemical coupling necessary for the transmission of contractile force. The function of ID in the intercellular communication and mechanical force transmission therefore suggests an important role of intercalated discs in the development of heart failure. These morphological changes have been described as membrane convolutions of IDs characteristic of the phenotype of cardiomyocytes in patients with dilated cardiomyopathy [26]. Remodeling of the intercalated disc has been furthermore observed in mice misexpressing cadherins [27], in aging mouse hearts [28], in tachypaced sheep [29], and in chronically pressure overloaded hearts of guinea pigs [30]. Staining of the ERM protein radixin indicated quite dramatic modifications in the structure of the area of IDs in patients with dilated cardiomyopathy and in cardiomyocyte cultures. Changes from accumulation of radixin into longitudinal directions paralleling the cellular axis result finally in elongated ends. We have previously demonstrated the enormous dynamic mobility of ERM proteins in cultures of adult cardiomyocytes when ezrin and radixin start to translocate from the area of the ID to different cellular locations [12]. There are a number of apparent reasons why remodeling of the area of the ID in the adult heart might be a major important adaptation to stress. The reduced ability to replace damaged myocytes by proliferation, the increasing stiffness through the rising number and maturation of sarcomeres, the oxygen-dependent energy metabolism, and the incapacity to reestablish cell contacts through migration render differentiated adult cardiomyocytes extremely vulnerable [12, 21, 26]. As a result, cardiomyocyte-cardiomyocyte contacts might be loosened or disrupted to a certain degree which can be analyzed in detail in cell cultures. However, we have at the present status also to address certain study limitations. Despite obvious localization of ERM proteins at the area of the intercalated disc, we are presently not able to distinguish whether radixin is indeed involved in remodeling of cardiomyocytes and their IDs or whether these observations are only side effects. The same applies to nonmuscle α-actinins. Furthermore, it is yet not clear whether the expression of Actn1 and Actn4 is indicative of an activation of the fetal gene program. Thus, further studies are needed utilizing cell cultures with siRNA knockdown of radixin and nonmuscle α-actinins as well as cardiomyocyte-restricted knockout mice which might also help to clarify their influence on the regenerative capacity of the diseased heart.

Generally, soluble secreted factors induce three patterns of adult cardiomyocyte remodeling in culture: first, decomposition of the area of the intercalated disc; second, formation of long extensions; and third, activation of fetal genes which can be recognized. Since all the three patterns were observed in patients, we assume that the underlying remodeling mechanisms are also valid for the development of dilated cardiomyopathy. Our cell culture data suggest furthermore that soluble secreted factors such as cytokines and growth factors play a major role in cardiomyocyte remodeling. Several important candidates have been shown to be involved in remodeling such as the interleukin-6 class of cytokines [8, 31], fibroblast growth factors [4, 32, 33], transforming growth factors [34], and insulin-like growth factors [25, 32]. However, it would be too simple to attribute the chronic and complex process of cardiac remodeling to the

activity of specific cytokines, hormones, or growth factors. Cardiac pathogenesis and regeneration are rather based on a complex interplay of different factors in a spatially and temporarily restricted way. Furthermore, secreted factors often exert completely different activities pointing to distinct functions which can be derived from the effects of serum and morphogens on cardiomyocyte cultures. Serum-stimulated hypertrophy and lateral spreading with very few extensions are reminiscent of the activity evoked by fibroblast growth factors and insulin-like growth factors [25, 32, 35]. In contrast, morphogens induced expression of fetal genes, atrophy, and an intense intercellular network through the formation of multiple long extensions reminiscent of a phenotype induced by members of the interleukin-6 family [8, 31].

A further important observation is the difference in remodeling capacity of neonatal and adult cardiomyocytes in culture. Neonatal cardiomyocytes grow rapidly within 2 days and develop hypertrophy after serum as well as morphogen stimulation. In contrast, there were very few differences until day 2 in protein synthesis as well as in the protein accumulation between controls and serum- and morphogen-stimulated adult cardiomyocytes, which became only apparent after a longer culture time. Furthermore, stimulation with morphogens induced atrophy in adult cardiomyocytes while neonatal myocytes developed hypertrophy. These data indicate intrinsic variances between neonatal and adult cardiomyocytes and might partly explain the different regenerative capacity of pediatric and adult patients.

5. Conclusions and Summary

Our results clearly demonstrate that soluble secreted factors induce remodeling of cultured adult cardiomyocytes similar to that observed in patients with dilated cardiomyopathy. Remodeling of cardiomyocytes involved radixin relocalization and nonmuscle α-actinin reexpression. In culture, dedifferentiated adult cardiomyocytes develop resistance to stress but do not regain the regenerative capacity of neonatal myocytes. Since changes in protein composition and cardiac morphology are quite dramatic during chronic heart failure, it is expected that the presently known structural proteins, signaling molecules, and cascades constitute only the "tip of an iceberg." Thus, an intense analysis of *in vitro* systems by "omics" technologies will accelerate the identification of molecules involved in cardiac regeneration and decipher hidden "survival programs" of neonatal hearts. Determination of these factors and their individual regenerative potential are of particular importance for a biomarker-guided therapy in adults.

Data Availability

The data as well as the images that support the findings of our study are available from the corresponding author Dr. Thomas Kubin (t.kubin@kerckhoff-klinik.de) upon reasonable request.

Ethical Approval

All studies with human material comply with the Declaration of Helsinki and were approved by the respective responsible ethical committees.

Conflicts of Interest

The authors have no conflict of interest to disclose.

Authors' Contributions

A.C., B.B., T.K., K.T., P.G., and N.K. performed the experiments and data analysis. A.C., T.K., H.A., Y.H.C., and M.R. designed and wrote the manuscript. D.S., B.S.H., K.V., and H.A. screened and analyzed the pediatric patients. M.R., Y.H.C., and M.S. screened and analyzed the adult patients. D.S., B.S.H., K.V., and M.S. organized, collected, and documented the tissue samples.

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References

[1] P. E. Kolattukudy, T. Quach, S. Bergese et al., "Myocarditis induced by targeted expression of the MCP-1 gene in murine cardiac muscle," *The American Journal of Pathology*, vol. 152, no. 1, pp. 101–111, 1998.

[2] N. Kubin, M. Richter, B. Sen-Hild et al., "Macrophages represent the major pool of IL-7Rα expressing cells in patients with myocarditis," *Cytokine*, vol. 130, p. 155053, 2020.

[3] I. Mozos, C. Malainer, J. Horbańczuk et al., "Inflammatory markers for arterial stiffness in cardiovascular diseases," *Frontiers in Immunology*, vol. 8, pp. 1058–1058, 2017.

[4] M. Richter, H.-J. Lautze, T. Walther, T. Braun, S. Kostin, and T. Kubin, "The failing heart is a major source of circulating FGF23 via oncostatin M receptor activation," *The Journal of Heart and Lung Transplantation*, vol. 34, no. 9, pp. 1211–1214, 2015.

[5] J. N. Cohn, R. Ferrari, and N. Sharpe, "Cardiac remodeling–concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. Behalf of an International Forum on Cardiac Remodeling," *Journal of the American College of Cardiology*, vol. 35, no. 3, pp. 569–582, 2000.

[6] S. Hein, E. Arnon, S. Kostin et al., "Progression from compensated hypertrophy to failure in the pressure-overloaded human heart: structural deterioration and compensatory mechanisms," *Circulation*, vol. 107, no. 7, pp. 984–991, 2003.

[7] S. Hein, T. Block, R. Zimmermann et al., "Deposition of nonsarcomeric alpha-actinin in cardiomyocytes from patients with dilated cardiomyopathy or chronic pressure overload,"

Experimental and Clinical Cardiology, vol. 14, no. 3, pp. e68–e75, 2009.

[8] T. Kubin, J. Pöling, S. Kostin et al., "Oncostatin M is a major mediator of cardiomyocyte dedifferentiation and remodeling," *Cell Stem Cell*, vol. 9, no. 5, pp. 420–432, 2011.

[9] M. Rajabi, C. Kassiotis, P. Razeghi, and H. Taegtmeyer, "Return to the fetal gene program protects the stressed heart: a strong hypothesis," *Heart Failure Reviews*, vol. 12, no. 3-4, pp. 331–343, 2007.

[10] M. Szibor, J. Poling, H. Warnecke, T. Kubin, and T. Braun, "Remodeling and dedifferentiation of adult cardiomyocytes during disease and regeneration," *Cellular and Molecular Life Sciences*, vol. 71, no. 10, pp. 1907–1916, 2014.

[11] A. E. Berezin, "Circulating biomarkers in heart failure," in *Heart Failure: From Research to Clinical Practice*, M. S. Islam, Ed., vol. 3, pp. 89–108, Springer International Publishing, Cham, 2018.

[12] Y. Hou, J. M. Adrian-Segarra, M. Richter et al., "Animal Models and "Omics" Technologies for Identification of Novel Biomarkers and Drug Targets to Prevent Heart Failure," *BioMed Research International*, vol. 2015, Article ID 212910, 10 pages, 2015.

[13] K. Kuwahara, T. Nishikimi, and K. Nakao, "Transcriptional regulation of the fetal cardiac gene program," *Journal of Pharmacological Sciences*, vol. 119, no. 3, pp. 198–203, 2012.

[14] A. Azakie, J. L. Russell, B. W. McCrindle et al., "Anatomic repair of anomalous left coronary artery from the pulmonary artery by aortic reimplantation: early survival, patterns of ventricular recovery and late outcome," *The Annals of Thoracic Surgery*, vol. 75, no. 5, pp. 1535–1541, 2003.

[15] S. Shiraishi, H. Uemura, K. Kagisaki et al., "Long-term results of total cavopulmonary connection with low ejection fraction," *General Thoracic and Cardiovascular Surgery*, vol. 59, no. 10, pp. 686–692, 2011.

[16] D. Schranz, A. Veldman, U. Bartram, I. Michel-Behnke, J. Bauer, and H. Akintürk, "Pulmonary artery banding for idiopathic dilative cardiomyopathy: a novel therapeutic strategy using an old surgical procedure," *The Journal of Thoracic and Cardiovascular Surgery*, vol. 134, no. 3, pp. 796-797, 2007.

[17] H. Lörchner, J. Pöling, P. Gajawada et al., "Myocardial healing requires Reg3β-dependent accumulation of macrophages in the ischemic heart," *Nature Medicine*, vol. 21, no. 4, pp. 353–362, 2015.

[18] T. Kubin, H. Ando, D. Scholz et al., "Microvascular endothelial cells remodel cultured adult cardiomyocytes and increase their survival," *The American Journal of Physiology*, vol. 276, no. 6, pp. H2179–H2187, 1999.

[19] T. Kubin, S. Vogen, J. Wetzel et al., "Porcine aortic endothelial cells show little effects on smooth muscle cells but are potent stimulators of cardiomyocyte growth," *Molecular and Cellular Biochemistry*, vol. 242, no. 1/2, pp. 39–45, 2003.

[20] S. Vogel, T. Kubin, D. von der Ahe, E. Deindl, W. Schaper, and R. Zimmermann, "MEK hyperphosphorylation coincides with cell cycle shut down of cultured smooth muscle cells," *Journal of Cellular Physiology*, vol. 206, no. 1, pp. 25–34, 2006.

[21] J. Poling, P. Gajawada, H. Lorchner et al., "The Janus face of OSM-mediated cardiomyocyte dedifferentiation during cardiac repair and disease," *Cell Cycle*, vol. 11, no. 3, pp. 439–445, 2014.

[22] E. Ruoslahti, "Stretching is good for a cell," *Science*, vol. 276, no. 5317, pp. 1345-1346, 1997.

[23] H.-Y. Kao, "The actinin family proteins: biological function and clinical implications," *Cell & Bioscience*, vol. 5, no. 1, 2015.

[24] C. H. Kos, T. C. Le, S. Sinha et al., "Mice deficient in α-actinin-4 have severe glomerular disease," *The Journal of Clinical Investigation*, vol. 111, no. 11, pp. 1683–1690, 2003.

[25] J. Pöling, P. Gajawada, M. Richter et al., "Therapeutic targeting of the oncostatin M receptor-β prevents inflammatory heart failure," *Basic Research in Cardiology*, vol. 109, no. 1, p. 396, 2014.

[26] J. C. Perriard, A. Hirschy, and E. Ehler, "Dilated cardiomyopathy: a disease of the intercalated disc?," *Trends in Cardiovascular Medicine*, vol. 13, no. 1, pp. 30–38, 2003.

[27] M. C. Ferreira-Cornwell, Y. Luo, N. Narula, J. M. Lenox, M. Lieberman, and G. L. Radice, "Remodeling the intercalated disc leads to cardiomyopathy in mice misexpressing cadherins in the heart," *Journal of Cell Science*, vol. 115, Part 8, pp. 1623–1634, 2002.

[28] T. A. Bonda, B. Szynaka, M. Sokołowska et al., "Remodeling of the intercalated disc related to aging in the mouse heart," *Journal of Cardiology*, vol. 68, no. 3, pp. 261–268, 2016.

[29] C. Pinali, H. J. Bennett, J. B. Davenport et al., "Three-dimensional structure of the intercalated disc reveals plicate domain and gap junction remodeling in heart failure," *Biophysical Journal*, vol. 108, no. 3, pp. 498–507, 2015.

[30] X. Wang, F. Li, and A. M. Gerdes, "Chronic pressure overload cardiac hypertrophy and failure in guinea pigs: I. regional hemodynamics and myocyte remodeling," *Journal of Molecular and Cellular Cardiology*, vol. 31, no. 2, pp. 307–317, 1999.

[31] K. C. Wollert, T. Taga, M. Saito et al., "Cardiotrophin-1 activates a distinct form of cardiac muscle cell hypertrophy," *Journal of Biological Chemistry*, vol. 271, no. 16, pp. 9535–9545, 1996.

[32] M. Y. Donath, J. Zapf, M. Eppenberger-Eberhardt, E. R. Froesch, and H. M. Eppenberger, "Insulin-like growth factor I stimulates myofibril development and decreases smooth muscle alpha-actin of adult cardiomyocytes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 5, pp. 1686–1690, 1994.

[33] C. Faul, A. P. Amaral, B. Oskouei et al., "FGF23 induces left ventricular hypertrophy," *The Journal of Clinical Investigation*, vol. 121, no. 11, pp. 4393–4408, 2011.

[34] T. Kubin, M. Tomars, C. Fach et al., "Transforming growth factor-β_1 downregulates beating frequency and remodeling of cultured rat adult cardiomyocytes," *Cell and Tissue Research*, vol. 321, no. 1, pp. 57–66, 2005.

[35] M. Eppenberger-Eberhardt, S. Aigner, M. Y. Donath et al., "IGF-I and bFGF differentially influence atrial natriuretic factor and α-smooth muscle actin expression in cultured atrial compared to ventricular adult rat cardiomyocytes," *Journal of Molecular and Cellular Cardiology*, vol. 29, no. 8, pp. 2027–2039, 1997.

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Review Article

Adverse Cardiac Remodelling after Acute Myocardial Infarction: Old and New Biomarkers

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The prevalence of heart failure (HF) due to cardiac remodelling after acute myocardial infarction (AMI) does not decrease regardless of implementation of new technologies supporting opening culprit coronary artery and solving of ischemia-relating stenosis with primary percutaneous coronary intervention (PCI). Numerous studies have examined the diagnostic and prognostic potencies of circulating cardiac biomarkers in acute coronary syndrome/AMI and heart failure after AMI, and even fewer have depicted the utility of biomarkers in AMI patients undergoing primary PCI. Although complete revascularization at early period of acute coronary syndrome/AMI is an established factor for improved short-term and long-term prognosis and lowered risk of cardiovascular (CV) complications, late adverse cardiac remodelling may be a major risk factor for one-year mortality and postponded heart failure manifestation after PCI with subsequent blood flow resolving in culprit coronary artery. The aim of the review was to focus an attention on circulating biomarker as a promising tool to stratify AMI patients at high risk of poor cardiac recovery and developing HF after successful PCI. The main consideration affects biomarkers of inflammation, biomechanical myocardial stress, cardiac injury and necrosis, fibrosis, endothelial dysfunction, and vascular reparation. Clinical utilities and predictive modalities of natriuretic peptides, cardiac troponins, galectin 3, soluble suppressor tumorogenicity-2, high-sensitive C-reactive protein, growth differential factor-15, midregional proadrenomedullin, noncoding RNAs, and other biomarkers for adverse cardiac remodelling are discussed in the review.

1. Introduction

Heart failure (HF) is a global health problem with serious economic burden that has been considered as the dominant cause of cardiovascular (CV) morbidity and mortality in the developed and developing countries [1, 2]. HF affects around 26 million people worldwide (including 5.7 million and 3.4 million people in the US and in the EU, respectively), and the estimated expenditures for HF care were around $31 billion [1]. It is expected that by 2030 more than 40 million people will have this condition, and the HF diagnosis and therapy will increase twice and even more [3]. The clinical outcomes remain poor with a five-year survival rate of approximately 50% regardless of phenotype of HF that completely correspond to the expected survival rate in non-metastatic cancer [3, 4]. Despite sufficient improvements in diagnosis, prevention and treatment of HF new incidences of HF with reduced ejection fraction (HFrEF) and midrange ejection fraction (HFmrEF) in contrast to HF with preserved ejection fraction (HFpEF) continue to occur as a need for heart transplantation and mechanical support device use [4]. Additionally, increased prevalence of HFpEF represents the most frequent cause of CV and sudden death, primary hospitalization, and readmission to the hospital due to acute decompensation of HF [5].

The most common primary causes of HFrEF/HFmrEF remain acute ST-segment elevation myocardial infarction (STEMI) and hypertension, while incidences of HFpEF were rather associated with hypertension, acute non-ST-segment elevation myocardial infarction (non-STEMI), and

alternative causes (atrial fibrillation, cardiomyopathy, myocarditis, valvular heart disease, and diabetes mellitus) compared with STEMI [6–8]. Contemporary strategy for the prevention of HF after acute STEMI is based on early complete cardiac revascularization and prevention of negative impact of comorbidities, such as diabetes mellitus, abdominal obesity, hypertension, thyroid dysfunction, kidney failure, and conventional CV risk factors (smoking, dyslipidaemia, insulin resistance, and hyperuricemia) [9, 10]. In fact, complete recovering of blood flow through culprit coronary artery and other ischemia-related stenosis with primary percutaneous coronary intervention (PCI) is not warranted for full prevention of late adverse cardiac remodelling [11, 12]. Although improvement of prognosis, increase in quality of life, and delay in progression or reversal of ischemia-induced cardiac remodelling and chronic HF remain prime targets for the treatment of AMI [13, 14], there are no clear approaches for risk stratification in AMI patients after successful PCI [15]. For instance, hyperemic microcirculatory resistance and no-reflow phenomenon were found as strong predictors for the extent of infarct size and early cardiac remodelling [16]. Additionally, optic coherent tomography or intravascular ultrasound performed over 3 months after initial major cardiac event frequently allows identifying several factors contributing advance in late cardiac remodelling, such as silent restenosis, progression of old stenotic lesions, late stent thrombosis, and several post-PCI technical problems with incomplete stent branches' expansion, stent malposition, and underpressed culprit plaque [17, 18]. Except for early revascularization, cardiac remodelling could be prevented by pharmacotherapy including complex neurohormonal blockade and device-based therapies, which are addressed in the improvement of ventricular dyssynchrony and prevention from fatal arrhythmias [19]. In this context, new diagnostic and predictive options are needed to prevent cardiac remodelling and HF. The aim of the review was focused on the circulating biomarker as a tool to stratify postmyocardial infarction patients at high risk of poor cardiac recovery after reperfusion with primary PCI and developing HF.

2. Adverse Cardiac Remodelling after Acute Myocardial Infarction: Definitions and Contributing Factors

2.1. Definition of Adverse Cardiac Remodelling. Adverse cardiac remodelling after AMI is defined as complex interactions between cellular and extracellular components of myocardium, which are neurohumoral and epigenetic regulations, leading to changes in the cardiac architectonics and geometry frequently affecting both ventricles and atrials, worsening diastolic filling and systolic function and associated with developing heart failure [17]. Additionally, there is a large number of definitions of cardiac remodelling after STEMI, which are based on multiple imaging modalities, such as presentation of akinesia area, left ventricle enlargement, reduced LVEF, and early diastolic dysfunction (including longitudinal strain increase, twist of LV apex, and tethering effect). In fact, an impact of passive mechanical constraint of surrounding myocardium on infarct zone mediates infarct expansion and decline in both regional and global systolic function [20].

Other criteria of cardiac remodelling affect shaping stunned and hibernated myocardium after incomplete revascularization or delay of PCI performing with inadequate perfusion recovery [21]. However, non-STEMI is also associated with cardiac remodelling, rather mild-to-moderate than severe, and frequently nondistinguished from STEMI-induced cardiac disorders in prognostic aspects, but the canonic model of pathogenesis of adverse cardiac remodelling was based on STEMI impact on cardiac architectonic. There is a sustainable option that STEMI-induced cardiac remodelling frequently relates to HFrEF/HFmrEF, but non-STEMI-induced cardiac remodelling is rather associated with developing HFpEF than HFrEF.

2.2. Contributing Factors of Adverse Cardiac Remodelling. In fact, there are at least two different variants of adverse cardiac remodelling after acute MI, which distinguished each other in pathogenesis, so called the early (at 2-3 weeks after initial event) and late (at 3-6 months after AMI) remodelling (see Figure 1).

Contemporary point of view is based on an idea that early complete primary revascularization of culprit artery and ischemia-induced stenosis/occlusions in other coronary arteries at first hours of STEMI is independent and the most powerful factor preventing early LV cavity dilation, declining LV pump function and the developing of HF. It has been postulated that preserved systolic function and LV dimensions at early stage of various revascularization procedures can accompany with myocardial biomechanical and energetics stress, mitochondrial dysfunction, and oxidative stress that lead to potent fatal arrhythmias even prior to diastolic dysfunction developing [22]. Over the next three months after restoring TIMI III blood flow through culprit artery with PCI, the primary causes inducing adverse cardiac remodelling can be different from the aforementioned. Indeed, other factors that may contribute to cardiac remodelling after successful primary PCI are arterial healing, vessel remodelling, stent restenosis, thrombosis, and incomplete expansion of stent branches (known as malposition), and stent fracture, which require ischemia-driven target vessel revascularization further [23]. Performing of optical coherence tomography (OCT) in STEMI patients presenting with late and very late stent thrombosis has yielded that stent malposition was determined in 55% cases, quarter of which had been found evidence of positive vessel remodelling [24]. Additionally, neoatherosclerosis and uncovered stent struts were reported as the primary cause of late thrombosis in 35% cases and 10% cases, respectively [24]. Although coronary stent fracture is an underrecognized event, it has been reported frequently in the drug-eluting stent era [25]. However, investigators have shown that technical problems with first-generation eluting stent implantations in STEMI patients were associated with higher in-hospital mortality and posthospital target vessel failure or cardiac death [24].

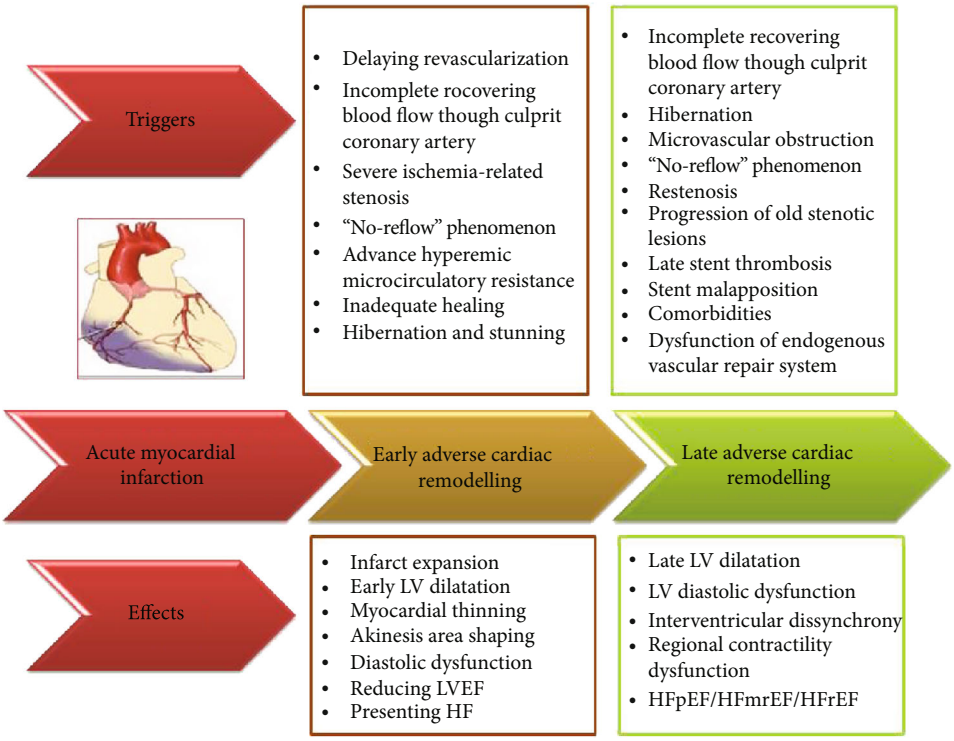

Figure 1: Adverse cardiac remodelling after AMI: the role of different triggers in development of cardiac architectonic disorders and heart failure. LV: left ventricular; HF: heart failure; HFpEF: HF with preserved ejection fraction; HFmrEF: HF with midrange ejection fraction; HFrEF: HF with reduced ejection fraction.

Endothelial shear stress, neointima formation, and late thrombosis can appear beyond inadequate PCI and stent positioning and are result of accelerating atherosclerosis and inadequate drug support, i.e., nonoptimal care with statins, refusal from dual antiplatelet therapy, effective anticoagulation if needed, and adenosine intracoronary for prevention no-reflow/slow-flow phenomena. Even a novel device (known as bioabsorbable cardiac matrix) was not able to attenuate adverse cardiac remodelling after AMI [26], while there were strong positive expectations regarding these devices [27]. Despite implantation of second-generation everolimus-eluting stent in STEMI appears to be better to first-generation eluting stents, there is evidence that even a small degree of chronic intrastent conditions may significantly influence on healing persistence [28]. Frequencies of uncovered and malapposed struts as well as percentage of stents fully covered with neointima were 1.2%, 0.4%, and 60.9%, respectively, for over a one-year period after PCI with second-generation everolimus-eluting stent implantation [28]. In fact, they were not associated with the incidence of clinical events and intrastent thrombus.

The next factor contributing to early and late cardiac remodelling is the "no-reflow" phenomenon. Indeed, the "no-reflow" phenomenon can be considered as a component of early cardiac remodelling after STEMI that relates to microvascular obstruction and dysfunction causing severe disturbance in regional perfusion [29]. In fact, the "no-reflow" phenomenon is a result in poor healing of the culprit artery and adverse cardiac remodelling, increasing the risk for major adverse cardiac events, such as recurrent MI, newly diagnosed HF, and sudden death, but the "slow-flow" phenomenon appears to be a serious factor contributing to both types of adverse cardiac remodelling [30, 31].

Additional factor that is involved onto a development of late adverse remodelling is epigenetically mediating disturbance of endogenous vascular repair system [32, 33]. It has been found that altered vascular repair has maintained vasoconstriction and vascular dysfunction that accelerated atherosclerosis and supported hibernation in the grey zone around myocardial infarction. Overall, the development of adverse cardiac remodelling after AMI regardless of initial cause (even in asymptomatic patients) was consistently associated with poor clinical outcomes, and it could be predicted and completely resolved [34, 35].

The factors preventing late adverse cardiac remodelling after successful reperfusion with primary PCI in STEMI patients are indicated in Figure 2. Recognition of the heterogeneous pathophysiology of adverse cardiac remodelling after AMI can create a powerful risk stratification score based on biomarkers reflecting various stages of pathogenesis of the condition [36].

3. Pathogenetic Mechanisms of Adverse Cardiac Remodelling after Acute Myocardial Infarction

Advances in our understanding of the molecular mechanisms of regulation toward late adverse cardiac remodelling were associated with the breakthrough in the recognition of

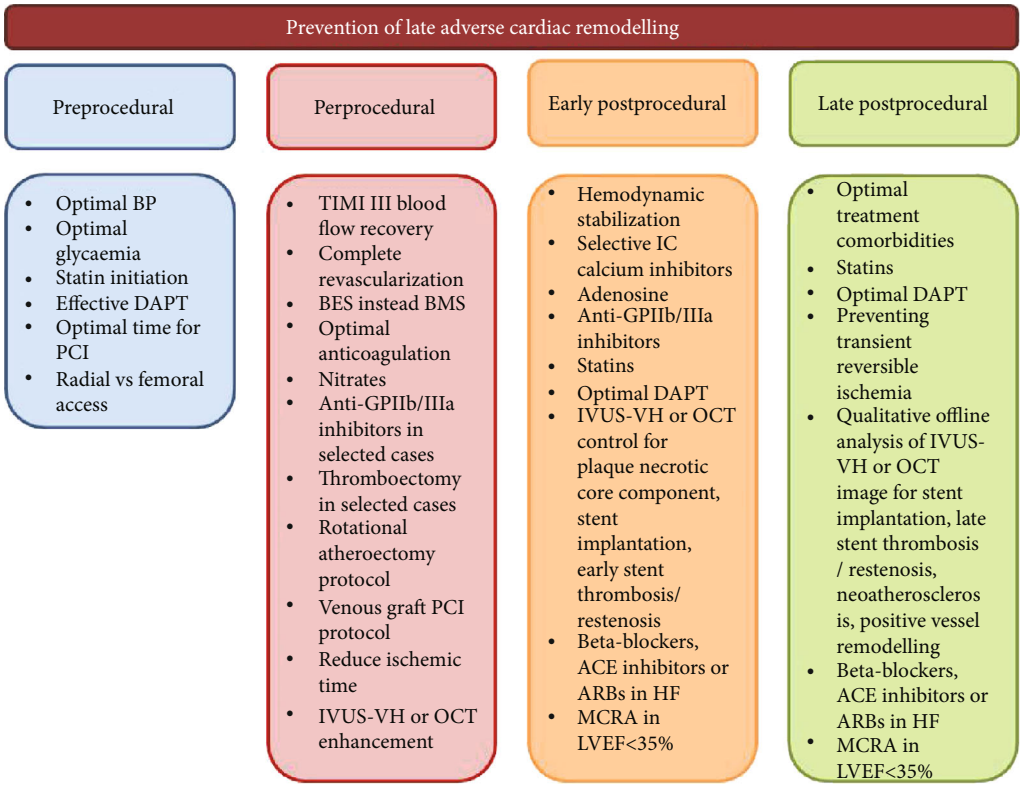

Figure 2: The factors preventing late adverse cardiac remodelling in AMI patients after successful reperfusion with PCI. IVUS-VH: intravascular ultrasound virtual-histology; BMS: bare metal stent; BES: biolimus eluting stent; OCT: optical coherence tomography; DAPT: dual antiplatelet therapy; ACE: angiotensin-converting enzyme; ARBs: angiotensin-II receptor antagonists; MCRA: mineralocorticoid receptor antagonists; IC: intracoronary.

interplaying between various processes translating ischemia/reperfusion injury on myocardium, such as disrupting nitric oxide (NO) and vascular endothelial growth factor (VEGF) signalling systems, p38 MAPK pathway and redox dysregulation, cytokine release, and activation of apoptotic and necrotic death pathways with subsequent stimulation of oxidative stress, mitochondrial dysfunction, altered myocardial cell metabolism, excessive fibrosis, and cardiac cell remodelling [37]. Therefore, preserved microvascular inflammation, small vessel obstruction, endothelial dysfunction, and atherosclerotic lesions mediate a remote effect on advance LV remodelling [38]. Additionally, there are new explanations regarding individual susceptibility to ischemia/reperfusion injury including early and remote ischemic preconditioning [39]. Figure 3 yields main pathogenetic mechanisms that are involved in the pathogenesis of late adverse cardiac remodelling.

In fact, restoration of adequate blood perfusion after a critical period of ischemia and prevention of reperfusion damage appear to be not the only protector over cardiac damage. Early irreversible cardiac myocyte injury leading to necrosis in the ischemic myocardium and expanding infarction zone are an attribute of susceptibility of cardiac cells to impaired metabolism, loss of structural integrity and selective permeability of the cell membranes, altered ultrastructure of cell organoids, such as sarcolemma disruption, deterioration of nucleus, ribosomes, mitochondria, and sarcoplasmic reticulum, the presentation of mitochondrial amorphous densities, and chromatin fragmentation [40]. During this early stage of AMI development, the mitochondrial dysfunction plays a pivotal role in cardiac myocyte apoptosis in the ischemic/reperfused heart, cardiac necrosis, and ischemia-induced preconditioning phenomenon [41, 42].

Numerous studies have shown that proapoptotic stimuli through involving cytokines, which belong to the B-cell lymphoma 2 (Bcl-2) super family, mediate the permeability of the mitochondrial membranes and stimulate the release of a wide spectrum of the active apoptogenic molecules (cytochrome c, Bax) into the cytoplasm. They cause the apoptotic response, peroxidation of membrane, and disruption of mitochondrial chromatin materials, including small interfering ribonucleic acid (RNA) and mitochondrial deoxyribonucleic acid (DNA) [43, 44]. Cytochrome C is able to bind to the adaptor protein apoptotic protease activating factor 1 (Apaf-1) and act as a trigger of its oligomerization that activates caspase cascade through initiating procaspase-9 recruitment. Caspases including caspase-6 and caspase-9 cleave cellular proteins and DNAs/RNAs emerging apoptosis [45]. This process is under the close epigenetic regulation of long noncoding RNAs (LncRNA) and microRNAs (miRNA-29b-1-5p, miRNA-195), which negatively regulate Bcl2l2 gene expression and participate in cardiac myocyte apoptosis, oxidative stress through inducing hydrogen peroxide (H_2O_2), and inflammation

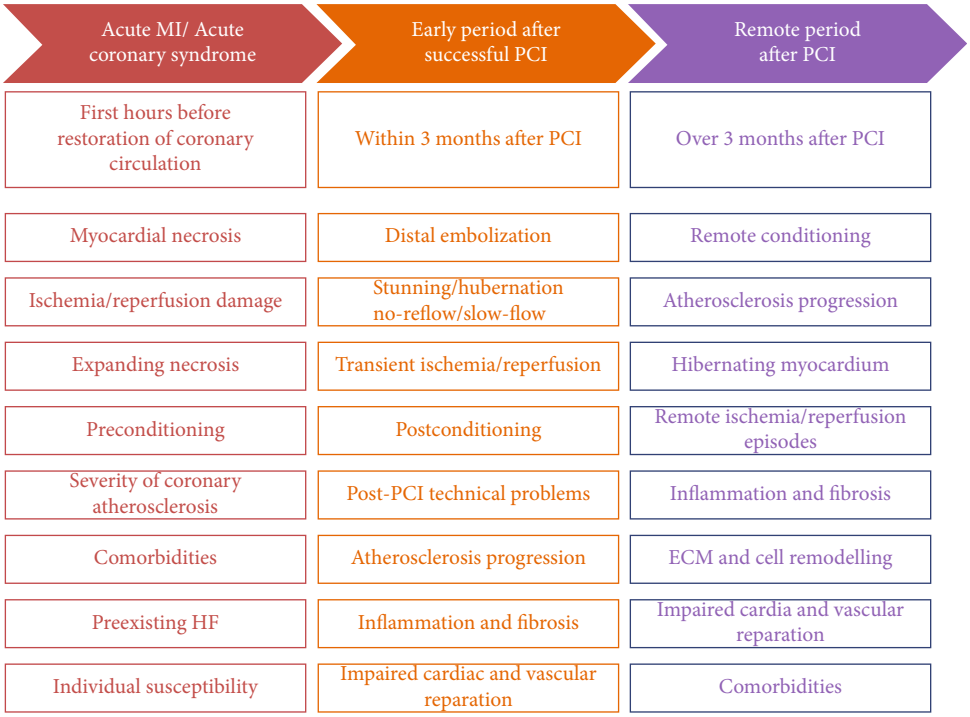

Figure 3: The main pathogenetic mechanisms underlying the initiation and progression of late adverse cardiac remodelling in AMI patients after successful reperfusion with PCI. HF: heart failure; ECM: extracellular matrix; PCI: percutaneous coronary intervention.

via triggering proinflammatory cytokine release [44, 46]. Therefore, downregulated miRNA-98 and miRNA-124 may attenuate cell survival through diminished levels of STAT3 and p-STAT3 in response to ischemia and over production of H_2O_2 [47, 48]. During ischemia/reperfusion episodes, oxidative stress, mitochondrial Ca^{2+} overload, proinflammatory cytokines (interleukin- (IL-) 2, IL-6, tumor necrosis factor-alpha, and interferon-gamma) stimulate the activity of the matrix metalloproteinases and suppress release of their tissue inhibitors [49]. MMPs (MMP-2, MMP-6, and MMP-9) directly contribute to global and local myocardial contractile dysfunction and induce cell death [50]. Other matricellular proteins, such as thrombospondin- (TSP-) 1 and TSP-2, as well as bone-related proteins (osteopontin, osteonectin, and osteoprotegerin), were found to regulate cardiac reparation and remodelling via activation of VEGF and transforming growth factor (TGF-β) by binding to the latency-associated propeptide, inhibition of MMP activity, and exertion of potent angiostatic actions of antigen-presenting cells and T-cells [51]. Moreover, they are triggers for accumulation, degradation and remodelling of extracellular matrix (ECM), cleaving big endothelin-1 and attenuating vasoconstriction, and modification of architectonics of myocardium leading to cardiac remodelling and HF development [51, 52].

Interestingly, susceptibility of myocardium to ischemia and reperfusion may relate to various inhered causes, such as mutations in genes encoding for angiotensin II, angiotensin-converting enzyme, osteopontin, osteoprotegerin, CC chemokine receptor 2, the members of the family of multidomain extracellular protease enzymes ADAMTS (A Disintegrin and Metalloproteinase with Thrombospondin motifs), predominantly ADAMTS-2, ADAMTS-4, ADAMTS-10, and ADAMTS-13, promoter region of endothelial NO synthase, apelin, TGF-β, VEGF, galectin-3, ficolin-1, S100 calcium-binding protein A9, and mitochondrial aldehyde dehydrogenase 2 (NDUFC2) [53–56]. Finally, susceptibility of cardiac cells to ischemia and reperfusion damage may relate to the capability of endogenous redox systems to protect cell membranes and cellular structures (mitochondria, cytoskeletal proteins, growth factor receptors, and microtubule-associated proteins) from the impaired effect of the deteriorating energetic metabolism and detergenting impact of oxidized lipids and proteins sustaining an effective work of transmembrane ionic pumps [57]. This phenomenon was called ischemic preconditioning, and now it is also recognized as an early (before AMI or during acute phase of AMI) and remote (overreparative period of AMI) phenomenon depending on a period of onset of ischemia-reperfusion episodes. However, previous studies have revealed a reduction of infarct size and peripheral area with hibernating/stunning myocardium with both types of preconditioning due to intracardiac protection that prevents cytosolic and mitochondrial Ca^{2+} overload, accumulation of reactive oxygen species (ROS), lysosomal/nonlysosomal enzyme releasing, and inflammatory reaction [58–60].

Recurrent episodes of ischemia/reperfusion induce cardioprotective mechanisms in failed heart named postconditioning and remote conditioning [61, 62], which are supported by various comorbidities (diabetes mellitus, insulin resistance, obesity, and inflammatory conditions) [63, 64]. The cardiac protective mechanisms may include upregulation of caveolin, resolvin D1/E1, ubiquinone, long pentraxin PTX3, apelin, glucocorticoids, and long noncoding RNAs expression for IL-19, VEGF, eNO synthase, haem

oxygenase-1 (HO-1), calcitonin gene-related peptide, and peroxisome proliferator-activated receptor gamma, and downregulation of β-adrenergic signalling, G protein-coupled receptor kinase-2, and β-arrestin 1 and 2 in cardiac myocytes, fibroblasts/myofibroblasts, tissue residence cells, and circulating progenitor cells as well as mononuclears [65–68]. These factors reduce inflammatory infiltrates, stabilize cell membrane, support membrane ionic channels, and suppress the formation of key proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), IL-1β, and IL-6. Additionally, IL-19 suppresses the polarization of proinflammatory subtype M1 macrophages and triggers M2 macrophage polarization in infarct myocardium that leads to inhibition of cardiac remodelling [68].

During AMI and recurrent episodes of micronecrosis in myocardium after PCI due to remote ischemia/reperfusion damage, the important role in the regulation of cardiac remodelling belongs to alarmins, which are released by necrotic myocardium and act as a powerful trigger of inflammatory cytokine synthesis [69]. Damaged and necrotic cardiac myocytes secrete wide-spectrum factors called DAMPs (Damage-Associated Molecular Patterns), such as high-mobility group 1B protein (HMGB1), RNA, nucleotides, heat shock proteins (HSP), members of the S100 family, and IL-1a, which potentiate the inflammatory response, attenuate oxidative stress, act as direct cytotoxic agents, and induce thrombus formation and circulating blood cell aggregation [70]. Numerous molecules, such as HMGB1, S100 family members, are able to induce apoptosis of circulating endothelial progenitor cells and tissue residence cells via a multiligand receptor for advanced glycation end products-(RAGE-) mediated activation of endoplasmic reticulum stress pathway [71]. Therefore, the DAMPs and other chemokines, such as CXC and CC (predominantly CCL2, CCR2, CCR5, and ELR+CXC chemokines), recruit various subpopulations of peripheral blood cells including proinflammatory mononuclears, regulatory T-cells, nature killers, and neutrophils in the infarcted myocardium and endothelium supporting inflammatory response [72].

Inflammation is a crucial element for clearance of cellular and matrix debris, while suppression of proinflammatory signalling is necessary to transform the inflammatory phase to the proliferative phase [73]. Indeed, proinflammatory mediators include uncoupling protein 2, superoxide dismutase- (SOD-) 1 and SOD-2, ROS, through the activation of mTOR, hypoxia-induced factor- (HIF-) 1, Toll-like receptor (TLR)/IL-1, and RAGE-dependent pathways in surviving border-zone fibroblasts, cardiac myocytes, endothelial cells, smooth muscle cells, mononuclears, and several residence and progenitor cells mediating reparative processes [74–76]. It relates to the modification of cardiac fibroblasts into myofibroblasts that are enriched in α-smooth muscle actin, accumulation of extracellular matrix, neovascularization, and angiogenesis. However, the proinflammatory cytokines may have a detrimental impact on cardiac remodelling and function directly maintaining repetitive ischemia/reperfusion episodes, suppressing reparation, supporting endothelial dysfunction, coagulation, and thrombosis [77–79].

Over a 3-month period after PCI, the extracellular matrix is continually being remodelled, and tissue fibroblasts, myofibroblasts, and antigen-presenting cells become quiescent and undergo apoptosis, and cell debris is cleared by macrophages [80–82]. The regulation of proliferative response and changing of cellular phases of tissue inflammation are mediated by the renin-angiotensin-aldosterone system (RAAS) and simpatico adrenal system, which also are central players in the endogenous repair system [83, 84]. Additionally, the autonomic nervous system may play a crucial role in the inflammatory and apoptotic remodelling following AMI [85]. Thus, late adverse cardiac remodelling is a sophisticated structural and functional response of failing heart to numerous triggers (inflammation, fibrosis, cell survival signalling, and β-adrenergic signalling) and damaged factors (ischemia, reperfusion, necrosis, and apoptosis) that appear consequently and mutually activate each other.

4. Biomarkers of Adverse Cardiac Remodelling

Although there are well-developed current clinical recommendations for HF provided by the experts of the European Society of Cardiology (2016) [86] and American College of Cardiology/American Heart Association (2017) [87], there is a lack of statements for the use of biomarker strategies for diagnosis, prediction, stratification, and prevention of adverse cardiac remodelling. In fact, cardiac remodelling after AMI regardless of PCI and other approaches for revascularization is strongly associated with the development and progress of HF. In this context, early biomarkers of myocardial injury and necrosis, as well as biomarkers of biomechanical stress, neurohumoral and inflammatory activation, and fibrosis, having predictive and diagnostic evidence for acute and chronic HF, are extrapolated over strategy regarding diagnosis, outcomes, and stratification of adverse cardiac remodelling (Table 1).

There is no complete agreement between experts from the European Society of Cardiology and American College of Cardiology/American Heart Association regarding the utility of biomarkers in HF [88]. Natriuretic peptides (NPs) are recommended by both guidelines for acute and chronic HF diagnosis, prediction of HF-relating outcomes, including death, and a risk stratification. In contrast, the European Society of Cardiology (2016) HF clinical recommendation does not consist the supporting evidence regarding other biomarkers for multitask strategy in HF, and HF-guided therapy is not routinely recommended, while the HF biomarker guidance was previously approved by the American College of Cardiology/American Heart Association. Additionally, there was poor discrimination when NPs were used in patients with HF at hospital discharge, which was inferior to its performance in patients with ambulatory HF regardless of severity cardiac dysfunction and phenotypes.

However, there is a large body of evidence that other biomarkers (growth/differential factor-15, MMP-2, MMP-6, MMP-9, adipocytokines (apelin, chemerin, and visfatin), circulating endothelial and mononuclear progenitor cells, activated and apoptotic endothelial cell-derived microvesicles, miRNAs, and bone-related proteins) reflecting different

Table 1: Clinical relevance of circulating biomarkers for late adverse cardiac remodelling: overlap with HF.

Biomarkers	Heart failure				Adverse cardiac remodelling		
	Diagnosis	Outcomes	Guided therapy	Risk stratification	Diagnosis	Outcomes	Risk stratification
Currently used or recommended biomarkers							
hs-troponin T/I[♯]	-	++	–	+	+	+	+
NPs[#♯]	++	+++	+	++	++	+++	++
MR-proADM	+	+++	-	++	+	+++	++
Galectin-3[♯]	-	+	-	+	-	++	+
sST2[♯]	–	++	+	-	-	+++	+
Promising biomarkers							
Copeptin	+	++	-	+	+	++	+
GDF15	–	++	-	+	-	++	++
hs-CRP	–	+	-	-	–	+	+
IL-1β	–	+	-	+	–	+	+
IL-6	–	+	-	+	–	+	+
MMP-2	–	+	–	–	+	+	+
MMP-9	–	+	–	–	+	+	+
CTPpC-I	–	+	–	+	-	+	++
APpC-III	–	+	–	+	-	+	++
miRNAs	–	+	+	+	-	+	+

[-]Mildly disagree; [–]moderately disagree; [+]mildly agree; [++]moderately agree; [+++]strongly agree; [#]approved by the European Society of Cardiology (2016); [♯]approved by the American College of Cardiology/American Heart Association (2017). hs: high sensitive; HF: heart failure; NPs: natriuretic peptides; sST2: soluble suppression of tumorigenicity-2; MR-proADM: midregional proadrenomedullin; GDF: growth/differential factor; CRP: C-reactive protein; miRNAs: microribonucleic acids; MMP: matrix metalloproteinase; CTPpC-I: carboxytelopeptides of procollagen type I; APpC-III: aminopeptide of procollagen type III.

stages of the pathogenesis of adverse cardiac remodelling after PCI can be considered as promising tools for further strategies to improve prediction of clinical outcomes, attenuate CV risk stratification, and develop personifying strategy for treatment [89, 90]. For instance, miRNAs are speculated to have crucial roles in the nature evolution of adverse cardiac remodelling after AMI, and identification of key genes associated with damaged heart response could improve prediction models for the patients [91]. Moreover, miRNA profiling and gene cards with information about a signature of mutations involved in the regulation of the transcription factors, which mediate cardiac remodelling, appear to be promising for further precise medicine after PCI [92].

5. Biomarkers of Cardiac Injury and Necrosis

Elevated levels of high-specific cardiac troponins T (hs-TnT) and I (hs-TnI) in peripheral blood are served as diagnostic and predictive biomarkers for acute coronary syndromes and AMI [93], as well as an independent prognosticator of CV risk in the general population [94]. Cardiac troponins are structure proteins of actin-myosin complex, which are released from the cells due to necrosis or leakage from cytosol through the permeable cell membrane [95]. High-sensitivity cardiac troponin assay allows diagnosing patients with minor myocardial injury and suggesting a size of infarction [96]. Cell-free pool of cardiac troponins was reported having a tendency to decrease after AMI, while peak concentrations of both hs-TnT and hs-TnI have strongly predicted major cardiovascular events including death, recurrent MI, need of PCI, and subsequent HF hospitalization [96, 97]. Moreover, elevated concentrations of circulating cardiac troponins remain useful independent predictive biomarkers of newly post-AMI HF [98, 99]. Interestingly, elevated levels of hs-TnI were associated with CV death, whereas hs-TnT has more strongly predicted the risk of non-CV death [100]. In fact, cardiac and noncardiac surgeries mediate the elevation of troponins in the peripheral blood postprocedurally. It requires specific approach to assay an impact of transient elevation of these findings on a risk of poor prognosis. Obviously, the combined biomarkers' models are necessary.

After a prolonged period of hopes regarding improvement of diagnostic and risk stratification in STEMI patients with subsequent PCI using the combined biomarker models (cardiac troponins, NPs, copeptin, choline, soluble ST2, GDF-15, high-sensitivity C-reactive protein, galectin-3, and lipoprotein-associated phospholipase A2) [101], it has clearly become what large clinical trials need to evaluate diagnostic and predictive values of various combinations of biomarkers, because the evidence of previous studies in AMI patient treated with PCI appeared to be controversial [102, 103]. Copeptin did not add diagnostic information to peak concentration of high-sensitive troponin T in STEMI patients with subsequent PCI [104, 105]. Yet, hs-TnT/hs-TnI and NT-probrain NP (NT-proBNP) were recognized to have similar predictive values for all-cause mortality and first readmission in HFpEF [106, 107], whereas NT-proBNP was superior to cardiac troponins for the prognostication of HFrEF clinical outcomes [108, 109]. It has been noted that the predictive value of hs-TnI for HF-related clinical outcome was strongest in men with HFpEF/HFrEF than in women [108]. Other biomarkers, including soluble ST2,

high-sensitivity C-reactive protein, galectin-3, midregional proadrenomedullin, and GDF-15, in combination with hs-TnI/hs-TnT did not represent superiority in comparison with the isolated use of hs-TnI/hs-TnT in HFpEF, whereas in patients with HFmrEF/HFrEF, multimarkers' strategy was better in the prognostication of poor prognosis [110, 111].

Although previous clinical trials did not find significant interactions between stable HFpEF and HFrEF when considering the prognostic value of the NT-proBNP, cystatin-C, hs-TnT, and soluble ST2 [112–114], it can be otherwise for HF that is associated with adverse cardiac remodelling after AMI with subsequent PCI. Thus, clinical prediction models for HF-related outcomes based on various biomarkers of biomechanical stress (NT-proBNP, copeptin, midregional proadrenomedullin (MR-proADM), and growth/differential factor- (GDF-) 15), inflammation (high-sensitivity C-reactive protein), and fibrosis (galectin-3, soluble ST2) were only improved marginally by the addition of hs-TnT/hs-TnI. Moreover, hs-TnT or hs-TnI added to NT-proBNP and sST2 appears to be emerging biomarkers in the prediction of adverse outcome of HF after AMI in a short-term period [115], but whether this combination is most suitable for remote prognostication in patients with known late adverse cardiac remodelling and different phenotypes of ischemia-induced HF is not fully clear.

6. Inflammatory Biomarkers

6.1. Interleukins. IL-1β, IL-6, and angiopoietin-like protein 2 (Angptl 2) are inflammatory cytokines that influence deleterious effects on myocardium structure and function unleashing to cardiac remodelling [116]. There is strong evidence clarifying that the myocardial expression levels of IL-1β, IL-6, and Angptl 2 were significantly higher in the AMI patients than in the healthy volunteers [117]. Moreover, the levels of Angptl 2 and IL-6 rather correlated with the severity of coronary atherosclerosis than the size of the infarct area and HF presence. In contrast, IL-1β levels were associated with prior HF admissions, functional cardiac impairment, and higher NT-proBNP, sST2, and hs-TnT concentrations [115]. In fact, circulating IL-1β levels had been clinically meaningful in HF patients interfering with the predictive ability of sST2. Indeed, regardless of LVEF, HF patients with low sST2 ($\geq$35.0 ng/ml) and also low IL-1β ($\geq$49.1 pg/ml) had significantly lower risk of CV death, HF-related outcomes including readmission, than among patients with high sST2 (>35.0 ng/ml) and also high IL-1β (<49.1 pg/ml) levels [115].

6.2. Soluble Suppression of Tumorigenicity-2. Serum levels of IL-33 and soluble suppression of tumorigenicity-2 (sST2), which is the soluble form of IL-1 receptor-like 1 (IL-33), were significantly higher in HF regardless of the presence HF phenotypes associated with HF symptom severity, LV hypertrophy, and the risk of CV death and hospitalization than in healthy volunteers [118, 119]. It was found that IL-33 improved cell viability after ischemia injury through ST2 signalling and suppression nuclear factor kappa-B that unleashed the upexpression of the antiapoptotic factors (XIAP, cIAP1, surviving) and HIF-1, preventing apoptosis [120]. In patients with AMI, serum levels of sST2 were found to be increased, and after adjustment for comorbidities, the Killip class and troponin T sST2 independently predicted the excess risk of death and HF [121]. Development of adverse cardiac remodelling due to AMI was strongly associated with the elevated levels of sST2 in the peripheral blood [122].

Serum sST2 served as a predictive biomarker in patients at risk of HF and in individuals with established chronic HF [123], but the prognostic value of the biomarker was diminished after adjusting for the clinical status including comorbidity presence (abdominal obesity, diabetes mellitus, and obstructive pulmonary disease) and NT-proBNP [124–126]. Additionally, sST2 was able to be helpful in short-term clinical outcome prognostication in acute HF and actually decompensated HF patients regardless of worsening kidney function, whereas renal failure was found to be a crucial factor for the NP predictive value [127, 128]. In-patients survived after acute HF have yielded the concentrations of sST2 at discharge which were independently associated with sudden death, CV death, HF-related death, and HF readmission during the 3-month period after discharge [127, 128]. Yet, sST2 yielded strong, independent predictive value for all-cause and cardiovascular mortality, and HF hospitalization in chronic HF, and deserves consideration to be part of a multimarker panel together with NT-proBNP and hs-TnT [129]. The PARADIGM-HF trial (Prospective Comparison of ARNI With ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure) has revealed the levels of sST2 increased at 1 month which were associated with worse subsequent HF clinical outcomes, and the decreased sST2 concentrations were related to better prognosis particularly related to declined CV death and HF admission [130].

6.3. C-Reactive Protein. High-sensitive C-reactive protein (hs-CRP) has also markedly improved the risk stratification of acute HF and acutely decompensated HF patients in multibiomarker models, which predominantly included MR-proADM and NT-proBNP [131, 132]. However, circulating levels of hs-CRP were associated with the New York Heart Association functional class of HF, primary hospitalizations and readmission predominantly in patients with HFrEF, but not HFpEF [133]. Unfortunately, hs-CRP did not add incremental value to NPs, sST2, and galectin-3 in patients with HFpEF rather than HFpEF [133, 134]. The ASCEND-HF trial has reported that the levels of hs-CRP at admission in acute HF patients were not associated with acute dyspnea improvement, in-hospital death, advancing HF, short-term (30 days) and long-term (180 days) mortality, and HF readmission [135, 136]. On the contrary, at 30 days, elevated levels of hs-CRP among survivors were associated with higher 180-day mortality and readmission [135]. Although hs-CRP is under ongoing investigations, potential treatment options and goals of the therapy among HF individuals are not fully determined.

6.4. Growth Differential Factor-15. Growth differentiation factor- (GDF-) 15 is determined as an inflammation and oxidative stress biomarker, which belongs to the TGF-β cytokine superfamily and is highly expressed in myocardium and endothelial cells in CV disease including HF [137]. Previous studies have shown that GDF-15 protected the myocardium from ischemia and reperfusion injury [138, 139]. Higher serum levels of GDF-15 were associated with poor prognosis in acute HF independent from concentrations of NPs [140] and chronic HF irrespective of LVEF [141, 142]. Moreover, the Valsartan Heart Failure Trial has shown that serial measurements of GDF-15 had increased the incremental predictive power to the only measure at baseline for the severity of HF and prognosis [143]. Additionally, the elevated serum level of GDF-15 was the most prognostic biomarker in comparison to NT-proBNP, hs-CRP, and hs-TnT, in predicting long-term mortality in advanced HF [144]. Overall, a multimarker model based on NT-proBNP, hs-CRP, GDF-15, and hs-TnT had more predictable HFrEF and HFpEF than the isolating biomarker [145, 146]. Probably, inflammatory mediators, such as sST2 and GDF-15, as it is expecting, can become molecular targets not only for the diagnosis but also for the treatment of adverse cardiac remodelling in the future.

7. Biomarkers of Cardiac Fibrosis

7.1. Galectin-3. Over the last decade, galectin-3 had been widely investigated as a biomarker of fibrosis and inflammation with a promising predictive value for HF development and CV events [147]. Galectin-3 is multifunction β-galactoside-binding protein, which belongs to lectin family and is expressed in several tissues and circulating cells, such as mononuclears, macrophages, progenitor cells, mast cells, and neutrophils [148]. Galectin-3 plays a pivotal role in inflammation, fibrosis, immunity, tissue repair, and cardiac remodelling and acts as a mediator of the development and progression of the diseases, for which these pathogenetic stages are crucial [149, 150]. Indeed, galectin-3 is expressed in myocardium releasing from activated macrophages and contributes cardiac dysfunction through the remodelling of ECM and accumulation of collagen [151]. Additionally, galectin-3 is able to mediate cardiac and vascular fibrosis induced by overexpressed aldosterone [152]. However, there is evidence confirming the role of polymorphism of galectin-3 gene in susceptibility to cardiac injury and fibrosis [153]. Being a mediator of both mutual relating processes—inflammation and fibrosis—galectin-3 was approved by the Food and Drugs Administration (USA) as a predictive biomarker for HF development and progression [87, 154]. In fact, elevated levels of galectin-3 were found in patients with adverse cardiac remodelling regardless of HF phenotypes and it ethnologies [155, 156]. Therefore, galectin-3 having some advantages to NPs (more stability and resistance against hemodynamic overload and unloading state) predicted CV mortality and rehospitalization in HFrEF and HFpEF [157, 158]. Moreover, the TRIUMPH (Translational Initiative on Unique and Novel Strategies for Management of Patients with Heart Failure) has shown that repeated measures of serum levels of galectin-3 could be useful in routine clinical practice for HF prognostication and treatment monitoring [159]. However, head-to-head comparison of sST2 and galectin3 has revealed the superiority of sST2 in long-term risk stratification in an ambulatory stable HF [160]. For future direction, these facts require to be investigated in detail in large clinical trials with large sample size, because a meta-analysis of a discriminative value of galectin-3 did not yield a confirmation of previously received data [161].

7.2. Biomarkers of Collagen Turnover. It has been postulated that biomarkers of collagen turnover, such as carboxy-terminal telopeptide of collagen type I, amino-terminal propeptide of type III procollagen, MMPs, and tissue inhibitors of MMPs, may be useful for risk stratification of cardiac remodelling associated with HFpEF and HFrEF [162, 163]. Indeed, myocardial fibrosis being a major cause of diastolic dysfunction contributes predominantly to the HFpEF [164]. The ECM rearrangement corresponds to an intensity of the inflammation in myocardium, and serum levels of biomarkers of collagen turnover are mediated by a balance between degradation of ECM components and synthesis. Proliferative phase complimented to myocardial fibrosis is considered a typical response during late adverse cardiac remodelling, whereas increased degradation of ECM is suitable for AMI and early cardiac dilatation [165]. In fact, MMP-2, MMP-9, carboxytelopeptides of procollagen type I, and aminopeptide of procollagen type III had a predictive value for HFpEF that was equal NT-proBNP [163], while discriminative ability of elevated serum levels of MMP-2 was superior to NT-proBNP for early HFpEF [162, 163, 166]. Whether emerging biomarkers of ECM rearrangement and collagen turnover is essential to identify asymptomatic patients with HFpEF after AMI with subsequent PCI is not fully clear, while a loss of myocardial collagen scaffolding plays a pivotal role in adverse cardiac remodelling with poor prognosis. Interestingly, elevated levels of C-terminal telopeptide were associated with global LVEF, the risk of CV death, and newly diagnosed or worsening HF due to various causes [167–169]. In this context, integrity of ECM biomarkers into personifying predictive strategy in AMI patients appears to be promised, because multiple biomarkers' approach with traditional biomarkers and indicators of ECM turnover may have increased the sensitivity and specificity of clinical outcomes in patients with adverse cardiac remodelling and isolated diastolic dysfunction.

8. Biomarkers of Biomechanical Myocardial Stress

8.1. Natriuretic Peptides. The physiologically natriuretic peptide (NP) system mediates water and sodium homeostasis playing a pivotal role in blood pressure enhancement, fluid retention, vascular function, structure remodelling of the heart, kidney, and vessels, and maintaining differentiation and repair tissue, and supports immunity, metabolic response, and inflammation [170]. There are at least four members of NP system, such as atrial NP (ANP), brain NP (BNP), C-type of NP, and D-type of NP [171]. Biological

Table 2: CV and non-CV causes of elevating NPs in peripheral blood.

CV causes	Non-CV causes
Acute and chronic HF	Sepsis/shock
LV hypertrophy	Severe infections
Pulmonary hypertension	Critical ill patients
ACS/AMI	Acute and chronic kidney failure
Stable CAD	Severe trauma/surgery
Multifocal atherosclerosis	Chronic obstructive pulmonary disease
Cardiomyopathies	Severe bronchial asthma
Myocarditis	Pneumonia
Atrial fibrillation and flutter	Large burns and frostbite
Hypertension	Stroke
Congenital and acquired valvular heart disease	Kidney amyloidosis
Pericardial disease	Diabetes mellitus
Cardiac toxicity due to tumoricidal therapy	Thyroid dysfunction
Electrical cardioversion/ablation	Anemia
Successful resuscitation	Pleural disease

HF: heart failure; LV: left ventricular; ACS: acute coronary syndrome; AMI: acute myocardial infarction; CAD: coronary artery disease.

effects of NPs are provided through interacting with appropriate receptors: NPR-A, NPR-B, and NPR-C. Kidney effects of NPs are diuresis and wateresis due to the decreasing tubular reabsorption of sodium and water, increasing glomerular filtration rate (GFR) in result of inducing afferent arteriole vasodilation, and protection of the kidney from metabolic and ischemia injury [172]. Vascular effects of NPs correspond to vasodilation, support, capillary permeability and vascular reparation, and antiproliferative and hypocoagulative effects [173]. NPs mediate cardiac protection with respect to decreasing preload and afterload, diminishing biomechanical stress, and maintaining anti-ischemic, antiproliferative, and antiapoptotic abilities. Therefore, NPs have direct inotropic and antiarrhythmic effects [174]. Overall, the NP system is a physiological antagonist of RAAS and the sympathoadrenal system. The main triggers for synthesis and release of NPs are myocardial stretching, fluid retention, increase of pre- and postload, BP elevation, decreasing GFR, and ischemia of target organs (kidney, heart, and brain). Therefore, adipocytes and glial cells can produce NPs as a result of proinflammatory stimulation [175].

Increased activity of a circulating and local NP system was determined in patients with CV disease including LV hypertrophy, AMI, stable coronary artery disease, hypertension, and HF [176]. However, there are large numbers of causes distinguishing from CV and accompanying elevation of circulating levels of NPs (see Table 2). There is a large body of evidence showing that NP production occurs in close relation to the severity of LV systolic dysfunction, and the circulating levels of BNP and ANP strongly correspond to the New York Heart Association functional class of HF [88]. However, the production of NPs in advanced HF became blunt and irrespective of how high concentration of NPs in peripheral blood fluid retention, vasoconstriction, and cardiac dysfunction appears to progressed. In contrast, adequate treatment of HF, which is associated with improvement of clinical status and increase of tolerance to physical exercise, corresponds to declining circulating levels of BNP and ANP [177].

Therefore, patients with abdominal obesity frequently present less levels of BNP that it is expected due to increased circulating levels of neprilysin, which degradates BNP [178]. Although older age and female sex are the most common reason association with increased levels of NPs in circulation beyond relative causes, some structural abnormalities corresponding to decreased mean e' velocity and increased mitral early flow velocity/early diastolic tissue velocity ratio can be found [179–181].

Current clinical recommendations are considered NPs predominantly BNP, NT-proBNP, and NT-proANP, as diagnostic and predictive biomarkers for HF regardless of LVEF, as well as a tool for risk stratification in general population [86, 87]. However, elevated levels of NPs (BNP $\geq$ 100 pg/ml or NT – proBNP $\geq$ 300 pg/ml; or BNP $\geq$ 300 pg/ml or NT – proBNP $\geq$ 900 pg/ml if in atrial fibrillation/flutter) in patients with suspected HFmrEF/HFpEF were found to confirm the diagnosis [182]. NPs are also excellent prognostic biomarkers of adverse cardiac remodelling after AMI, whereas the clinical value of such discriminative ability is less clear than established acute and chronic HF [183]. Therefore, decreased levels of NT – proBNP < 1000 pg/ml as a result of HF therapy was associated with lower 180-day mortality and readmission in comparison with NT – proBNP $\geq$ 1000 pg/ml, whereas NT-proBNP reduction of >30% from initial levels did not improve 6-month outcomes and was not more effective than a traditional treatment [184–186]. Overall, elevated levels of NPs including NT-proBNP and NT-proANP had higher negative diagnostic value than the positive diagnostic value for HF, while the positive predictive ability of NPs in elevating concentrations was superior to the negative predictive value for asymptomatic cardiac remodelling, as well as HF regardless of LVEF. In fact, high individual variability, depending on the serum levels of NPs on comorbidities, including GFR, abdominal obesity, and older age and female sex, gives more opportunities to rule out major structural cardiac abnormalities and HF, when NP levels are normal or near normal. Confirmation of the HF and cardiac remodelling with isolating diastolic dysfunction requires more predictive information including clinical conditions, diastolic characteristics, measure of LVEF, and other biomarker assay.

8.2. Copeptin. Copeptin is a stable 39-aminoacid glycopeptide derived from C-terminal portion of the precursor of arginine vasopressin, which is a key regulator of water homeostasis and plasma osmolality [187]. Serum levels of copeptin have exhibited close linear correlation with concentrations of arginine vasopressin and are use as surrogate biomarker of its secretion [188]. There is evidence that elevated serum levels of copeptin are a diagnostic biomarker of

asymptomatic cardiac remodelling, HF, sepsis, acute kidney injury, insulin resistance, and metabolic syndrome [189]. Several trials have yielded that increased levels of copeptin were strong predictor of mortality in patients with acute and chronic HF [189, 190], stroke [191], end stage of renal disease [192], stable CAD [193], and diabetes mellitus [194]. However, there is a large number of confounding factors (hydration status, gender, blood pressure, GFR, and body mass), which make it difficult to interpret data of copeptin levels in patients with known CV disease, as well as in healthy individuals [195]. Additionally, copeptin was not better than the NPs in the diagnosis and prognosis of HF as well as in prognostication of adverse cardiac remodelling after AMI [196].

8.3. Midregional Proadrenomedullin. Midregional proadrenomedullin (MR-proADM) is stable peptide fragment that is precursor for adrenomedullin (ADM) and generated through posttranslational processing from preproadrenomedullin [197]. ADM is expressed in several tissues (adrenal medulla, brain, kidney, lung, spleen, liver, and vasculature) and cells (endothelial cells, cardiac myocytes, vascular smooth muscle cells, and epithelial cells) and mediates natriuresis, diuresis, vasodilation, positive inotropic effect, and hypotension [198].

Early clinical trials have shown that circulating levels of MR-proADM were significantly increased in patients with acute HF and STEMI [199, 200], and a cut-off value of 0.79 nmol/l has been yielded to be associated with adverse outcomes including death [201, 202]. Additionally, serum levels of MR-proADM >0.70 nmol/l were proposed to be the rule-in criteria of AMI [203].

The MR-proADM has become a biomarker that was specifically investigated as a possible prognosticator of acute HF and early outcomes in STEMI patients undergoing PCI. The BACH (Biomarkers in Acute Heart Failure) study revealed that increased serum levels of MR-proANP were a useful diagnostic biomarker as BNP for acute HF in patients with acute dyspnoe [204]. The results of the DANAMI-3 (The Danish Study of Optimal Acute Treatment of Patients with ST-segment-elevation myocardial infarction) study have shown that elevated levels of MR-proADM were strong predictor of short- and long-term mortality and hospital admission for HF after AMI [205]. Unfortunately, MR-proADM has demonstrated predictive ability with high similarity to BNP, MR-proANP, and copeptin for one-year all-cause mortality in acute HF [206]. However, the measure of MR-proADM may give additional diagnostic and prognostic information for incident CV events associated with advanced atherosclerosis that is useful for risk stratification among patients with adverse cardiac remodelling after AMI with subsequent PCI [207, 208]. Therefore, MR-proADM was able to predict major adverse cardiac events in patients suspecting AMI regardless of HF [209]. Moreover, in contrast to NPs, MR-proADM did not exhibit lowered concentration in obese patients with known HF that may facilitate diagnosis and prognosis of HF in this patient population [210].

9. Other Biomarkers of Cardiac Remodelling

9.1. Noncoding RNAs. Noncoding RNAs are powerful epigenetic regulators of cardiac gene expression and mediators of cardiac homeostasis and functions [211]. There are several types of noncoding RNAs, such as microRNAs (miRNAs), long noncoding RNAs, and circular RNAs, which play a central role in the regulation of numerous pathogenetic mechanisms and coordinate coupling of morbidity state with susceptibility to inflammatory and proliferative response [212]. Among these types of noncoding RNAs, various miRNAs are widely investigated (see Figure 4). Although there is a large body of evidence regarding up- and downregulation of genes for potassium channels, SERCA, subunits of receptors, signal molecules, proinflammatory cytokines, apoptotic mediators (Bax, caspase-9) in myocardium [213–216], and miRNAs are considered rather targets for personifying intervention and translational therapy, as well as prognosticators than diagnostic biomarkers for adverse cardiac remodelling and HF [217]. However, having signatures of miRNAs, which correspond to adverse cardiac remodelling, HF, sudden death, and cardiac abnormalities with established poor prognosis, such as concentric LV hypertrophy, fibrosis, and inflammation, it has not completely understood whether the "miRNA card" personally created for each patient will have clinical significance in the prediction of HF [33, 218].

9.2. Circulating Mononuclear and Endothelial Progenitor Cells. Mononuclears (MPCs) and endothelial progenitor cells (EPCs) are essential components of endogenous vascular repair system that is activated as a result of several triggers, such as ischemia/hypoxia, inflammation, shear stress, thrombosis, infiltration of lipids, direct injury of vasculature, and endothelium [32].

It has been hypothesized that mobilization of MPCs/EPCs and increase in growth and differentiation into mature cells in vasculature accompany acute events, including AMI and acute HF, and are associated with vascular reparation [219, 220]. However, previous acute CV events and chronic metabolic and CV diseases, such as diabetes mellitus, abdominal obesity, and hypertension, were reported to be causes of an exhausting pool of circulating angiopoetic MPCs/EPCs with immune phenotypes CD45+CD34+, CD45+CD34+CD133+, and CD45+CD34+CD133+CD184+ [221]. Consequently, advanced HF and progression of AMI-induced adverse cardiac remodelling were related to impaired vascular repair, vascularization, and angiogenesis due to a declined number of circulating precursors and lowered their function and survival [222]. This phenomenon is known as progenitor cell dysfunction and considered a promising predictive biomarker for CV mortality and HF progression and admission [223, 224], as well as in patient population with AMI submitted to PCI [225]. Probably, coronary circulating proangiogenic MPCs/EPCs collected from coronary sinus in AMI patients with subsequent PCI can become a powerful biomarker with increased accuracy in the prediction of adverse cardiac remodelling. However, the number and functionality of proangiogenic circulating precursors appear as promising biomarkers for the

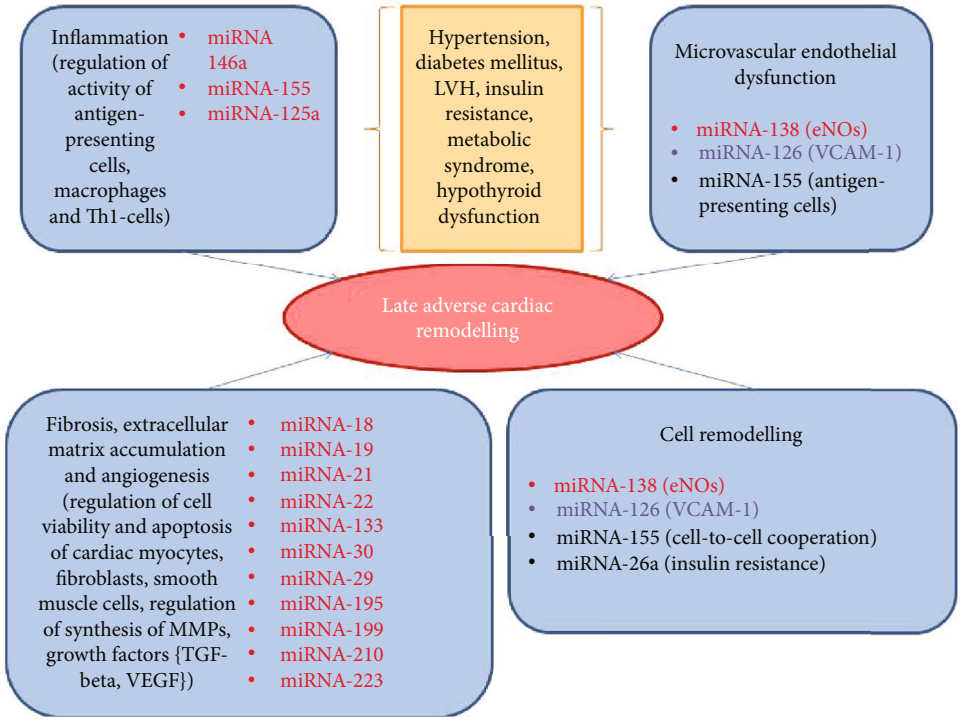

Figure 4: The role of miRNAs in the pathogenesis of late adverse cardiac remodelling in AMI. VEGF: vascular endothelial growth factor; TGF: transforming growth factor; NO: nitric oxide; eNOs: endothelial NO synthase; MMP: matrix metalloproteinase; VCAM: vascular adhesive molecule.

prediction of cardiac remodelling and HF development. Large clinical trials are required to clearly understand the role of new biomarkers in the diagnostic and predictive strategies among AMI patients with PCI.

9.3. Future Perspectives. There are numerous biomarkers, which were investigated as candidates for risk stratification and prognosis in AMI patients with PCI, such as activated and apoptotic endothelial cell-derived microvesicles, bone-related proteins (osteopontin, osteoprotegerin, and osteonectin), adipokines, gastrointestinal hormones, apelin, cardiotrophin-1, defensin-1 and defensin-2, macrophage inhibitory cytokine-1, circular RNAs, and gene card. Although the data received appear to be promising, there is no clear understanding whether diagnostic and predictive abilities of these biomarkers will be better than the conventional biomarkers of biomechanical stress, inflammation, fibrosis, and cardiac injury.

10. Conclusions

Circulating biomarkers are a promising tool to stratify AMI patients undergoing PCI at high risk of poor cardiac recovery and HF development. NPs are traditionally recommended as diagnostic and predictive biomarkers for acute HF and chronic HF regardless of LVEF, whereas sST2, galectin-3, and cardiac troponins can be used optionally. Previous clinical studies have yielded that multimarker models, which were based on the combination of biomarkers of several pathological axes involved in the nature evolution of adverse cardiac remodelling (biomechanical myocardial stress, necrosis and injury of cardiac myocytes, and inflammation), have provided incremental prognostic information for prediction of CV death or HF in AMI patients with subsequent PCI. Future clinical trials with larger sample sizes are required to elucidate the role of personifying biomarker-based strategy for diagnostic, prediction, and treatment among patients suspecting adverse cardiac remodelling and HF.

Abbreviations

ACE: Angiotensin-converting enzyme
ADAMTS: A Disintegrin and Metalloproteinase with Thrombospondin motifs
ADM: adrenomedullin
AMI: Acute myocardial infarction
Apaf-1: Adaptor protein apoptotic protease activating factor 1
ARBs: Angiotensin-II receptor antagonists
Bcl-2: B-cell lymphoma 2
BES: Biolimus eluting stent
BMS: Bare metal stent
CRP: C-reactive protein
CV: Cardiovascular
DAMPs: Damage-Associated Molecular Patterns
DAPT: Dual antiplatelet therapy
DNA: Deoxyribonucleic acid
ECM: Extracellular matrix
EF: Ejection fraction
GDF15: Growth/differential factor-15

H_2O_2: Hydrogen peroxide
HF: Heart failure
HFmrEF: Heart failure with midrange ejection fraction
HFpEF: Heart failure with preserved ejection fraction
HFrEF: Heart failure with reduced ejection fraction
HIF: Hypoxia-induce factor
HMGB1: High-mobility group 1B protein
HO-1: Haem oxygenase-1
HSP: Heat shock proteins
IC: Intracoronary
IL: Interleukin
IVUS-VH: Intravascular ultrasound virtual-histology
LncRNA: Long noncoding RNA
LV: Left ventricle
MAPK: Mitogen-activated protein kinase
MCRA: Mineralocorticoid receptor antagonists
miRNA: MicroRNA
MMP: Matrix metalloproteinase
NO: Nitric oxide
NPs: Natriuretic peptides
OCT: Optical coherence tomography
PCI: Percutaneous coronary intervention
RAAS: Renin-angiotensin-aldosterone system
RNA: Ribonucleic acid
ROS: Reactive oxygen species
SOD: Superoxide dismutase
sST2: Soluble suppression of tumorigenicity-2
STEMI: ST-segment elevation myocardial infarction
TIMI score: Thrombolysis in Myocardial Infarction score
TGF: Transforming growth factor
TLR: Toll-like receptor
TNF: Tumor necrosis factor
TSP: Thrombospondin
VEGF: Vascular endothelial growth factor.

Data Availability

This is a narrative review, so dataset was not created.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

[1] H. E. Carter, D. Schofield, and R. Shrestha, "Productivity costs of cardiovascular disease mortality across disease types and socioeconomic groups," *Open Heart*, vol. 6, no. 1, article e000939, 2019.

[2] J. Yap, S. Y. Chia, F. Y. Lim et al., "The Singapore Heart Failure Risk Score: prediction of survival in Southeast Asian patients," *Annals of the Academy of Medicine of Singapore*, vol. 48, no. 3, pp. 86–94, 2019.

[3] X. Chen, G. Savarese, U. Dahlström, L. H. Lund, and M. Fu, "Age-dependent differences in clinical phenotype and prognosis in heart failure with mid-range ejection compared with heart failure with reduced or preserved ejection fraction," *Clinical Research in Cardiology*, vol. 108, no. 12, pp. 1394–1405, 2019, [Epub ahead of print].

[4] P. M. Seferović, M. Polovina, J. Bauersachs et al., "Heart failure in cardiomyopathies: a position paper from the Heart Failure Association of the European Society of Cardiology," *European Journal of Heart Failure*, vol. 21, no. 5, pp. 553–576, 2019.

[5] K. Dharmarajan and M. W. Rich, "Epidemiology, pathophysiology, and prognosis of heart failure in older adults," *Heart Failure Clinics*, vol. 13, no. 3, pp. 417–426, 2017.

[6] A. Slee, M. Saad, and S. Saksena, "Heart failure progression and mortality in atrial fibrillation patients with preserved or reduced left ventricular ejection fraction," *Journal of Interventional Cardiac Electrophysiology*, vol. 55, no. 3, pp. 325–331, 2019, [Epub ahead of print].

[7] Y. Sato, A. Yoshihisa, M. Oikawa et al., "Prognostic Impact of Worsening Renal Function in Hospitalized Heart Failure Patients With Preserved Ejection Fraction: A Report From the JASPER Registry," *Journal of Cardiac Failure*, vol. 25, no. 8, pp. 631–642, 2019, [Epub ahead of print].

[8] M. Nakamura and J. Sadoshima, "Cardiomyopathy in obesity, insulin resistance and diabetes," *The Journal of Physiology*, 2019, [Epub ahead of print].

[9] L. Shen, P. S. Jhund, K. F. Docherty et al., "Prior pacemaker implantation and clinical outcomes in patients with heart failure and preserved ejection fraction," *JACC: Heart Failure*, vol. 7, no. 5, pp. 418–427, 2019, [Epub ahead of print].

[10] H. Seligman, M. J. Shun-Shin, A. Vasireddy et al., "Fractional flow reserve derived from microcatheters versus standard pressure wires: a stenosis-level meta-analysis," *Open Heart*, vol. 6, no. 1, article e000971, 2019.

[11] K. V. Patel, R. Mauricio, J. L. Grodin et al., "Identifying a low-flow phenotype in heart failure with preserved ejection fraction: a secondary analysis of the RELAX trial," *ESC Heart Failure*, vol. 6, no. 4, pp. 613–620, 2019.

[12] G. V. Halade, V. Kain, B. Tourki, and J. K. Jadapalli, "Lipoxygenase drives lipidomic and metabolic reprogramming in ischemic heart failure," *Metabolism*, vol. 96, pp. 22–32, 2019, [Epub ahead of print].

[13] J. P. Tsai, K. T. Sung, C. H. Su et al., "Diagnostic accuracy of left atrial remodelling and natriuretic peptide levels for preclinical heart failure," *ESC Heart Failure*, vol. 6, no. 4, pp. 723–732, 2019, [Epub ahead of print].

[14] A. Burlacu, P. Simion, I. Nistor, A. Covic, and G. Tinica, "Novel percutaneous interventional therapies in heart failure with preserved ejection fraction: an integrative review," *Heart Failure Reviews*, vol. 24, no. 5, pp. 793–803, 2019, [Epub ahead of print].

[15] H. Zeng and J. X. Chen, "Microvascular rarefaction and heart failure with preserved ejection fraction," *Frontiers in Cardiovascular Medicine*, vol. 6, 2019.

[16] R. Scarsini, G. L. de Maria, A. Borlotti et al., "Incremental value of coronary microcirculation resistive reserve ratio in predicting the extent of myocardial infarction in patients with STEMI. Insights from the Oxford Acute Myocardial Infarction (OxAMI) study," *Cardiovascular Revascularization Medicine*, vol. 20, no. 12, pp. 1148–1155, 2019, [Epub ahead of print].

[17] B. Ky, B. French, A. May Khan et al., "Ventricular-Arterial Coupling, Remodeling, and Prognosis in Chronic Heart Failure," *Journal of the American College of Cardiology*, vol. 62, no. 13, pp. 1165–1172, 2013.

[18] B. He, L. Gai, J. Gai et al., "Correlation between major adverse cardiac events and coronary plaque characteristics,"

Experimental & Clinical Cardiology, vol. 18, no. 2, pp. e71–e76, 2013.

[19] A. S. Bhatt, A. P. Ambrosy, and E. J. Velazquez, "Adverse remodeling and reverse remodeling after myocardial infarction," *Current Cardiology Reports*, vol. 19, no. 8, 2017.

[20] A. S. Blom, J. J. Pilla, R. C. Gorman III et al., "Infarct size reduction and attenuation of global left ventricular remodeling with the CorCap cardiac support device following acute myocardial infarction in sheep," *Heart Failure Reviews*, vol. 10, no. 2, pp. 125–139, 2005.

[21] A. W. Schoenenberger, P. Jamshidi, R. Kobza et al., "Progression of coronary artery disease during long-term follow-up of the Swiss Interventional Study on Silent Ischemia Type II (SWISSI II)," *Clinical Cardiology*, vol. 33, no. 5, pp. 289–295, 2010.

[22] P. Erne, A. W. Schoenenberger, D. Burckhardt et al., "Effects of percutaneous coronary interventions in silent ischemia after myocardial infarction: the SWISSI II randomized controlled trial," *JAMA*, vol. 297, no. 18, pp. 1985–1991, 2007.

[23] G. A. Sgueglia, F. D'Errico, G. Gioffrè et al., "Angiographic and clinical performance of polymer-free biolimus-eluting stent in patients with ST-segment elevation acute myocardial infarction in a metropolitan public hospital: the BESAMI MUCHO study," *Catheterization and Cardiovascular Interventions*, vol. 91, no. 5, pp. 851–858, 2018.

[24] C. R. Jones, S. J. Khandhar, M. Ramratnam et al., "Identification of intrastent pathology associated with late stent thrombosis using optical coherence tomography," *Journal of Interventional Cardiology*, vol. 28, no. 5, pp. 439–448, 2015.

[25] A. Al Mamary, G. Dariol, and M. Napodano, "Late stent fracture – A potential role of left ventricular dilatation," *Journal of the Saudi Heart Association*, vol. 26, no. 3, pp. 162–165, 2014.

[26] S. V. Rao, U. Zeymer, P. S. Douglas et al., "A randomized, double-blind, placebo-controlled trial to evaluate the safety and effectiveness of intracoronary application of a novel bioabsorbable cardiac matrix for the prevention of ventricular remodeling after large ST- segment elevation myocardial infarction: Rationale and design of the PRESERVATION I trial," *American Heart Journal*, vol. 170, no. 5, pp. 929–937, 2015, Epub 2015 Aug 24.

[27] S. V. Rao, U. Zeymer, P. S. Douglas et al., "Bioabsorbable intracoronary matrix for prevention of ventricular remodeling after myocardial infarction," *Journal of the American College of Cardiology*, vol. 68, no. 7, pp. 715–723, 2016.

[28] T. Mizoguchi, T. Sawada, T. Shinke et al., "Detailed comparison of intra-stent conditions 12 months after implantation of everolimus-eluting stents in patients with ST-segment elevation myocardial infarction or stable angina pectoris," *International Journal of Cardiology*, vol. 171, no. 2, pp. 224–230, 2014, Epub 2013 Dec 21.

[29] S. H. Rezkalla, R. V. Stankowski, J. Hanna, and R. A. Kloner, "Management of no-reflow phenomenon in the catheterization laboratory," *JACC: Cardiovascular Interventions*, vol. 10, no. 3, pp. 215–223, 2017.

[30] J. Barrabes, "Comments on the 2015 ESC Guidelines for the Management of Acute Coronary Syndromes in Patients Presenting Without Persistent ST-segment Elevation," *Revista Española de Cardiología (English Edition)*, vol. 68, no. 12, pp. 1061–1067, 2015.

[31] T. K. Steigen, C. E. Buller, G. B. John Mancini et al., "Myocardial perfusion grade after late infarct artery recanalization is associated with global and regional left ventricular function at one year: analysis from the Total Occlusion Study of Canada-2," *Circulation: Cardiovascular Interventions*, vol. 3, no. 6, pp. 549–555, 2010.

[32] A. E. Berezin, "Endogenous vascular repair system in cardiovascular disease: the role of endothelial progenitor cells," *Australasian Medical Journal*, vol. 12, no. 2, pp. 42–48, 2019.

[33] A. Berezin, "Epigenetics in heart failure phenotypes," *BBA Clinical*, vol. 6, pp. 31–37, 2016.

[34] A. Celik, N. Kalay, H. Korkmaz et al., "Short-term left ventricular remodeling after revascularization in subacute total and subtotal occlusion with the infarct-related left anterior descending artery," *Cardiology Research*, vol. 2, no. 5, pp. 229–235, 2011.

[35] M. E. Pfusterer, P. Buser, S. Osswald, P. Weiss, J. Bremerich, and F. Burkart, "Time dependence of left ventricular recovery after delayed recanalization of an occluded infarct-related coronary artery: findings of a pilot study," *Journal of the American College of Cardiology*, vol. 32, no. 1, pp. 97–102, 1998.

[36] N. Reifart, "Challenges in complicated coronary chronic total occlusion recanalisation," *Interventional Cardiology Review*, vol. 8, no. 2, pp. 107–111, 2013.

[37] N. G. Frangogiannis, "Pathophysiology of myocardial infarction," *Comprehensive Physiology*, vol. 20, pp. 1841–1875, 2015.

[38] M. Neri, I. Riezzo, N. Pascale, C. Pomara, and E. Turillazzi, "Ischemia/reperfusion injury following acute myocardial infarction: a critical issue for clinicians and forensic pathologists," *Mediators Inflamm*, vol. 2017, article 7018393, 14 pages, 2017, Epub 2017 Feb 13.

[39] D. Y. Fuhrman and J. A. Kellum, "Remote ischemic preconditioning in the PICU: a simple concept with a complex past," *Pediatric Critical Care Medicine*, vol. 17, no. 8, pp. e371–e379, 2016.

[40] S. Hernandez-Resendiz, K. Chinda, S. B. Ong, H. Cabrera-Fuentes, C. Zazueta, and D. Hausenloy, "The role of redox dysregulation in the inflammatory response to acute myocardial ischaemia-reperfusion injury - adding fuel to the fire," *Current Medicinal Chemistry*, vol. 25, no. 11, pp. 1275–1293, 2018.

[41] K. Przyklenk and P. Whittaker, "Remote ischemic preconditioning: current knowledge, unresolved questions, and future priorities," *Journal of Cardiovascular Pharmacology and Therapeutics*, vol. 16, no. 3-4, pp. 255–259, 2016.

[42] D. M. Yellon and D. J. Hausenloy, "Myocardial reperfusion injury," *The New England Journal of Medicine*, vol. 357, no. 11, pp. 1121–1135, 2007.

[43] P. Pagliaro, F. Moro, F. Tullio, M.-G. Perrelli, and C. Penna, "Cardioprotective pathways during reperfusion: focus on redox signaling and other modalities of cell Signaling," *Antioxidants & Redox Signaling*, vol. 14, no. 5, pp. 833–850, 2011.

[44] B. Long, N. Li, X. X. Xu et al., "Long noncoding RNA FTX regulates cardiomyocyte apoptosis by targeting miR-29b-1-5p and Bcl2l2," *Biochemical and Biophysical Research Communications*, vol. 495, no. 1, pp. 312–318, 2018.

[45] T. Kalogeris, Y. Bao, and R. J. Korthuis, "Mitochondrial reactive oxygen species: a double edged sword in ischemia/reperfusion vs preconditioning," *Redox Biology*, vol. 2, no. 1, pp. 702–714, 2014.

[46] N. Zhang, X. Meng, L. Mei, J. Hu, C. Zhao, and W. Chen, "The long non-coding RNA SNHG1 attenuates cell apoptosis by regulating miR-195 and BCL2-like protein 2 in human cardiomyocytes," *Cellular Physiology and Biochemistry*, vol. 50, no. 3, pp. 1029–1040, 2018.

[47] F. He, H. Liu, J. Guo et al., "Inhibition of microRNA-124 reduces cardiomyocyte apoptosis following myocardial infarction via targeting STAT3," *Cellular Physiology and Biochemistry*, vol. 51, no. 1, pp. 186–200, 2018.

[48] C. Sun, H. Liu, J. Guo et al., "MicroRNA-98 negatively regulates myocardial infarction-induced apoptosis by down-regulating Fas and caspase-3," *Scientific Reports*, vol. 7, no. 1, p. 7460, 2017.

[49] T. Okamoto, T. Akaike, T. Sawa, Y. Miyamoto, A. Van der Vliet, and H. Maeda, "Activation of matrix metalloproteinases by peroxynitrite-induced protein S-glutathiolation via disulfide S-oxide formation," *The Journal of Biological Chemistry*, vol. 276, no. 31, pp. 29596–29602, 2001.

[50] P.-Y. Cheung, G. Sawicki, M. Wozniak, W. Wang, M. W. Radomski, and R. Schulz, "Matrix metalloproteinase-2 contributes to ischemia-reperfusion injury in the heart," *Circulation*, vol. 101, no. 15, pp. 1833–1839, 2000.

[51] A. E. Berezin and T. A. Samura, "Prognostic value of biological markers in myocardial infarction patients," *Asian Cardiovascular and Thoracic Annals*, vol. 21, no. 2, pp. 142–150, 2013.

[52] C. Fernandez-Patron, M. W. Radomski, and S. T. Davidge, "Vascular matrix metalloproteinase-2 cleaves big endothelin-1 yielding a novel vasoconstrictor," *Circulation Research*, vol. 85, no. 10, pp. 906–911, 1999.

[53] D. Edwards, M. Handsley, and C. Pennington, "The ADAM metalloproteinases," *Molecular Aspects of Medicine*, vol. 29, no. 5, pp. 258–289, 2008.

[54] O. V. Petyunina, M. P. Kopytsya, and A. E. Berezin, "Biomarker-based prognostication of adverse cardiac remodeling after STEMI: the role of single nucleotide polymorphism T786C in endothelial NO-synthase gene," *Journal of Cardiology and Therapy*, vol. 6, no. 1, pp. 768–774, 2019.

[55] S. Cardin, M. P. Scott-Boyer, S. Praktiknjo et al., "Differences in cell-type-specific responses to angiotensin II explain cardiac remodeling differences in C57BL/6 mouse substrains," *Hypertension*, vol. 64, no. 5, pp. 1040–1046, 2014.

[56] Y. Li, X._. He, C. Li, L. Gong, and M. Liu, "Identification of candidate genes and microRNAs for acute myocardial infarction by weighted gene coexpression network analysis," *BioMed Research International*, vol. 2019, Article ID 5742608, 11 pages, 2019.

[57] L. M. Buja, "Myocardial ischemia and reperfusion injury," *Cardiovascular Pathology*, vol. 14, no. 4, pp. 170–175, 2005.

[58] C. P. Baines, "How and when do myocytes die during ischemia and reperfusion: the late phase," *Journal of Cardiovascular Pharmacology and Therapeutics*, vol. 16, no. 3-4, pp. 239–243, 2016.

[59] Z. Zhao, "Oxidative stress-elicited myocardial apoptosis during reperfusion," *Current Opinion in Pharmacology*, vol. 4, no. 2, pp. 159–165, 2004.

[60] A. Prasad, G. W. Stone, D. R. Holmes, and B. Gersh, "Reperfusion injury, microvascular dysfunction, and cardioprotection: the 'dark side' of reperfusion," *Circulation*, vol. 120, no. 21, pp. 2105–2112, 2009.

[61] P. Ferdinandy, D. J. Hausenloy, G. Heusch, G. F. Baxter, and R. Schulz, "Interaction of risk factors, comorbidities, and comedications with ischemia/reperfusion injury and cardioprotection by preconditioning, postconditioning, and remote conditioning," *Pharmacological Reviews*, vol. 66, no. 4, pp. 1142–1174, 2014.

[62] R. S. Vander Heide and C. Steenbergen, "Cardioprotection and Myocardial Reperfusion," *Circulation Research*, vol. 113, no. 4, pp. 464–477, 2013.

[63] K. McCafferty, S. Forbes, C. Thiemermann, and M. M. Yaqoob, "The challenge of translating ischemic conditioning from animal models to humans: the role of comorbidities," *Disease Models & Mechanisms*, vol. 7, no. 12, pp. 1321–1333, 2014.

[64] A. Bonaventura, F. Montecucco, F. Dallegri et al., "Novel findings in neutrophil biology and their impact on cardiovascular disease," *Cardiovascular Research*, vol. 115, no. 8, pp. 1266–1285, 2019, [Epub ahead of print].

[65] M. A. Razzaque, X. Xu, M. Han, A. Badami, and S. A. Akhter, "Inhibition of postinfarction ventricular remodeling by high molecular weight polyethylene glycol," *Journal of Surgical Research*, vol. 232, pp. 171–178, 2018.

[66] W. An, Y. Yu, Y. Zhang, Z. Zhang, Y. Yu, and X. Zhao, "Exogenous IL-19 attenuates acute ischaemic injury and improves survival in male mice with myocardial infarction," *British Journal of Pharmacology*, vol. 176, no. 5, pp. 699–710, 2019.

[67] M. Arita, T. Ohira, Y.-P. Sun, S. Elangovan, N. Chiang, and C. N. Serhan, "Resolvin E1 selectively interacts with leukotriene B4Receptor BLT1 and ChemR23 to regulate inflammation," *The Journal of Immunology*, vol. 178, no. 6, pp. 3912–3917, 2007.

[68] V. Kain, K. A. Ingle, R. A. Colas et al., "Resolvin D1 activates the inflammation resolving response at splenic and ventricular site following myocardial infarction leading to improved ventricular function," *Journal of Molecular and Cellular Cardiology*, vol. 84, pp. 24–35, 2015.

[69] L. A. Grisanti, T. P. Thomas, R. L. Carter et al., "Pepducin-mediated cardioprotection via β-arrestin-biased β2-adrenergic receptor-specific signaling," *Theranostics*, vol. 8, no. 17, pp. 4664–4678, 2018.

[70] Z. Guo, N. Liu, L. Chen, X. Zhao, and M. R. Li, "Independent roles of CGRP in cardioprotection and hemodynamic regulation in ischemic postconditioning," *European Journal of Pharmacology*, vol. 828, pp. 18–25, 2018.

[71] Q. Huang, Z. Yang, J. P. Zhou, and Y. Luo, "HMGB1 induces endothelial progenitor cells apoptosis via RAGE-dependent PERK/eIF2α pathway," *Molecular and Cellular Biochemistry*, vol. 431, no. 1-2, pp. 67–74, 2017, Epub 2017 Mar 1.

[72] S. Frankenreiter, P. Bednarczyk, A. Kniess et al., "cGMP-elevating compounds and ischemic conditioning provide cardioprotection against ischemia and reperfusion injury via cardiomyocyte-specific BK channels," *Circulation*, vol. 136, no. 24, pp. 2337–2355, 2017.

[73] S. Raffa, X. L. D. Chin, R. Stanzione et al., "The reduction of NDUFC2 expression is associated with mitochondrial impairment in circulating mononuclear cells of patients with acute coronary syndrome," *International Journal of Cardiology*, vol. 286, pp. 127–133, 2019.

[74] J. Wang, M. Liu, Q. Wu et al., "Human embryonic stem cell-derived cardiovascular progenitors repair infarcted hearts through modulation of MacrophagesviaActivation of signal transducer and activator of transcription 6," *Antioxidants &*

Redox Signaling, vol. 31, no. 5, pp. 369–386, 2019, [Epub ahead of print].

[75] Z. Zhong, J. Hou, Q. Zhang et al., "Differential expression of circulating long non-coding RNAs in patients with acute myocardial infarction," *Medicine*, vol. 97, no. 51, article e13066, 2018.

[76] Z. A. Ibrahim, C. L. Armour, S. Phipps, and M. B. Sukkar, "RAGE and TLRs: relatives, friends or neighbours?," *Molecular Immunology*, vol. 56, no. 4, pp. 739–744, 2013.

[77] J. J. De Haan, M. B. Smeets, G. Pasterkamp, and F. Arslan, "Danger signals in the initiation of the inflammatory response after myocardial infarction," *Mediators of Inflammation*, vol. 2013, Article ID 206039, 13 pages, 2013.

[78] A. Micera, B. O. Balzamino, A. D. Zazzo, F. Biamonte, G. Sica, and S. Bonini, "Toll-like receptors and tissue remodeling: the pro/cons recent findings," *Journal of Cellular Physiology*, vol. 231, no. 3, pp. 531–544, 2016.

[79] K. Fujiu, J. Wang, and R. Nagai, "Cardioprotective function of cardiac macrophages," *Cardiovascular Research*, vol. 102, no. 2, pp. 232–239, 2014.

[80] B. Tourki and G. Halade, "Leukocyte diversity in resolving and nonresolving mechanisms of cardiac remodeling," *The FASEB Journal*, vol. 31, no. 10, pp. 4226–4239, 2017.

[81] O. Dewald, P. Zymek, K. Winkelmann et al., "CCL2/monocyte chemoattractant protein-1 regulates inflammatory responses critical to healing myocardial infarcts," *Circ. Res.*, vol. 96, no. 8, pp. 881–889, 2005.

[82] P. M. Henson, D. L. Bratton, and V. A. Fadok, "Apoptotic cell removal," *Current Biology*, vol. 11, no. 19, pp. R795–R805, 2001.

[83] S. Iravanian and S. C. Dudley Jr., "The renin-angiotensin-aldosterone system (RAAS) and cardiac arrhythmias," *Heart Rhythm*, vol. 5, no. 6, pp. S12–S17, 2008.

[84] M. Massa, V. Rosti, M. Ferrario et al., "Increased circulating hematopoietic and endothelial progenitor cells in the early phase of acute myocardial infarction," *Blood*, vol. 105, no. 1, pp. 199–206, 2005.

[85] C. Gao, K. Howard-Quijano, C. Rau et al., "Inflammatory and apoptotic remodeling in autonomic nervous system following myocardial infarction," *PLoS One*, vol. 12, no. 5, article e0177750, 2017.

[86] P. Ponikowski, A. A. Voors, S. D. Anker et al., "2016 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure: the task force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC," *European Journal of Heart Failure*, vol. 18, no. 8, pp. 891–975, 2016.

[87] C. W. Yancy, M. Jessup, B. Bozkurt et al., "2017 ACC/AHA/HFSA focused update of the 2013 ACCF/AHA Guideline for the management of heart failure: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society of America," *Circulation*, vol. 136, no. 6, pp. e137–e161, 2017.

[88] B. Bozkurt, "What is new in heart failure management in 2017? Update on ACC/AHA Heart Failure Guidelines," *Current Cardiology Reports*, vol. 20, no. 6, 2018.

[89] A. E. Berezin, "Circulating biomarkers in heart failure," *Advances in Experimental Medicine and Biology*, vol. 1067, pp. 89–108, 2018.

[90] A. E. Berezin, "Prognostication in different heart failure phenotypes: the role of circulating biomarkers," *Journal of Circulating Biomarkers*, vol. 5, no. 6, p. 6, 2016.

[91] M. Cheng, S. An, and J. Li, "Identifying key genes associated with acute myocardial infarction," *Medicine*, vol. 96, no. 42, article e7741, 2017.

[92] Y. Cao, R. Li, Y. Li et al., "Identification of transcription factor-gene regulatory network in acute myocardial infarction," *Heart, Lung and Circulation*, vol. 26, no. 4, pp. 343–353, 2017, Epub 2016 Jul 26.

[93] B. Ibanez, S. James, S. Agewall et al., "2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: the Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC)," *European Heart Journal*, vol. 39, no. 2, pp. 119–177, 2018.

[94] M. N. Lyngbakken, H. Røsjø, O. L. Holmen, H. Dalen, K. Hveem, and T. Omland, "Temporal changes in cardiac troponin I are associated with risk of cardiovascular events in the general population: the Nord-Trøndelag Health Study," *Clinical Chemistry*, vol. 65, no. 7, pp. 871–881, 2019, [Epub ahead of print].

[95] D. Soetkamp, K. Raedschelders, M. Mastali, K. Sobhani, C. N. Bairey Merz, and J. Van Eyk, "The continuing evolution of cardiac troponin I biomarker analysis: from protein to proteoform," *Expert Review of Proteomics*, vol. 14, no. 11, pp. 973–986, 2017.

[96] A. S. Shah, A. Anand, Y. Sandoval et al., "High-sensitivity cardiac troponin I at presentation in patients with suspected acute coronary syndrome: a cohort study," *Lancet*, vol. 386, no. 10012, pp. 2481–2488, 2015.

[97] D. Stelzle, A. S. V. Shah, A. Anand et al., "High-sensitivity cardiac troponin I and risk of heart failure in patients with suspected acute coronary syndrome: a cohort study," *European Heart Journal - Quality of Care and Clinical Outcomes*, vol. 4, no. 1, pp. 36–42, 2018.

[98] A. E. Berezin, "Circulating biomarkers in heart failure: diagnostic and prognostic importance," *Journal of Laboratory and Precision Medicine*, vol. 3, article 36, 2018.

[99] X. Jia, W. Sun, R. C. Hoogeveen et al., "High-Sensitivity Troponin I and Incident Coronary Events, Stroke, Heart Failure Hospitalization, and Mortality in the ARIC Study," *Circulation*, vol. 139, no. 23, pp. 2642–2653, 2019.

[100] P. Welsh, D. Preiss, C. Hayward et al., "Cardiac troponin T and troponin I in the general Population," *Circulation*, vol. 139, no. 24, pp. 2754–2764, 2019, [Epub ahead of print].

[101] T. Raskovalova, R. Twerenbold, P. O. Collinson et al., "Diagnostic accuracy of combined cardiac troponin and copeptin assessment for early rule-out of myocardial infarction: a systematic review and meta-analysis," *European Heart Journal: Acute Cardiovascular Care*, vol. 3, no. 1, pp. 18–27, 2014.

[102] J. Searle, O. Danne, C. Müller, and M. Mockel, "Biomarkers in acute coronary syndrome and percutaneous coronary intervention," *Minerva Cardioangiologica*, vol. 59, no. 3, pp. 203–223, 2011.

[103] C. Mueller, "Biomarkers and acute coronary syndromes: an update," *European Heart Journal*, vol. 35, no. 9, pp. 552–556, 2014, Epub 2013 Dec 18.

[104] M. Möckel and J. Searle, "Copeptin-marker of acute myocardial infarction," *Current Atherosclerosis Reports*, vol. 16, no. 7, p. 421, 2014.

[105] M. Karakas, J. L. Januzzi Jr., J. Meyer et al., "Copeptin does not add diagnostic information to high-sensitivity troponin T in low- to intermediate-risk patients with acute chest pain: results from the rule out myocardial infarction by computed tomography (ROMICAT) study," *Clinical Chemistry*, vol. 57, no. 8, pp. 1137–1145, 2011.

[106] S. Suzuki, H. Motoki, M. Minamisawa et al., "Prognostic significance of high-sensitivity cardiac troponin in patients with heart failure with preserved ejection fraction," *Heart Vessels*, vol. 34, no. 10, pp. 1650–1656, 2019, [Epub ahead of print].

[107] R. A. de Boer, M. Nayor, C. R. deFilippi et al., "Association of cardiovascular biomarkers with incident heart failure with preserved and reduced ejection fraction," *JAMA Cardiology*, vol. 3, no. 3, pp. 215–224, 2018.

[108] A. Gohar, J. P. C. Chong, O. W. Liew et al., "The prognostic value of highly sensitive cardiac troponin assays for adverse events in men and women with stable heart failure and a preserved vs. reduced ejection fraction," *European Journal of Heart Failure*, vol. 19, no. 12, pp. 1638–1647, 2017, Epub 2017 Aug 28.

[109] R. Santhanakrishnan, J. P. C. Chong, T. P. Ng et al., "Growth differentiation factor 15, ST2, high-sensitivity troponin T, and N-terminal pro brain natriuretic peptide in heart failure with preserved vs. reduced ejection fraction," *European Journal of Heart Failure*, vol. 14, no. 12, pp. 1338–1347, 2012.

[110] P. Moliner, J. Lupón, J. Barallat et al., "Bio-profiling and bio-prognostication of chronic heart failure with mid-range ejection fraction," *International Journal of Cardiology*, vol. 257, pp. 188–192, 2018.

[111] P. Welsh, L. Kou, C. Yu et al., "Prognostic importance of emerging cardiac, inflammatory, and renal biomarkers in chronic heart failure patients with reduced ejection fraction and anaemia: RED-HF study," *European Journal of Heart Failure*, vol. 20, no. 2, pp. 268–277, 2018.

[112] S. Sanders-van Wijk, V. van Empel, N. Davarzani et al., "Circulating biomarkers of distinct pathophysiological pathways in heart failure with preserved vs. reduced left ventricular ejection fraction," *European Journal of Heart Failure*, vol. 17, no. 10, pp. 1006–1014, 2015.

[113] S. L. Seliger, J. de Lemos, I. J. Neeland et al., "Older Adults, "Malignant" Left Ventricular Hypertrophy, and Associated Cardiac-Specific Biomarker Phenotypes to Identify the Differential Risk of New-Onset Reduced Versus Preserved Ejection Fraction Heart Failure: CHS (Cardiovascular Health Study)," *JACC: Heart Failure*, vol. 3, no. 6, pp. 445–455, 2015, Epub 2015 May 14.

[114] C. Sinning, T. Kempf, M. Schwarzl et al., "Biomarkers for characterization of heart failure – Distinction of heart failure with preserved and reduced ejection fraction," *International Journal of Cardiology*, vol. 227, pp. 272–277, 2017.

[115] D. A. Pascual-Figal, S. Manzano-Fernández, M. Boronat et al., "Soluble ST2, high-sensitivity troponin T- and N-terminal pro-B-type natriuretic peptide: complementary role for risk stratification in acutely decompensated heart failure," *European Journal of Heart Failure*, vol. 13, no. 7, pp. 718–725, 2011.

[116] D. A. Pascual-Figal, A. Bayes-Genis, M. C. Asensio-Lopez et al., "The interleukin-1 axis and risk of death in patients with acutely decompensated heart failure," *Journal of the American College of Cardiology*, vol. 73, no. 9, pp. 1016–1025, 2019.

[117] Y. Cao, R. Li, F. Zhang, Z. Guo, S. Tuo, and Y. Li, "Correlation between angiopoietin-like proteins in inflammatory mediators in peripheral blood and severity of coronary arterial lesion in patients with acute myocardial infarction," *Experimental and Therapeutic Medicine*, vol. 17, no. 5, pp. 3495–3500, 2019.

[118] E. O. Weinberg, M. Shimpo, S. Hurwitz, S. Tominaga, J. L. Rouleau, and R. T. Lee, "Identification of serum soluble ST2 receptor as a novel heart failure biomarker," *Circulation*, vol. 107, no. 5, pp. 721–726, 2003.

[119] J. L. Januzzi, W. F. Peacock, A. S. Maisel et al., "Measurement of the Interleukin Family Member ST2 in Patients With Acute Dyspnea: Results From the PRIDE (Pro-Brain Natriuretic Peptide Investigation of Dyspnea in the Emergency Department) Study," *Journal of the American College of Cardiology*, vol. 50, no. 7, pp. 607–613, 2007.

[120] K. Seki, S. Sanada, A. Y. Kudinova et al., "Interleukin-33 prevents apoptosis and improves survival after experimental myocardial infarction through ST2 signaling," *Circulation: Heart Failure*, vol. 2, no. 6, pp. 684–691, 2009.

[121] W. S. Jenkins, V. L. Roger, A. S. Jaffe et al., "Prognostic value of soluble ST2 after myocardial infarction: a community perspective," *The American Journal of Medicine*, vol. 130, no. 9, pp. 1112.e9–1112.e15, 2017, Epub 2017 Mar 23.

[122] R. A. P. Weir, A. M. Miller, G. E. J. Murphy et al., "Serum soluble ST2: a potential novel mediator in left ventricular and infarct remodeling after acute myocardial infarction," *Journal of the American College of Cardiology*, vol. 55, no. 3, pp. 243–250, 2010.

[123] W. H. W. Tang, Y. Wu, J. L. Grodin et al., "Prognostic value of baseline and changes in circulating soluble ST2 levels and the effects of nesiritide in acute decompensated heart failure," *JACC: Heart Failure*, vol. 4, no. 1, pp. 68–77, 2016.

[124] S. Manzano-Fernández, J. L. Januzzi, F. J. Pastor-Pérez et al., "Serial monitoring of soluble interleukin family member ST2 in patients with acutely decompensated heart failure," *Cardiology*, vol. 122, no. 3, pp. 158–166, 2012.

[125] T. Mueller, A. Gegenhuber, W. Poelz, and M. Haltmayer, "Diagnostic accuracy of B type natriuretic peptide and amino terminal proBNP in the emergency diagnosis of heart failure," *Heart*, vol. 91, no. 5, pp. 606–612, 2005.

[126] A. E. Berezin, "Biomarkers for cardiovascular risk in patients with diabetes," *Heart*, vol. 102, no. 24, pp. 1939–1941, 2016.

[127] M. S. Kim, T. D. Jeong, S. B. Han, W. K. Min, and J. J. Kim, "Role of soluble ST2 as a prognostic marker in patients with acute heart failure and renal insufficiency," *Journal of Korean Medical Science*, vol. 30, no. 5, pp. 569–575, 2015.

[128] D. A. Pascual-Figal, J. Ordoñez-Llanos, P. L. Tornel et al., "Soluble ST2 for predicting sudden cardiac death in patients with chronic heart failure and left ventricular systolic dysfunction," *Journal of the American College of Cardiology*, vol. 54, no. 23, pp. 2174–2179, 2009.

[129] M. Emdin, A. Aimo, G. Vergaro et al., "sST2 Predicts Outcome in Chronic Heart Failure Beyond NT–proBNP and High- Sensitivity Troponin T," *Journal of the American College of Cardiology*, vol. 72, no. 19, pp. 2309–2320, 2018.

[130] E. O'Meara, M. F. Prescott, B. Claggett et al., "Independent prognostic value of serum soluble ST2 measurements in patients with heart failure and a reduced ejection fraction in

the PARADIGM-HF trial (Prospective Comparison of ARNI With ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure)," *Circulation: Heart Failure*, vol. 11, no. 5, article e004446, 2018.

[131] J. Lassus, E. Gayat, C. Mueller et al., "Incremental value of biomarkers to clinical variables for mortality prediction in acutely decompensated heart failure: the Multinational Observational Cohort on Acute Heart Failure (MOCA) study," *International Journal of Cardiology*, vol. 168, no. 3, pp. 2186–2194, 2013.

[132] W. P. Huang, X. Zheng, L. He, X. Su, C. W. Liu, and M. X. Wu, "Role of soluble ST2 levels and beta-blockers dosage on cardiovascular events of patients with unselected ST-segment elevation myocardial infarction," *Chinese Medical Journal*, vol. 131, no. 11, pp. 1282–1288, 2018.

[133] Y. Michowitz, Y. Arbel, D. Wexler et al., "Predictive value of high sensitivity CRP in patients with diastolic heart failure," *International Journal of Cardiology*, vol. 125, no. 3, pp. 347–351, 2008.

[134] J. P. Araújo, P. Lourenço, A. Azevedo et al., "Prognostic value of high-sensitivity C-reactive protein in heart failure: a systematic review," *Journal of Cardiac Failure*, vol. 15, no. 3, pp. 256–266, 2009.

[135] A. P. Kalogeropoulos, W. H. W. Tang, A. Hsu et al., "High-sensitivity C-reactive protein in acute heart failure: insights from the ASCEND-HF trial," *Journal of Cardiac Failure*, vol. 20, no. 5, pp. 319–326, 2014.

[136] K. Huynh, B. Van Tassell, and S. L. Chow, "Predicting therapeutic response in patients with heart failure: the story of C-reactive protein," *Expert Review of Cardiovascular Therapy*, vol. 13, no. 2, pp. 153–161, 2015, Epub 2015 Jan 12.

[137] K. C. Wollert, T. Kempf, and L. Wallentin, "Growth differentiation factor 15 as a biomarker in cardiovascular disease," *Clinical Chemistry*, vol. 63, no. 1, pp. 140–151, 2017.

[138] T. Kempf, M. Eden, J. Strelau et al., "The transforming growth factor-beta superfamily member growth-differentiation factor-15 protects the heart from ischemia/reperfusion injury," *Circulation Research*, vol. 98, no. 3, pp. 351–360, 2006.

[139] A. Rohatgi, P. Patel, S. R. Das et al., "Association of growth differentiation factor-15 with coronary atherosclerosis and mortality in a young, multiethnic population: observations from the Dallas Heart Study," *Clinical Chemistry*, vol. 58, no. 1, pp. 172–182, 2012.

[140] P. Bettencourt, J. Ferreira-Coimbra, P. Rodrigues et al., "Towards a multi-marker prognostic strategy in acute heart failure: a role for GDF-15," *ESC Heart Failure*, vol. 5, no. 6, pp. 1017–1022, 2018.

[141] T. Kempf, S. von Haehling, T. Peter et al., "Prognostic utility of growth differentiation factor-15 in patients with chronic heart failure," *Journal of the American College of Cardiology*, vol. 50, no. 11, pp. 1054–1060, 2007.

[142] M. M. Y. Chan, R. Santhanakrishnan, J. P. C. Chong et al., "Growth differentiation factor 15 in heart failure with preserved vs. reduced ejection fraction," *European Journal of Heart Failure*, vol. 18, no. 1, pp. 81–88, 2016.

[143] I. S. Anand, T. Kempf, T. S. Rector et al., "Serial measurement of growth-differentiation factor-15 in heart failure: relation to disease severity and prognosis in the Valsartan Heart Failure Trial," *Circulation*, vol. 122, no. 14, pp. 1387–1395, 2010.

[144] D. J. Lok, I. J. T. Klip, S. I. Lok et al., "Incremental Prognostic Power of Novel Biomarkers (Growth-Differentiation Factor-15, High-Sensitivity C-Reactive Protein, Galectin-3, and High- Sensitivity Troponin-T) in Patients With Advanced Chronic Heart Failure," *The American Journal of Cardiology*, vol. 112, no. 6, pp. 831–837, 2013.

[145] B. G. Demissei, G. Cotter, M. F. Prescott et al., "A multimarker multi-time point-based risk stratification strategy in acute heart failure: results from the RELAX-AHF trial," *European Journal of Heart Failure*, vol. 19, no. 8, pp. 1001–1010, 2017.

[146] J. Li, Y. Cui, A. Huang et al., "Additional diagnostic value of growth differentiation factor-15 (GDF-15) to N-terminal B-type natriuretic peptide (NT-proBNP) in patients with different stages of heart failure," *Medical Science Monitor*, vol. 24, pp. 4992–4999, 2018.

[147] L. B. Daniels and Q. M. Bui, "Should a high Gal-3 have us scared stiff?," *Journal of the American College of Cardiology*, vol. 73, no. 18, pp. 2296–2298, 2019.

[148] J. Dumic, S. Dabelic, and M. Flogel, "Galectin-3: an open-ended story," *Biochim Biophys Acta*, vol. 1760, no. 4, pp. 616–635, 2006.

[149] H. Sano, D. K. Hsu, J. R. Apgar et al., "Critical role of galectin-3 in phagocytosis by macrophages," *Journal of Clinical Investigation*, vol. 112, no. 3, pp. 389–397, 2003.

[150] A. Krzeslak and A. Lipinska, "Galectin-3 as a multifunctional protein," *Cellular & Molecular Biology Letters*, vol. 9, no. 2, pp. 305–328, 2004.

[151] U. C. Sharma, S. Pokharel, T. J. van Brakel et al., "Galectin-3 marks activated macrophages in failure-prone hypertrophied hearts and contributes to cardiac dysfunction," *Circulation*, vol. 110, no. 19, pp. 3121–3128, 2004.

[152] L. Calvier, M. Miana, P. Reboul et al., "Galectin-3 mediates aldosterone-induced vascular fibrosis," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 33, no. 1, pp. 67–75, 2013.

[153] L. Yu, W. P. T. Ruifrok, M. Meissner et al., "Genetic and pharmacological inhibition of galectin-3 prevents cardiac remodeling by interfering with myocardial fibrogenesis," *Circulation: Heart Failure*, vol. 6, no. 1, pp. 107–117, 2013.

[154] R. A. de Boer, A. A. Voors, P. Muntendam, W. H. van Gilst, and D. J. van Veldhuisen, "Galectin-3: a novel mediator of heart failure development and progression," *European Journal of Heart Failure*, vol. 11, no. 9, pp. 811–817, 2009.

[155] K. Karatolios, G. Chatzis, V. Holzendorf et al., "Galectin-3 as a Predictor of Left Ventricular Reverse Remodeling in Recent- Onset Dilated Cardiomyopathy," *Disease Markers*, vol. 2018, Article ID 2958219, 7 pages, 2018.

[156] R. A. de Boer, L. Yu, and D. J. van Veldhuisen, "Galectin-3 in cardiac remodeling and heart failure," *Current Heart Failure Reports*, vol. 7, no. 1, pp. 1–8, 2010.

[157] R. A. de Boer, D. J. A. Lok, T. Jaarsma et al., "Predictive value of plasma galectin-3 levels in heart failure with reduced and preserved ejection fraction," *Annals of Medicine*, vol. 43, no. 1, pp. 60–68, 2010.

[158] D. J. Lok, S. I. Lok, P. W. Bruggink-André de la Porte et al., "Galectin-3 is an independent marker for ventricular remodeling and mortality in patients with chronic heart failure," *Clinical Research in Cardiology*, vol. 102, no. 2, pp. 103–110, 2013.

[159] L. C. van Vark, I. Lesman-Leegte, S. J. Baart et al., "Prognostic value of serial galectin-3 measurements in patients with acute

heart failure," *Journal of the American Heart Association*, vol. 6, no. 12, article e003700, 2017.

[160] A. Bayes-Genis, M. de Antonio, J. Vila et al., "Head-to-head comparison of 2 myocardial fibrosis biomarkers for long-term heart failure risk stratification: ST2 versus galectin-3," *Journal of the American College of Cardiology*, vol. 63, no. 2, pp. 158–166, 2014, Epub 2013 Sep 24.

[161] A. Chen, W. Hou, Y. Zhang, Y. Chen, and B. He, "Prognostic value of serum galectin-3 in patients with heart failure: A meta- analysis," *International Journal of Cardiology*, vol. 182, pp. 168–170, 2015.

[162] J. Meluzin, J. Tomandl, H. Podrouzkova et al., "Can markers of collagen turnover or other biomarkers contribute to the diagnostics of heart failure with normal left ventricular ejection fraction?," *Biomedical Papers*, vol. 157, no. 4, pp. 331–339, 2013, Epub 2013 Feb 14.

[163] R. Martos, J. Baugh, M. Ledwidge et al., "Diagnosis of heart failure with preserved ejection fraction: improved accuracy with the use of markers of collagen turnover," *European Journal of Heart Failure*, vol. 11, no. 2, pp. 191–197, 2009.

[164] F. H. Rutten, M. J. Cramer, and W. J. Paulus, "Heart failure with preserved ejection fraction: diastolic heart failure," *Nederlands Tijdschrift voor Geneeskunde*, vol. 156, no. 45, p. A5315, 2012.

[165] S. de Denus, J. Lavoie, A. Ducharme et al., "Differences in biomarkers in patients with heart failure with a reduced vs a preserved left ventricular ejection fraction," *Canadian Journal of Cardiology*, vol. 28, no. 1, pp. 62–68, 2012.

[166] N. Maharaj, B. K. Khandheria, E. Libhaber et al., "Relationship between left ventricular twist and circulating biomarkers of collagen turnover in hypertensive patients with heart failure," *Journal of the American Society of Echocardiography*, vol. 27, no. 10, pp. 1064–1071, 2014.

[167] T. A. Zelniker, P. Jarolim, B. M. Scirica et al., "Biomarker of collagen turnover (C-terminal telopeptide) and prognosis in patients with non-ST-elevation acute coronary syndromes," *Journal of the American Heart Association*, vol. 8, no. 9, article e011444, 2019.

[168] K. Nagao, T. Inada, A. Tamura et al., "Circulating markers of collagen types I, III, and IV in patients with dilated cardiomyopathy: relationships with myocardial collagen expression," *ESC Heart Failure*, vol. 5, no. 6, pp. 1044–1051, 2018.

[169] A. M. Dupuy, N. Kuster, C. Curinier et al., "Exploring collagen remodeling and regulation as prognosis biomarkers in stable heart failure," *Clinica Chimica Acta*, vol. 490, pp. 167–171, 2019.

[170] M. Volpe, M. Carnovali, and V. Mastromarino, "The natriuretic peptides system in the pathophysiology of heart failure: from molecular basis to treatment," *Clinical Science*, vol. 130, no. 2, pp. 57–77, 2016.

[171] M. Volpe, S. Rubattu, and J. Burnett, "Natriuretic peptides in cardiovascular diseases: current use and perspectives," *European Heart Journal*, vol. 35, no. 7, pp. 419–425, 2014.

[172] R. Kerkelä, J. Ulvila, and J. Magga, "Natriuretic peptides in the regulation of cardiovascular physiology and metabolic events," *Journal of the American Heart Association*, vol. 4, no. 10, article e002423, 2015.

[173] C. Calvieri, S. Rubattu, and M. Volpe, "Molecular mechanisms underlying cardiac antihypertrophic and antifibrotic effects of natriuretic peptides," *Journal of Molecular Medicine*, vol. 90, no. 1, pp. 5–13, 2012.

[174] D. K. Gupta and T. J. Wang, "Natriuretic peptides and cardiometabolic health," *Circulation Journal*, vol. 79, no. 8, pp. 1647–1655, 2015.

[175] L. R. Potter, "Natriuretic peptide metabolism, clearance and degradation," *FEBS Journal*, vol. 278, no. 11, pp. 1808–1817, 2011.

[176] R. D'Alessandro, D. Masarone, A. Buono et al., "Natriuretic peptides: molecular biology, pathophysiology and clinical implications for the cardiologist," *Future Cardiology*, vol. 9, no. 4, pp. 519–534, 2013.

[177] Z. Cao, Y. Jia, and B. Zhu, "BNP and NT-proBNP as diagnostic biomarkers for cardiac dysfunction in both clinical and forensic medicine," *International Journal of Molecular Sciences*, vol. 20, no. 8, p. 1820, 2019.

[178] M. A. Alpert, C. J. Lavie, H. Agrawal, K. B. Aggarwal, and S. A. Kumar, "Obesity and heart failure: epidemiology, pathophysiology, clinical manifestations, and management," *Translational Research*, vol. 164, no. 4, pp. 345–356, 2014.

[179] E. H. Nah, S. Y. Kim, S. Cho, S. Kim, and H. I. Cho, "Plasma NT-proBNP levels associated with cardiac structural abnormalities in asymptomatic health examinees with preserved ejection fraction: a retrospective cross-sectional study," *BMJ Open*, vol. 9, no. 4, article e026030, 2019.

[180] U. L. Faxén, L. H. Lund, N. Orsini et al., "N-terminal pro-B-type natriuretic peptide in chronic heart failure: the impact of sex across the ejection fraction spectrum," *International Journal of Cardiology*, vol. 287, pp. 66–72, 2019, Epub 2019 Apr 11.

[181] F. S. Gaborit, C. Kistorp, T. Kümler et al., "Prevalence of early stages of heart failure in an elderly risk population: the Copenhagen Heart Failure Risk Study," *Open Heart*, vol. 6, no. 1, article e000840, 2019.

[182] Z. J. Han, X. D. Wu, J. J. Cheng et al., "Diagnostic accuracy of natriuretic peptides for heart failure in patients with pleural effusion: a systematic review and updated meta-analysis," *PLoS One*, vol. 10, no. 8, article e0134376, 2015.

[183] H.-P. B.-L. Rocca and S. S.-v. Wijk, "Natriuretic peptides in chronic heart failure," *Cardiac Failure Review*, vol. 5, no. 1, pp. 44–49, 2019.

[184] P. L. Myhre, M. Vaduganathan, B. Claggett et al., "B-type natriuretic peptide during treatment with sacubitril/valsartan: the PARADIGM-HF Trial," *Journal of the American College of Cardiology*, vol. 73, no. 11, pp. 1264–1272, 2019.

[185] S. Stienen, K. Salah, A. H. Moons et al., "NT-proBNP (N-terminal pro-B-type natriuretic peptide)-guided therapy in acute decompensated heart failure: PRIMA II randomized controlled trial (can NT-proBNP-guided therapy during hospital admission for acute decompensated heart failure reduce mortality and readmissions?)," *Circulation*, vol. 137, no. 16, pp. 1671–1683, 2018, Epub 2017 Dec 14.

[186] G. M. Felker, K. J. Anstrom, K. F. Adams et al., "Effect of natriuretic peptide-guided therapy on hospitalization or cardiovascular mortality in high-risk patients with heart failure and reduced ejection fraction: a randomized clinical trial," *JAMA*, vol. 318, no. 8, pp. 713–720, 2017.

[187] N. G. Morgenthaler, J. Struck, C. Alonso, and A. Bergmann, "Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin," *Clinical Chemistry*, vol. 52, no. 1, pp. 112–119, 2006.

[188] L. Dobša and K. C. Edozien, "Copeptin and its potential role in diagnosis and prognosis of various diseases," *Biochemia Medica*, vol. 23, no. 2, pp. 172–192, 2013.

[189] D. Bolignano, A. Cabassi, E. Fiaccadori et al., "Copeptin (CTproAVP), a new tool for understanding the role of vasopressin in pathophysiology," *Clinical Chemistry and Laboratory Medicine (CCLM)*, vol. 52, no. 10, pp. 1447–1456, 2014.

[190] N. G. Morgenthaler, "Copeptin: a biomarker of cardiovascular and renal function," *Congestive Heart Failure*, vol. 16, Suppl 1, pp. S37–S44, 2010.

[191] W. J. Tu, G. Z. Ma, Y. Ni et al., "Copeptin and NT-proBNP for prediction of all-cause and cardiovascular death in ischemic stroke," *Neurology*, vol. 88, no. 20, pp. 1899–1905, 2017.

[192] W. Fenske, C. Wanner, B. Allolio et al., "Copeptin levels associate with cardiovascular events in patients with ESRD and type 2 diabetes mellitus," *Journal of the American Society of Nephrology*, vol. 22, no. 4, pp. 782–790, 2011.

[193] I. Tasevska, S. Enhorning, M. Persson, P. M. Nilsson, and O. Melander, "Copeptin predicts coronary artery disease cardiovascular and total mortality," *Heart*, vol. 102, no. 2, pp. 127–132, 2016.

[194] S. S. Bhandari, I. Loke, J. E. Davies, I. B. Squire, J. Struck, and L. L. Ng, "Gender and renal function influence plasma levels of copeptin in healthy individuals," *Clinical Science*, vol. 116, no. 3, pp. 257–263, 2009.

[195] G. Velho, S. Ragot, R. el Boustany et al., "Plasma copeptin, kidney disease, and risk for cardiovascular morbidity and mortality in two cohorts of type 2 diabetes," *Cardiovascular Diabetology*, vol. 17, no. 1, p. 110, 2018.

[196] A. E. Berezin, "Up-to-date clinical approaches of biomarkers' use in heart failure," *Biomedical Research and Therapy*, vol. 4, no. 6, pp. 1344–1370, 2017.

[197] K. Kitamura, K. Kangawa, and T. Eto, "Adrenomedullin and PAMP: discovery, structures, and cardiovascular functions," *Microscopy Research and Technique*, vol. 57, no. 1, pp. 3–13, 2002.

[198] T. Nishikimi and Y. Nakagawa, "Adrenomedullin as a biomarker of heart failure," *Heart Failure Clinics*, vol. 14, no. 1, pp. 49–55, 2018, Epub 2017 Oct 7.

[199] K. Kobayashi, K. Kitamura, N. Hirayama et al., "Increased plasma adrenomedullin in acute myocardial infarction," *American Heart Journal*, vol. 131, no. 4, pp. 676–680, 1996.

[200] Y. Miyao, T. Nishikimi, Y. Goto et al., "Increased plasma adrenomedullin levels in patients with acute myocardial infarction in proportion to the clinical severity," *Heart*, vol. 79, no. 1, pp. 39–44, 1998.

[201] O. S. Dhillon, S. Q. Khan, H. K. Narayan et al., "Prognostic Value of Mid-Regional Pro-Adrenomedullin Levels Taken on Admission and Discharge in Non–ST-Elevation Myocardial Infarction: The LAMP (Leicester Acute Myocardial Infarction Peptide) II Study," *Journal of the American College of Cardiology*, vol. 56, no. 2, pp. 125–133, 2010.

[202] I. T. Klip, A. A. Voors, S. D. Anker et al., "Prognostic value of mid-regional pro-adrenomedullin in patients with heart failure after an acute myocardial infarction," *Heart*, vol. 97, no. 11, pp. 892–898, 2011.

[203] S. Q. Khan, R. J. O'Brien, J. Struck et al., "Prognostic value of midregional pro-adrenomedullin in patients with acute myocardial infarction: the LAMP (Leicester Acute Myocardial Infarction Peptide) study," *Journal of the American College of Cardiology*, vol. 49, no. 14, pp. 1525–1532, 2007.

[204] A. Maisel, C. Mueller, R. Nowak et al., "Mid-region prohormone markers for diagnosis and prognosis in acute dyspnea: results from the BACH (Biomarkers in Acute Heart Failure) trial," *Journal of the American College of Cardiology*, vol. 55, no. 19, pp. 2062–2076, 2010.

[205] A. C. Falkentoft, R. Rørth, K. Iversen et al., "MR-proADM as a prognostic marker in patients with ST-segment-elevation myocardial infarction-DANAMI-3 (a Danish Study of Optimal Acute Treatment of Patients With STEMI) Substudy," *Journal of the American Heart Association*, vol. 7, no. 11, article e008123, 2018.

[206] A. Gegenhuber, J. Struck, B. Dieplinger et al., "Comparative evaluation of B-type natriuretic peptide, mid-regional pro-A-type natriuretic peptide, mid-regional pro-adrenomedullin, and copeptin to predict 1-year mortality in patients with acute destabilized heart failure," *Journal of Cardiac Failure*, vol. 13, no. 1, pp. 42–49, 2007.

[207] O. Melander, C. Newton-Cheh, P. Almgren et al., "Novel and conventional biomarkers for prediction of incident cardiovascular events in the community," *JAMA*, vol. 302, no. 1, pp. 49–57, 2009.

[208] J. T. Neumann, S. Tzikas, A. Funke-Kaiser et al., "Association of MR-proadrenomedullin with cardiovascular risk factors and subclinical cardiovascular disease," *Atherosclerosis*, vol. 228, no. 2, pp. 451–459, 2013.

[209] K. S. Shah, N. A. Marston, C. Mueller et al., "Midregional proadrenomedullin predicts mortality and major adverse cardiac events in patients presenting with chest pain: results from the CHOPIN trial," *Academic Emergency Medicine*, vol. 22, no. 5, pp. 554–563, 2015.

[210] C. Sinning, F. Ojeda, P. S. Wild et al., "Midregional proadrenomedullin and growth differentiation factor-15 are not influenced by obesity in heart failure patients," *Clinical Research in Cardiology*, vol. 106, no. 6, pp. 401–410, 2017.

[211] S. Dangwal, K. Schimmel, A. Foinquinos, K. Xiao, and T. Thum, "Noncoding RNAs in heart failure," in *Heart Failure*, vol. 243 of Handbook of Experimental Pharmacology, pp. 423–445, Springer, Cham, 2017.

[212] E. L. Vegter, P. van der Meer, L. J. de Windt, Y. M. Pinto, and A. A. Voors, "MicroRNAs in heart failure: from biomarker to target for therapy," *European Journal of Heart Failure*, vol. 18, no. 5, pp. 457–468, 2016, Epub 2016 Feb 11.

[213] W. Li, M. Liu, C. Zhao et al., "MiR-1/133 attenuates cardiomyocyte apoptosis and electrical remodeling in mice with viral myocarditis," *Cardiology Journal*, 2013, [Epub ahead of print].

[214] A. Jodati, S. M. Pirouzpanah, N. Fathi Maroufi et al., "Different expression of Micro RNA-126, 133a and 145 in aorta and saphenous vein samples of patients undergoing coronary artery bypass graft surgery," *Journal of Cardiovascular and Thoracic Research*, vol. 11, no. 1, pp. 43–47, 2019.

[215] Y. Xiao, J. Zhao, J. P. Tuazon, C. V. Borlongan, and G. Yu, "MicroRNA-133a and myocardial infarction," *Cell Transplantation*, vol. 28, no. 7, pp. 831–838, 2019, [Epub ahead of print].

[216] J. Song, Q. Xie, L. Wang et al., "The TIR/BB-loop mimetic AS-1 prevents Ang II-induced hypertensive cardiac hypertrophy via NF-κB dependent downregulation of miRNA-143," *Scientific Reports*, vol. 9, no. 1, p. 6354, 2019.

[217] P. Shah, M. R. Bristow, and J. D. Port, "MicroRNAs in heart failure cardiac transplantation, and myocardial recovery:

biomarkers with therapeutic potential," *Current Heart Failure Reports*, vol. 14, no. 6, pp. 454–464, 2017.

[218] R. Verjans, W. J. A. Derks, K. Korn et al., "Functional screening identifies microRNAs as multi-cellular regulators of heart failure," *Scientific Reports*, vol. 9, no. 1, p. 6055, 2019.

[219] A. Samman Tahhan, M. Hammadah, M. Raad et al., "Progenitor cells and clinical outcomes in patients with acute coronary syndromes," *Circulation Research*, vol. 122, no. 11, pp. 1565–1575, 2018.

[220] E. Shantsila, B. J. Wrigley, A. Shantsila, L. D. Tapp, P. S. Gill, and G. Y. H. Lip, "Monocyte-derived and CD34+/KDR+ endothelial progenitor cells in heart failure," *Journal of Thrombosis and Haemostasis*, vol. 10, no. 7, pp. 1252–1261, 2012.

[221] E. Nollet, V. Y. Hoymans, I. R. Rodrigus et al., "Bone marrow-derived progenitor cells are functionally impaired in ischemic heart disease," *Journal of Cardiovascular Translational Research*, vol. 9, no. 4, pp. 266–278, 2016.

[222] A. E. Berezin and A. A. Kremzer, "Circulating endothelial progenitor cells as markers for severity of ischemic chronic heart failure," *Journal of Cardiac Failure*, vol. 20, no. 6, pp. 438–447, 2014.

[223] A. E. Berezin, A. A. Kremzer, Y. V. Martovitskaya et al., "The utility of biomarker risk prediction score in patients with chronic heart failure," *International Journal of Clinical and Experimental Medicine*, vol. 8, no. 10, pp. 18255–18264, 2015.

[224] A. E. Berezin, A. A. Kremzer, T. A. Samura et al., "Predictive value of apoptotic microparticles to mononuclear progenitor cells ratio in advanced chronic heart failure patients," *Journal of Cardiology*, vol. 65, no. 5, pp. 403–411, 2015.

[225] J. A. Suárez-Cuenca, R. Robledo-Nolasco, M. A. Alcántara-Meléndez et al., "Coronary circulating mononuclear progenitor cells and soluble biomarkers in the cardiovascular prognosis after coronary angioplasty," *Journal of Cellular and Molecular Medicine*, vol. 23, no. 7, 2019.

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Research Article

Short-Term Prognosis Value of sST2 for an Unfavorable Outcome in Hypertensive Patients

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Background. sST2 represents a useful biomarker for the diagnosis and prognosis of patients with heart failure, but limited data is available on its role in patients with hypertension. The aim of this study is to evaluate the short-term prognosis value of sST2 for an unfavorable outcome in hypertensive patients. *Methods*. This was a prospective observational study which enrolled 80 patients with hypertension, who were followed for one year. All patients underwent clinical, laboratory (including sST2), and echocardiographic assessment at baseline. The patients were grouped according to the cardiovascular (CV) events reported during the follow-up: group A (with CV events) and group B (without CV events). *Results*. Overall, 59 CV events were reported during the follow-up period. Compared to group B, the patients in group A had significantly higher sST2 levels, a higher number of CV risk factors, and a higher left ventricle mass. Except for the diastolic dysfunction parameters, the echocardiographic findings were similar in the two groups. Patients in group A had a lower E/A ratio, larger deceleration time, and increased telediastolic pressure as quantified by the E/E' ratio than those in group B. Multivariate logistic regression analysis showed that sST2 and fasting plasma glucose at baseline were independent predictors for the CV events reported during the follow-up period. sST2 levels > 28.5 ng/mL were associated with poor clinical outcomes ($p = 0.006$, Kaplan-Meier analysis). *Conclusions*. sST2 levels were correlated with the risk of adverse CV outcomes in hypertensive patients and may represent a useful prognostic marker in these patients.

1. Introduction

The receptor of suppression of tumorigenicity 2 (ST2) (also known as IL-1R4, DER4, Fit-1, or T1) is a type 1 transmembrane protein encoded by the *IL-1RL1* gene, and the symbol for ST2 is approved by the Human Gene Nomenclature Database [1]. The gene is located on chromosome 2.12. The protein product of the ST2 gene encodes three isoforms identified in human tissues: a released soluble form (sST2, acting as a decoy receptor for IL-33, inhibiting IL-33/ST2L signalling) which can be detected in human serum, a transmembrane receptor (ST2L or ST2) discovered to be IL-33 in 2005, and a variant of ST2 (ST2V) [2].

Serum levels of sST2 are increased in conditions of ventricular biomechanical overload such as acute myocardial infarction (AMI) and proved to be useful in predicting mortality and heart failure (HF) in these patients [3]. Moreover, sST2 levels predict an outcome in patients with HF, and a variation of sST2 concentration over time is associated with prognosis [4]. Several other studies showed a correlation

between levels of sST2 and the severity of HF, the left ventricular (LV) systolic function associated with valvular diseases [5], renal impairment, and other biomarkers of cardiac dysfunction such as B-type natriuretic peptide and C-reactive protein [6, 7].

Such data suggest that measurement of serum levels of sST2 may provide insight into the hemodynamic burden of the myocardium and might be useful for the early detection of cardiac disease, both systolic and diastolic. Taking these into consideration, investigating sST2 in patients with high blood pressure (HBP) was a logical stepwise approach as HBP affects the myocardium leading to left ventricular hypertrophy (LVH), left ventricular diastolic dysfunction (LVDD), and heart failure with preserved ejection fraction (HFpEF) [8, 9]. Actually, hypertension is the most important risk factor for HFpEF, with 75% of HFpEF patients being hypertensive [10]. The diagnosis of different pathogenic stages leading to HFpEF in hypertensive patients is based on ECG, which has low sensitivity but high specificity. Echocardiography, on the other hand, even though it is a sensitive and specific tool, lacks great accessibility, requires qualified personnel, and has low accuracy in obese or respiratory patients [9]. In the light of these findings, the identification of biomarkers useful for the early diagnosis and for prognosis of LVH, LVDD, and HFpEF in hypertensive patients is imperative and the data regarding this subject are scarce.

Thus, the present study was aimed at analyzing the relationship between serum levels of sST2 and the presence of LVH and LVDD in hypertensive patients, assessing at the same time the potential short-term prognosis value off sST2 for cardiovascular (CV) events in these patients.

2. Patients and Methods

2.1. Study Population. 80 hypertensive patients (mean age 54.7 ± 13.5 years; 47.5% men and 52.5% women) were enrolled in a prospective observational study and followed for 1 year. The diagnosis of HBP was established according to the recommendations of the ESH/ESC guidelines [11]: SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg or if patients received treatment for HBP. Patients with chronic inflammatory or acute infectious diseases, heart disease (ischemic, congenital, and valvular heart disease, myocarditis, severe acute or chronic heart failure, and acute coronary syndrome), or pulmonary disease (COPD, asthma, and sleep apnea) were excluded. Clinical examination was performed, and demographic data, risk factors, and previous medical history were collected for all patients. Upon enrolment, all patients signed an informed consent and respected national and international legislation regarding clinical studies [12, 13].

2.2. Study Protocol. At baseline, the patients were examined clinically and fasting blood was collected by venous puncture, in the morning, after a rest of 5-10 minutes. Serum was obtained by centrifuging the coagulated blood for 15 minutes at $1000 \times g$ and stored at -20°C until ST2 measurement, performed with the soluble ST2/IL-1R4 (human) and ELISA Tecan Sunrise reader. For the ST2 assay, the analytical limit of detection (sensitivity) was 5 pg/mL, intra-assay coefficients of variation (%) were 4-6%, and interassay coefficients of variation (%) were 8-10%.

Echocardiography was performed by an experienced sonographer using the 4-2 MHz probe on a Philips Affiniti 50 machine and measured the parameters for cardiac remodeling and diastolic and systolic function. The dimensions of the walls and heart cavities were measured in the M-mode, and the ejection fraction was estimated using Simpson's biplane method. The LV mass was determined using the modified Devereaux's formula [11]: values $> 95\,\mathrm{g/m^2}$ in women and $>115\,\mathrm{g/m^2}$ in men were considered diagnostic for the presence of LV hypertrophy.

Diastolic function was assessed using mitral diastolic flow parameters (A wave, E wave, E/A ratio, E-wave DT, and IVRT) and mitral annular (septal and lateral) tissue Doppler—mean value of e' (e'm) and E/e(m). Patients were followed for 1 year, and all CV events, such as hypertension emergencies, episodes of LV failure, and unstable angina, were registered. Hypertensive emergencies were defined as severe hypertension ($\geq$180/110 mmHg) in patients presenting to the emergency department in whom there is no clinical evidence of acute organ damage, in accordance with ESC Guidelines [11]. LV failure was considered in the presence of cardiac failure clinical signs (more than 2 symptoms from the Framingham score) and echocardiographic signs of diastolic and/or systolic LV dysfunction as described above. The diagnosis of unstable angina was established in accordance with the current criteria of the ESC Guidelines [14]: retrosternal pain with characteristic angina (de novo, aggravated by effort, and occurring at an early stage after percutaneous or surgical myocardial revascularization), the presence of electrocardiographic changes, and the absence of a myocardial enzymatic reaction (CK, CK-MB, and troponin). The following ECG changes were considered diagnosis criteria for unstable angina: ST depression ≥ 0.05 mV in two or more contiguous leads, ST depression combined with transient ST elevation (not fulfilling the STEMI criteria mentioned above), and T-wave inversion [14]. The ST and/or T variability on different ECG was considered another important diagnosis criterion for unstable angina.

2.3. Statistical Analysis. Statistical analyses were performed with IBM® SPSS® package version 19 (IBM Corporation, Armonk, NY, USA). Normally distributed continuous variables were expressed as mean and standard deviation and compared using the Student test. Continuous variables with abnormal distribution and ordinal variables were compared using the Mann-Whitney U test. Categorical variables were expressed as numbers and percentages, and the group comparison was done using the χ^2 test. Correlation coefficients were calculated by linear regression analysis, while multiple regression analysis was applied for analysis of the dependency between variables. $p < 0.05$ was considered statistically significant. The predictive capacity of ST2 was evaluated using computed areas under the receiver operating curve (AUC). A value of $p < 0.05$ was deemed significant; confidence intervals (CI) were calculated for $p = 0.05$ as the threshold.

Table 1: Baseline characteristics of all patients, group A, and group B.

Characteristics	All patients 80 (%)		Group A (HBP with CV events) 36 patients	Group B (HBP without CV events) 44 patients	p
Age	54.7 ± 13.5		55.23 ± 14.47	52.68 ± 11.53	0.06
Sex					
Men	38 (47.5%)	0.051	18 (50%)	20 (45.45%)	0.061
Women	42 (52.5%)		18 (50%)	24 (54.54%)	0.052
Hypertension (HBP)					
Stage I	20 (25%)	<0.001[a]	10 (27.77%)	10 (22.72%)	0.028
Stage II	34 (42.5%)	0.002[b]	12 (27.77%)	22 (50%)	0.001
Stage III	26 (32.5%)	0.023[c]	14 (38.88%)	12 (27.27%)	0.005
Additional cardiovascular risk					
Moderate	15 (18.75%)	0.002[a]	3 (8.34%)	12 (27.27%)	0.03
High	33 (41.25%)	0.023[b]	12 (33.3%)	21 (47.72%)	0.008[b]
Very high	21 (26.25%)	<0.001[c]		11 (25%)	0.000[c]
Controlled hypertension					
No	25 (31.25%)		21 (58.33%)	4 (9.09%)	<0.001
Yes	55 (68.75%)	<0.001	15 (41.66%)	40 (90.90%)	<0.001
Dyslipidaemia					
Without	8 (10%)		3 (8.33%)	5 (11.36%)	0.04
Hypercholesterolemia	42 (52.5%)		22 (61.11%)	20 (45.45%)	0.002
Mixed	26 (32.5%)		11 (30.55%)	15 (34.09%)	0.05
Hypertriglyceridemia	4 (5%)		—	4 (9.1%)	—
Smoke					
No	58 (72.5%)		18 (50%)	40 (90.9%)	0.001
Yes	22 (27.5%)	0.001	18 (50%)	4 (9.1%)	0.001
BMI					
Normal weight	32 (40%)	0.04[a]	5 (13.88%)	27 (61.63%)	0.003
Overweight	28 (35%)	0.45[b]	16 (44.44%)	12 (27.27%)	0.026
Obese	20 (25%)	0.03[c]	15 (41.66%)	5 (11.36%)	0.018
Diabetes mellitus (DM)					
No	54 (67.5%)		20 (55.55%)	34 (77.27%)	0.021
Yes	26 (32.5%)	0.001	16 (44.44%)	10 (22.72%)	<0.001

[a]Comparisons between 1 and 2; [b]comparisons between 2 and 3; [c]comparisons between 1 and 3.

3. Results

Baseline clinical and demographic characteristics of the 80 patients are shown in Table 1. 30% of the patients had grade 3 HBP and 45% had grade 2 HBP. In 68.75% of the patients, BP values were correctly controlled at the first examination, in accordance with the recommendations of the European guidelines [15]. Patients had several modifiable or nonmodifiable risk factors. Dyslipidaemia was found in 90% of the patients (hypercholesterolemia in 52.5% of them, mixed in 32.5% of them, and hypertriglyceridemia in 5% of them). 27.5% of the patients were smokers, and 51.25% of them had weight problems (27.5% were overweight and 25% obese). Diabetes was associated with HBP in 35% of cases. During the one-year study follow-up, 36 patients (45%) presented a total number of 59 CV events. Patients with CV events were included in group A (36 patients), and those without were included in group B (44 patients).

Table 1 shows the characteristics of the patients in the two groups. There was no significant difference regarding gender and age of the patients, but patients in group A had a higher proportion of stage 3 HBP with very high additional CV risk (e.g., 10-year cardiovascular risk categories using European guideline recommendations for hypertension [15]) and worse BP control rates than those in group B. In group A, we found more patients presenting dyslipidaemia (with hypercholesterolemia and mixed dyslipidaemia), smokers with higher BMI (overweight and obese), and diabetics compared with group B. Serum sST2 levels were higher in patients in group A compared to group B.

There were no significant differences between the two groups except for the parameters of diastolic dysfunction.

Table 2: Clinical, laboratory, and echocardiographic data of patients in the two groups.

Variables	Group A (HBP with CV events) 36 patients	Group B (HBP without CV events) 44 patients	p
Age	55.23 ± 14.47	52.68 ± 11.53	0.06
Men	18 (50%)	20 (45.45%)	0.061
Women	18 (50%)	24 (54.54%)	0.052
SBP (mmHg)	156.00 ± 24.56	136.63 ± 26.71	0.023
DBP (mmHg)	95.22 ± 28.61	84.76 ± 12.92	0.024
BMI (kg/m^2)	32.42 ± 6.71	27.84 ± 7.11	0.03
Uric acid (mg/dL)	6.75 ± 1.65	4.98 ± 2.13	0.079
Creatinine (mg/dL)	0.9 ± 0.51	0.79 ± 0.38	0.246
Serum glucose (mg/dL)	110.55 ± 31.64	104.36 ± 33.78	0.338
Total cholesterol (mg/dL)	160.72 ± 86.81	144.05 ± 58.84	0.57
HDL cholesterol (mg/dL)	47.05 ± 8.94	48.59 ± 13.46	0.48
LDL cholesterol (mg/dL)	104.81 ± 49	103.97 ± 52.88	0.49
Triglycerides (mg/dL)	128.70 ± 87.53	119.58 ± 83.52	0.08
ST2 (ng/mL)	52.71 (41.7–99.45)	21.34 (15.17–44.24)	0.002
Ascending aorta (mm)	30.92 ± 3.90	29.46 ± 8.45	0.002
Left atrium size (mm)	33.63 ± 9.56	34.04 ± 5.71	0.056
Left atrium area (mm)	10.65 ± 5.39	10.42 ± 6.92	0.053
End-systolic interventricular septum (mm)	12.19 ± 2.05	12.00 ± 2.26	0.051
End-diastolic interventricular septum (mm)	12.19 ± 2.05	12.00 ± 2.26	0.188
End-systolic LV posterior wall (mm)	13.07 ± 5.14	12.99 ± 5.344	0.051
End-diastolic LV posterior wall (mm)	11.68 ± 1.79	11.33 ± 2.64	0.052
End-systolic LV size (mm)	27.73 ± 12.29	27.04 ± 11.97	0.224
End-diastolic LV size (mm)	45.26 ± 6.04	42.36 ± 9.89	0.306
Right ventricle size (mm)	27.19 ± 7.08	25.14 ± 9.16	0.074
Stroke volume (mL)	34.83 ± 12.86	32.60 ± 11.70	0.001
LV mass (g/m^2)	176.4 ± 26.2	155.13 ± 83.6	<0.001
LV mass index	146.95 ± 75.80	140.15 ± 82.93	<0.001
IVRT (ms)	112.12 ± 46.7	104.56 ± 57.03	0.009
E/A	0.78 ± 0.43	0.97 ± 0.59	0.0043
EDT (ms)	225.86 ± 75.06	204.64 ± 99.24	0.0032
E/E'm	10.64 ± 2.33	8.24 ± 3.56	0.0035

Patients in group A had a lower E/A ratio, a longer E-wave DT, and significantly higher end-diastolic pressure (quantified by the E'/E ratio) (Table 2).

Admission sST2 levels were significantly correlated with CV events ($p < 0.001$). Table 3 shows the univariate association between CV events and log-transformed sST2 and clinical or echocardiographic parameters. CV event risk increases with increasing sST2 levels and glycemia. 80.7% of CV event number variability is determined by the sST2 level and glycemia. Regression analysis showed that 80.7% of CV event number variability can be explained by a 10 ng/mL increase in sST2 level and a 10 mg/dL increase in glycemia.

Diastolic function parameters—E/A ratio and E/em ratio—and LV mass were correlated with the incidence and number of CV events. Multivariate analysis showed that sST2 and fasting glucose independently increased the risk of CV events over a period of 1 year of follow-up (Table 4).

The receiver operating characteristic curve (ROC) for sST2 concentration identified 28.5 ng/mL as the optimal cut-off value to predict CV events with sensitivity and specificity of 94.4% and 69.1%, respectively ($p = 0.000$). An area under the ROC curve (AUC) was 0.84 (95% CI 0.78-0.89) which indicates the discriminative potential of this value of sST2 between high- and low-risk patients. Kaplan-Meier curve analysis showed that patients with sST2 > 28.5 ng/mL had a higher occurrence of CV events (HR 2.43, $p < 0.001$) (Figures 1 and 2).

Table 3: Univariate and multivariate analyses showing association between CV events and log-transformed ST2.

	Univariate analysis		Multivariate analysis	
	Coefficient of correlation	p	Coefficient of correlation	p
ST2	*0.696*	*<0.001*	*0.64*	*0.002*
Fasting glucose	*0.380*	*0.020*	*0.34*	*0.0032*
LV mass (g/m^2)	0.44	*<0.001*	0.38	0.051
E/A	0.28	0.027	0.34	0.07
E/e'm	0.31	0.021	0.45	0.06
TEj	0.289	0.006		

Table 4: Multivariate analysis showing sST2 and fasting glucose independent correlation with CV events on the short term.

Variable	Hazard ratio (95% CI) CV events	p
sST2	2.43 (1.32–7.24)	0.005
Fasting glucose	1.43 (1.041–1.732)	0.0023

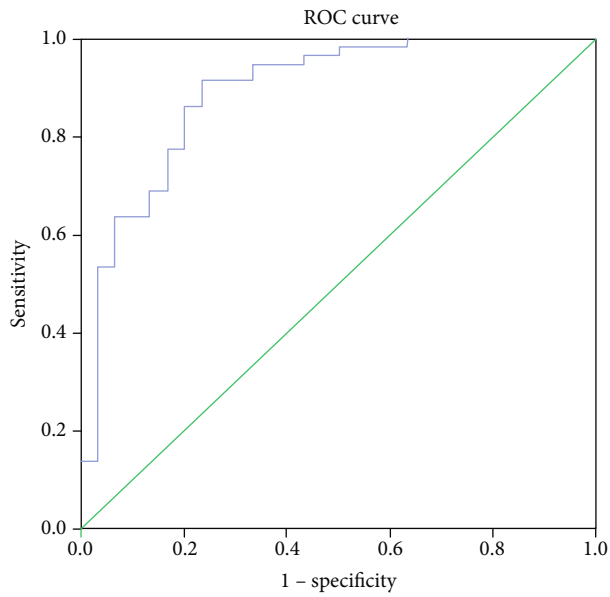

Figure 1: ROC analysis showing the sST2 sensitivity and specificity for predicting the CV events during one year after hospitalization.

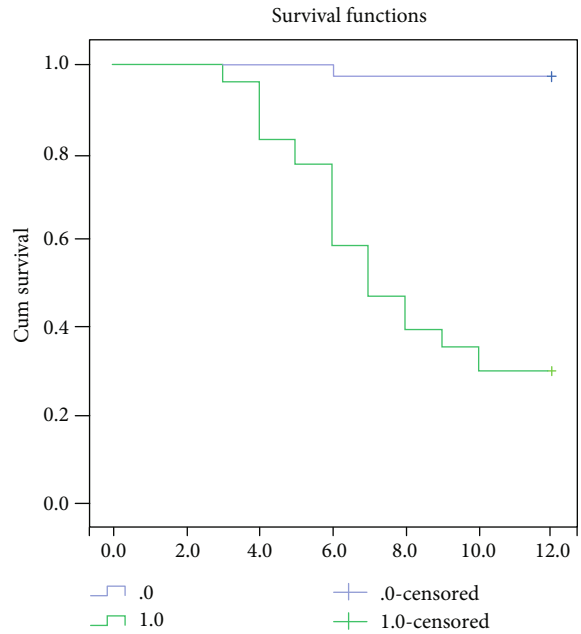

Figure 2: Kaplan-Meier curve analysis revealed that patients with sST2 > 28.5 ng/mL had a higher occurrence of CV events.

4. Discussions

Our study showed that sST2 levels are higher in hypertensive patients with CV events than in those free of CV events. sST2 levels are correlated with higher LVM, a number of CV risk factors, and the presence of LVDD. Fasting glucose and sST2 are correlated with CV events on the short term.

4.1. sST2, HBP, and LVDD. Other small studies of sST2 variations were performed in hypertensive patients, with similar results. Ojji et al. in 2013 suggested a link between LV geometry and sST2. The authors raised the hypothesis that sST2 level was not only affected by hypertensive LVH but might be a future biomarker in differentiating concentric hypertrophy from normal geometry in HBP [16]. The study comprised 133 consecutive patients diagnosed with HBP, with 37% presenting LVH [16]. Later on, the same group showed that sST2 serum levels correlated strongly with clinical and echocardiographic parameters and correlated well with NT-pro-BNP [17]. So sST2 might be useful to distinguish between hypertensive patients with and without LVH. Our group (Farcas et al.) has previously shown that sST2 could be useful as an early diagnostic biomarker for cardiac remodeling and altered diastolic performance in HBP, providing additional data to echocardiography. It could represent a milestone in early detection of cardiac performance alteration [18]. Furthermore, Wang et al. performed a larger study on 344 patients with HBP and HFpEF and showed that sST2 measurement provides diagnostic aid of stable HFpEF, correlated with NYHA class and LVDD [19]. In these patients, combined measurement provided an advanced risk stratification value compared to one biomarker measurement alone [19]. As a physiopathological explanation of the link between altered diastolic performance and high sST2 levels in hypertensive patients, Bartunek et al. showed that in humans with cardiac hypertrophy and heart failure, serum sST2 correlates with the diastolic load and has an extramyocardial source [20]. Furthermore, Zile et al. discovered that HBP in the presence of HFpEF alters passive myocardial stiffness with simultaneous increase in inflammation and fibrosis biomarkers, such as sST2, sustaining the hypothesis that the development of HFpEF depends on changes in both collagen and titin homeostasis [21].

4.2. sST2 and Short-Term Outcomes. In our study, we found that sST2 correlated with short-term prognosis for CV events

in hypertensive patients. Released as a consequence of myocardial strain, influenced by inflammation and imbalance of the extracellular matrix, sST2 may be a suitable biomarker for prognosis, i.e., LVDD progression to HF [22]. Most of the large studies focused on the prognosis value of sST2 in HFrEF [23–25] and in acute coronary syndromes [3], rather than in ambulatory patients. In a multicentric study of 447 HFpEF patients admitted for acute HF, the authors demonstrated a comparable prognostic value of sST2 in both HFpEF and HFrEF [26]. When evaluated in ambulatory patients with HF, sST2 provided "valuable long-term risk stratification information in HF beyond that reported by other biomarkers of stretch, inflammation, necrosis, and remodeling" [27].

4.3. sST2 Relation to Other Imaging Methods. One of the main findings in our study is the relations of sST2 levels and LVDD diagnosed by echocardiography. The potential diagnosis capacity of sST2 in LVDD and its correlations with echocardiographic findings were elegantly reviewed by DeFilippi et al. [28]. In brief, large studies such as the Cardiovascular Health Study, comprising older patients (>65 years old) with LVDD ≥ grade 1 present in 24.1%, showed that sST2 was strongly associated with LVDD (OR 1.35 (95% confidence interval 1.06-1.72)) and especially with echocardiographic criteria (*E*/*A* mitral inflow) and had the capacity to improve the diagnosis accuracy [29]. On the other hand, the Framingham study showed no correlation between sST2 and echocardiographic criteria for LVDD, supporting the idea that sST2 might be more suitable for a risk screening strategy in large cohorts, rather than a screening tool for structural heart disease [30]. Moreover, Daniels et al. evaluated 588 ambulatory patients with HF and found that sST2 was predominantly associated with right ventricle and not LV structural alterations [31]. The question remains whether this strong association between sST2 and LVDD in elders is a result of a cardiac-specific effect or it is influenced by general factors such as inflammation or vascular stiffness, which are common findings in aging patients [28].

4.4. sST2 in comparison to Other Biomarkers. Our study focused on single-marker evaluation, rather than on a multimarket strategy, for the diagnosis of LVDD. In our previous work, we assessed the potential of N-terminal pro-B-type natriuretic peptide as a diagnosis biomarker for LVDD in hypertensive patients with metabolic syndrome [32]. As in patients with metabolic syndrome, in our study group, 34% of the patients were diagnosed with diabetes mellitus (DM) and fasting glucose, along with higher sST2 levels, is associated with higher risk of CV events. In this respect, Ruocco et al. showed that ST2 was higher in patients with LVDD and DM, it significantly correlated with glycosylated haemoglobin (HbA1c), and it was related to an adverse event occurrence within 6 months and with poor prognosis [33]. Miller et al. have recently demonstrated that sST2 is related to DM and inflammation rather than CV risk factors, blood pressure, or smoking [34]. The pathogenic link between circulating sST2 and DM is still not clear but could be causal. The authors hypothesized that sST2 not only is a biomarker but also may contribute to the pathogenesis of diabetes via IL-33 interactions. The IL-33/ST2 pathway may participate in the inflammatory and remodeling processes of various tissues in patients with DM [34]. The potential diagnosis biomarkers in cardiac remodeling after myocardial infarction in patients with HBP and DM were a subject of a previous study published by our group. We showed that lower leptin levels were associated with reduced values of echocardiographic parameters of ventricular remodeling [35]. Further research should focus on sST2 and cardiac remodeling after myocardial infarction.

Other studies showed sST2 to improve discrimination when adjusted to multivariable models comprising N-terminal pro-B-type natriuretic peptide or galectin-3 [27]. sST2 as compared to other biomarkers has the advantage not to be influenced by confounders (renal function, BMI, or age), and its levels are modified by the progressing disease.

4.5. Limitations of the Study. There are some limitations to our study that are worth taking into consideration. First of all, our study is a cross-sectional study with a single measurement of biomarker levels and is limited by the small number of patients. As such, the study does not benefit from the inherent variability in time of the tested biomarkers. As outpatients were included, the study group consisted of rather young patients, equally distributed by sex. Thus, these results cannot necessarily be extrapolated to older populations. Also, the study population was not tested for underlying coronary artery disease, which could also present as LVDD. Moreover, the interaction between medical therapy and the serum levels of the studied biomarkers was not addressed in this analysis, which was more focused on the links between sST2 and echocardiographic parameters of LVDD. Finally, we have to acknowledge that the number of participants was relatively small because of the multiple exclusion criteria, but it was enough to give a study power of over 0.80. Larger clinical trials will be needed for the validation of an ST2-predictive value in clinical practice.

5. Conclusions

Serum levels of sST2 are strongly correlated with higher CV risk in hypertensive patients and have a predictive potential for poor prognosis in these patients. Fasting glucose and sST2 are correlated with CV events on the short term.

Data Availability

The clinical data used to support the findings of this study are included within the article.

Disclosure

Parts of this manuscript were presented at the European Congress of Cardiology 2018 (Abstract P1866 European Heart Journal (2018) 39 (Supplement), 396).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Anca Daniela Farcaş, Mihaela Mocan, Florin Petru Anton, and Luminița Animarie Vida-Simiti contributed equally to this work. Anca Daniela Farcaş and Luminița Animarie Vida-Simiti designed the research study. Florin Petru Anton, Diana Larisa Mocan-Hognogi, Roxana Mihaela Chiorescu, and Mirela Anca Stoia performed the research. Anca Daniela Farcaş, Mihaela Mocan, Camelia Larisa Vonica, and Cerasela Mihaela Goidescu analyzed the data. Mihaela Mocan and Anca Daniela Farcaş wrote the paper.

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References

[1] X. Liu, Y. Xiao, Y. Pan, H. Li, G. Zheng, and W. Su, "The role of the IL-33/ST2 axis in autoimmune disorders: friend or foe?," *Cytokine & Growth Factor Reviews*, vol. 50, pp. 60–74, 2019.

[2] A. Dattagupta and S. Immaneni, "ST2: current status," *Indian Heart Journal*, vol. 70, pp. S96–S101, 2018.

[3] M. Shimpo, D. A. Morrow, E. O. Weinberg et al., "Serum levels of the interleukin-1 receptor family member ST2 predict mortality and clinical outcome in acute myocardial infarction," *Circulation*, vol. 109, no. 18, pp. 2186–2190, 2004.

[4] L. C. VarkVan, I. Lesman-leegte, S. J. Baart et al., "Prognostic Value of Serial ST2 Measurements in Patients With Acute Heart Failure," *Journal of the American College of Cardiology*, vol. 70, no. 19, pp. 2378–2388, 2017.

[5] I. Fabiani, L. Conte, N. Pugliese et al., "The integrated value of sST2 and global longitudinal strain in the early stratification of patients with severe aortic valve stenosis: a translational imaging approach," *The International Journal of Cardiovascular Imaging*, vol. 33, no. 12, pp. 1915–1920, 2017.

[6] W. Meijers, A. van der Velde, and R. DeBoer, "Biomarkers in heart failure with preserved ejection fraction," *Netherlands Heart Journal*, vol. 24, no. 4, pp. 252–258, 2016.

[7] M. Mocan, L. Diana, M. Hognogi et al., "Biomarkers of inflammation in left ventricular diastolic dysfunction," *Disease Markers*, vol. 2019, 14 pages, 2019.

[8] M. Santos and A. Shah, "Alterations in cardiac structure and function in hypertension," *Current Hypertension Reports*, vol. 16, no. 5, pp. 1–10, 2014.

[9] P. Palmiero, A. Zito, M. Maiello et al., "Left ventricular diastolic function in hypertension: methodological considerations and clinical implications," *Journal of Clinical Medicine Research*, vol. 7, no. 3, pp. 137–144, 2014.

[10] A. S. Go, D. Mozaffarian, V. L. Roger et al., "Heart Disease and Stroke Statistics—2013 Update," *Circulation*, vol. 127, no. 1, pp. e6–e245, 2013.

[11] H. Marwick, T. C. Gillebert, G. Aurigemma, and E. Al, "Recommendations on the use of echocardiography in adult hypertension: a report from the European Association of Cardiovascular Imaging (EACVI) and the American Society of Echocardiography (ASE)†," *European Heart Journal - Cardiovascular Imaging*, vol. 16, pp. 577–605, 2015.

[12] S. I. Vida, "Interventional clinical and chemical studies in Romania, legal guarantees," *Revista de Chimie*, vol. 69, pp. 267–270, 2018.

[13] I. Vida-Simiti, "Medical liability for off label use of drugs in Romania," *Revista de Chimie*, vol. 68, pp. 755–758, 2018.

[14] M. Roffi, C. Patrono, and J. Collet, "2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation," *European Heart Journal*, vol. 37, no. 3, pp. 267–315, 2016.

[15] G. Mancia, R. Fagard, K. Narkiewicz et al., "2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC)," *European Heart Journal*, vol. 34, no. 28, pp. 2159–2219, 2013.

[16] D. B. Ojji, L. H. Opie, S. Lecour, L. Lacerda, O. Adeyemi, and K. Sliwa, "Relationship between left ventricular geometry and soluble ST2 in a cohort of hypertensive patients," *Journal of Clinical Hypertension*, vol. 15, no. 12, pp. 899–904, 2013.

[17] D. B. Ojji, L. H. Opie, S. Lecour, L. Lacerda, O. M. Adeyemi, and K. Sliwa, "The effect of left ventricular remodelling on soluble ST2 in a cohort of hypertensive subjects," *Journal of Human Hypertension*, vol. 28, no. 7, pp. 432–437, 2014.

[18] A. D. Farcas, F. P. Anton, C. M. Goidescu, I. L. Gavrilă, L. A. Vida-Simiti, and M. A. Stoia, "Serum soluble ST2 and diastolic dysfunction in hypertensive patients," *Disease Markers*, vol. 2017, 8 pages, 2017.

[19] Y. Wang, C. Yu, F. Chiu et al., "Soluble ST2 as a biomarker for detecting stable heart failure with a normal ejection fraction in hypertensive patients," *Journal of Cardiac Failure*, vol. 19, no. 3, pp. 163–168, 2013.

[20] J. Bartunek, L. Delrue, F. Van Durme et al., "Nonmyocardial production of ST2 protein in human hypertrophy and failure is related to diastolic load," *Journal of the American College of Cardiology*, vol. 52, no. 25, pp. 2166–2174, 2008.

[21] M. R. Zile, C. F. Baicu, J. S. Ikonomidis et al., "Myocardial stiffness in patients with heart failure and a preserved ejection fraction: contributions of collagen and titin," *Circulation*, vol. 131, no. 14, pp. 1247–1259, 2015.

[22] A. Bayés-Genís, J. Núñez, and J. Lupón, "Soluble ST2 for Prognosis and Monitoring in Heart Failure: The New Gold Standard?," *Journal of the American College of Cardiology*, vol. 70, no. 19, pp. 2389–2392, 2017.

[23] C. G. Bahuleyan, G. K. Alummoottil, J. Abdullakutty et al., "Prognostic value of soluble ST2 biomarker in heart failure patients with reduced ejection fraction – a multicenter study," *Indian Heart Journal*, vol. 70, pp. S79–S84, 2018.

[24] I. S. Anand, T. S. Rector, M. Kuskowski, J. Snider, and J. N. Cohn, "Prognostic value of soluble ST2 in the Valsartan Heart Failure Trial," *Circulation: Heart Failure*, vol. 7, no. 3, pp. 418–426, 2014.

[25] J. L. Januzzi, A. Mebazaa, and S. Disomma, "ST2 and prognosis in acutely decompensated heart failure: the International ST2 Consensus Panel," *The American Journal of Cardiology*, vol. 115, no. 7, pp. 26B–31B, 2015.

[26] S. Manzano-Fernndez, T. Mueller, D. Pascual-Figal, Q. A. Truong, and J. L. Januzzi, "Usefulness of soluble concentrations of interleukin family member ST2 as predictor of mortality in patients with acutely decompensated heart failure relative to left ventricular ejection fraction," *The American Journal of Cardiology*, vol. 107, no. 2, pp. 259–267, 2011.

[27] A. Bayes-Genis, J. Januzzi, H. Gaggin et al., "ST2 pathogenetic profile in ambulatory heart failure patients," *Journal of Cardiac Failure*, vol. 21, no. 4, pp. 355–361, 2014.

[28] C. DeFilippi, L. B. Daniels, and A. Bayes-Genis, "Structural Heart Disease and ST2: Cross-Sectional and Longitudinal Associations With Echocardiography," *The American Journal of Cardiology*, vol. 115, no. 7, pp. 59B–63B, 2015.

[29] S. Seliger, E. Ginsberg, J. Gottdiener, R. Christenson, and C. DeFilippi, "Soluble ST2 and galectin-3 are associated with subclinical diastolic dysfunction in older adults," *Journal of the American College of Cardiology*, vol. 63, no. 12, p. A769, 2014.

[30] T. J. Wang, K. C. Wollert, M. G. Larson et al., "Prognostic utility of novel biomarkers of cardiovascular stress: the Framingham Heart Study," *Circulation*, vol. 126, no. 13, pp. 1596–1604, 2012.

[31] L. Daniels, P. Clopton, N. Iqbal, K. Tran, and A. Maisel, "Association of ST2 levels with cardiac structure and function and mortality in outpatients," *American Heart Journal*, vol. 160, no. 4, pp. 721–728, 2010.

[32] M. Mocan, F. Anton, S. Suciu, R. Rahaian, S. N. Blaga, and A. D. Farcas, "Multimarker assessment of diastolic dysfunction in metabolic syndrome patients," *Metabolic Syndrome and Related Disorders*, vol. 15, no. 10, pp. 507–514, 2017.

[33] G. Ruocco, I. Evangelista, B. Franci et al., "Combination of ST2 and B-type natriuretic peptide in diabetic patients with acute heart failure," *Journal of Cardiovascular Medicine*, vol. 20, no. 2, pp. 81–90, 2019.

[34] A. M. Miller, D. Purves, A. Mcconnachie et al., "Soluble ST2 Associates with Diabetes but Not Established Cardiovascular Risk Factors: A New Inflammatory Pathway of Relevance to Diabetes?," *PLoS One*, vol. 7, no. 10, article e47830, 2012.

[35] A. D. Farcas, A. Rusu, M. A. Stoia, and L. A. Vida-Simiti, "Plasma leptin, but not resistin, TNF-α and adiponectin, is associated with echocardiographic parameters of cardiac remodeling in patients with coronary artery disease," *Cytokine*, vol. 103, pp. 46–49, 2018.

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Research Article

ADAMTS-5 Decreases in Coronary Arteries and Plasma from Patients with Coronary Artery Disease

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The current study demonstrates that a disintegrin and metalloproteinase with thrombospondin type 1 motif- (ADAMTS-) 5 is a key extracellular matrix protease and associated with cardiovascular diseases. However, the plasma ADAMTS-5 levels and relevance of coronary artery disease (CAD) remain largely unknown. This study is aimed at examining the relationship between the plasma ADAMTS-5 levels and the severity of coronary stenosis in patients with CAD. In the present study, the expression of ADAMTS-5 was analyzed in coronary artery samples and blood. The results showed that the plasma ADAMTS-5 levels were lower in the CAD group than in the control group. In addition, significantly higher matrix metalloproteinase- (MMP-) 2 and MMP-9 levels were also observed in the patients with CAD, and the ADAMTS-5 levels were negatively correlated with the MMP-2 and MMP-9 levels. Spearman's correlation analysis showed that the Gensini score was negatively correlated with the ADAMTS-5 levels but was positively correlated with the MMP-2 and MMP-9 levels. Receiver-operating characteristic (ROC) analysis revealed that ADAMTS-5, MMP-2, and MMP-9 may have a certain diagnostic value in CAD and that the combination of all three metalloproteinases had a higher diagnostic value. The findings provided a better understanding of the role of ADAMTS-5 in the diagnosis of CAD.

1. Introduction

Coronary atherosclerotic disease (CAD) is the most common cardiovascular disease and remains a leading cause of morbidity and mortality worldwide. Narrowing of the arterial lumen and rupture of coronary atherosclerotic plaques with or without luminal thrombosis and vasospasm are currently considered to be the main causes of CAD [1–5]. Several pathophysiologic mechanisms are involved in the process of plaque rupture, including coronary atherosclerotic plaque instability, inflammation, and circumferential wall shear stress. The arteries are composed of three layers: the endothelium, the media consist of smooth muscle cells (SMCs) and elastin, and the adventitia. The extracellular matrix (ECM) of the vasculature is primarily produced by cells originally residing in the cardiovascular tissue, namely, endothelial cells (ECs), SMCs, fibroblasts, and cardiomyocytes [6–8]. Additionally, the ECM is also the most abundant component of normal vessels and atherosclerotic plaque, including fibrous caps. Plaque rupture is increased by a weakened fibrous cap, promoted by the loss of function of the vascular smooth muscle cells (VSMCs) and the breakdown of collagen and ECM that may subsequently lead to acute myocardial infarction (AMI) or stroke [9, 10].

The metalloproteinase superfamily comprises several subfamilies, including the matrix metalloproteinase (MMP), a disintegrin and metalloproteinase (ADAM), and ADAM with thrombospondin type 1 motif (ADAMTS) families. These metalloproteinases are capable of degrading the ECM and play an important role in the development and progression of cardiovascular diseases. The ADAMTS proteases are multidomain proteins that are composed of 19 members,

which share a similar structural motif and substrate range. Accumulating evidence suggests that ADAMTS are key ECM proteases associated with ECM turnover, which showed a close association with CAD [11–13]. Previous studies have demonstrated that the plasma ADAMTS-7 level was significantly increased in patients with AMI and was positively correlated with ventricular function after AMI [12, 14]. Similarly, low ADAMTS-13 levels were associated with an increased risk of myocardial infarction [13, 15].

ADAMTS-5, known as aggrecanase-2, has the highest aggrecanase activity and contributes to vascular remodeling [16, 17]. Suna et al. reported that the expression level of ADAMTS-5 was markedly decreased in stent-induced vascular injury, associated with the accumulation of proteoglycans, notably aggrecan and versican [16]. In addition, decreased ADAMTS-5 expression was found in atherosclerosis of apolipoprotein E (ApoE) null mice, associated with the accumulation of aggrecan and versican [18]. However, direct reports regarding circulating ADAMTS-5 levels in patients with CAD is still lacking.

In this study, we detected the expression and localization of ADAMTS-5 in humans with CAD. Furthermore, we also tested the plasma levels of ADAMTS-5 and assayed its correlation with CAD as well as its predictive power for the severity of coronary stenosis.

2. Methods

2.1. Human Coronary Artery Samples. The study was performed at Renmin Hospital of Wuhan University. Atherosclerotic plaque samples were obtained from the right coronary artery (RCA) of patients with CAD ($n = 6$) who underwent heart transplantation surgery. Normal human coronary arteries were collected from healthy donors ($n = 6$) who were declared brain-dead, but which were not suitable for transplantation as a result of noncardiac reasons. The donor had no apparent history of cardiovascular disease, and the coronary arteries were not damaged in car accident and without pathology. The protocol conformed with the Declaration of Helsinki principles and was approved by the Ethics Committee of Renmin Hospital of Wuhan University. Written informed consent was obtained from each patient.

2.2. Western Blotting. Total proteins were extracted from the coronary artery in RIPA lysis buffer as described in our previous study [19]. Protein concentrations were determined using a BCA Protein Assay kit (23227, Thermo Fisher Scientific, USA). Total proteins were separated via sodium dodecyl sulphate polyacrylamide gel electrophoresis and then transferred to a polyvinylidene fluoride membrane (IPFL00010, Millipore, USA). Thereafter, the membranes were blocked with 5% skim milk in Tris-buffered saline for 60 min at 37°C. The membranes were incubated with anti-ADAMTS-5 (GTX100332, 1 : 1000 dilution, GeneTex, USA) and anti-β-tubulin (2128S, 1 : 1000 dilution, Cell Signaling Technology, USA) antibodies overnight at 4°C, followed by incubation with secondary antibody at room temperature for 60 min. The blots were detected using a two-color infrared imaging system (Odyssey; LICOR) to quantify protein expression. Specific protein expression levels were normalized to the corresponding β-tubulin protein.

2.3. Histologic Analysis. The coronary artery tissues were fixed with 4% neutral paraformaldehyde, embedded in paraffin, cut into 4-5 μm slices, and mounted onto slides. Hematoxylin and eosin staining was performed according to a standard process.

Immunohistochemistry staining was also performed as previously described [20]. Briefly, the sections were deparaffinized and blocked with 10% bovine serum albumin, followed by incubation with an anti-ADAMTS-5 antibody (GTX100332, 1 : 100 dilution, GeneTex, USA) for 12 h at 4°C. Thereafter, the sections were incubated with anti-rabbit HRP reagent for 1 h at 37°C and visualized with diaminobenzidine. Finally, the sections were mounted with neutral gums and assessed via light microscopy (Nikon H550L, Tokyo, Japan).

For immunofluorescence staining, the sections were blocked with 10% goat serum for 10 min and subsequently incubated with anti-ADAMTS-5 (GTX100332, 1 : 100 dilution, GeneTex, USA), anti-α-smooth muscle cell (α-SMA, GTX89701, 1 : 100 dilution, GeneTex, USA), CD31 (ab24590, 1 : 100 dilution, Abcam, USA), and CD68 (ab31630, 1 : 100 dilution, Abcam, USA) overnight at 4°C. Next, the sections were incubated with the appropriate secondary antibodies and DAPI solution at room temperature.

2.4. Collection of Human Blood Samples. The study was performed at Renmin Hospital of Wuhan University. Between July 2015 and September 2017, patients who experienced chest pain and underwent coronary angiography (CAG) were recruited. The major exclusion criteria were as follows: (1) age of >80 years, (2) severe hepatic or renal insufficiency, (3) malignancy, (4) condition complicated with infectious diseases, (5) autoimmune diseases, and (6) unclear history of drug use. The 159 remaining patients were divided into the non-CAD (NCAD) ($n = 36$) and CAD groups ($n = 123$) according to clinical symptoms and results of CAG. The CAD group was defined as the presence of luminal diameter equal to or more than 50% in one or more epicardial coronary arteries, and the NCAD group was defined as the normal coronary arteries without any stenosis [21, 22]. Demographic data, body weight, height, biochemical parameters, and medication use were recorded on admission to the hospital.

2.5. Blood Sample Measurement. Fasting venous peripheral blood samples were obtained at 6:30–7:30 am and collected into sodium heparin vacutainers following admission of all participants. All plasma samples were centrifuged for 20 min at 4000×g, and the plasma was collected. Thereafter, all samples were stored at −80°C for further analyses.

All the samples were thawed, and the plasma ADAMTS-5 (DY2198-05, R&D Systems, USA), MMP-2 (DY902, R&D Systems, USA), and MMP-9 (DMP900, R&D Systems, USA) levels were measured using the commercially available enzyme-linked immune sorbent assay (ELISA) kits according to the manufacturer's instructions.

2.6. Gensini Score. The degree of coronary stenosis was estimated using the Gensini score based on the results of CAG [23]. The method was as follows: ≤25% narrowing was assigned scores of 1; 26-50% narrowing, scores of 2; 51-75% narrowing, scores of 4; 76-90% narrowing, scores of 8; 91-99% narrowing, scores of 16; and total occlusion, scores of 32. For localization, the left main coronary artery was assigned a score of 5; proximal left anterior descending (LAD) and left circumflex (LCX), a score of 2.5; midsegment of the LAD, a score of 1.5; and RCA, distal segment of the LAD, and midsegment of the LCX, a score of 1. Finally, the Gensini scores of the patients were calculated via summation of each coronary stenosis.

2.7. Statistical Analysis. The plasma cytokine concentrations and clinical characteristics were presented as median values (with interquartile ranges) and compared using the Mann-Whitney *U* tests. Spearman's correlation was used to calculate the correlations between the plasma ADAMTS-5, MMP-2, and MMP-9 levels and the Gensini score. The diagnostic sensitivity and specificity for plasma cytokines in diagnosing CAD were calculated from the receiver-operating characteristic (ROC) curves, using the subjects without CAD as negative controls. For the establishment of the model with the combination of all three cytokines, the probability value was obtained from logistic regression analysis and then used as a new indicator for the diagnosis of CAD based on ROC curve analysis. The data from western blotting were expressed as means ± SDs and compared using Student's *t*-test. A P value of <0.05 was considered to indicate statistical significance. Statistical analyses were performed using SPSS 21.0 and GraphPad Prism7.

3. Results

3.1. Basic Clinical Characteristics of Patients Who Provided Coronary Tissue Samples. Among the patients who provided coronary tissue, the levels of C-reactive protein (CRP) were higher in the patients with CAD. No differences were found between normal donors and patients with CAD for other clinical characteristics, including gender, age, smoking, glucose (Glu), total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), systolic blood pressure (SBP), and diastolic blood pressure (DBP). The clinical data for all patients are listed in Table 1.

3.2. Expression of ADAMTS-5 in Human Atherosclerotic Plaque Tissue. To investigate the potential role of ADAMTS-5 in the progression of CAD, we examined whether ADAMTS-5 expression levels were altered in atheromatous plaques. The western blotting and immunohistochemistry results showed that the expression level of ADAMTS-5 significantly decreased in the coronary arteries of the patients with CAD (Figures 1(a) and 1(b)). To further investigate the source of ADAMTS-5 in coronary arteries of the patients with CAD, double-immunofluorescence staining was performed. The results showed that ADAMTS-5 was expressed in VSMCs and macrophages, while VSMCs were the major source of ADAMTS-5 in human CAD atherosclerotic plaques (Figure 1(c)).

Table 1: Clinical characteristics in patients who provide coronary tissue samples.

Characteristics	NCAD ($n = 6$)	CAD ($n = 6$)	P
Gender (M/F)	3/3	5/1	0.546
Age (years)	57 (46.5, 62)	58 (51.5, 66)	0.485
Smoking (n, %)	2 (33.3%)	4 (75%)	0.568
Glu (mmol/L)	5.30 (4.95, 5.75)	5.60 (5.10, 6.35)	0.394
TG (mmol/L)	1.41 (1.04, 1.81)	1.46 (1.32, 1.98)	0.485
TC (mmol/L)	3.35 (2.58, 3.66)	3.87 (3.31, 4.93)	0.240
HDL-C (mmol/L)	0.78 (0.71, 1.18)	0.80 (0.72, 0.99)	0.818
LDL-C (mmol/L)	1.72 (1.29, 1.91)	2.09 (1.27, 3.00)	0.290
SBP (mmHg)	120 (104, 131)	125 (108, 128)	0.589
DBP (mmHg)	72 (64, 85)	68 (62, 77)	0.485
CRP (mg/L)	0.66 (0.49, 0.93)	3.06 (0.77, 8.70)	0.026

Data are expressed as median (interquartile range) or the number (percentage) for category variables. Glu: fasting glucose; TG: total triglycerides; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; SBP: systolic blood pressure; DBP: diastolic blood pressure; CRP: C-reactive protein.

3.3. Baseline Characteristics of Patients Who Provided Plasma Samples. Baseline clinical characteristics of the two groups are presented in Table 2. There were no significant differences in mean age, gender, smoking, drinking, hypertension, hyperlipidemia, diabetes, Glu, TG, TC, HDL-C, and LDL-C. The incidence rate of the Gensini score and CRP was higher in the CAD group when compared with the NCAD group. Baseline characteristics of the patients are shown in Table 2.

3.4. Plasma Cytokine Concentrations in CAD Patients. Plasma levels of ADAMTS-5, MMP-2, and MMP-9 of each patient were detected by ELISA. The plasma ADAMTS-5 levels were lower in the CAD group than in the control group. The plasma MMP-2 and MMP-9 levels, by contrast, were higher in patients with CAD (Figures 2(a)–2(c)). Spearman's correlation analysis reported that the plasma ADAMTS-5 levels were negatively correlated with the MMP-2 levels ($r = -0.3042$, $P = 0.0006$) and MMP-9 levels ($r = -0.3399$, $P = 0.0001$) (Figures 2(c) and 2(d)), whereas the MMP-2 levels were positively correlated with MMP-9 levels ($r = 0.2201$, $P = 0.0144$) (Figures 2(e) and 2(f)). To analyze whether the plasma cytokine levels were associated with the severity of CAD, the correlations among Gensini score, ADAMTS-5, MMP-2, and MMP-9 levels in CAD patients were assessed. Spearman's correlation analysis showed that the Gensini score was negatively correlated with the ADAMTS-5 levels ($r = -0.4663$, $P < 0.0001$) (Figure 3(a)) but was positively correlated with the MMP-2 ($r = 0.3135$, $P = 0.0004$) and MMP-9 ($r = 0.3504$, $P < 0.0001$) levels (Figures 3(b) and 3(c)). In addition, the circulating CRP were negatively correlated with the ADAMTS-5 levels ($r = -0.2587$, $P = 0.0039$) (Figure 4(a)), whereas positively correlated with MMP-2 levels ($r = 0.2937$, $P = 0.0010$)

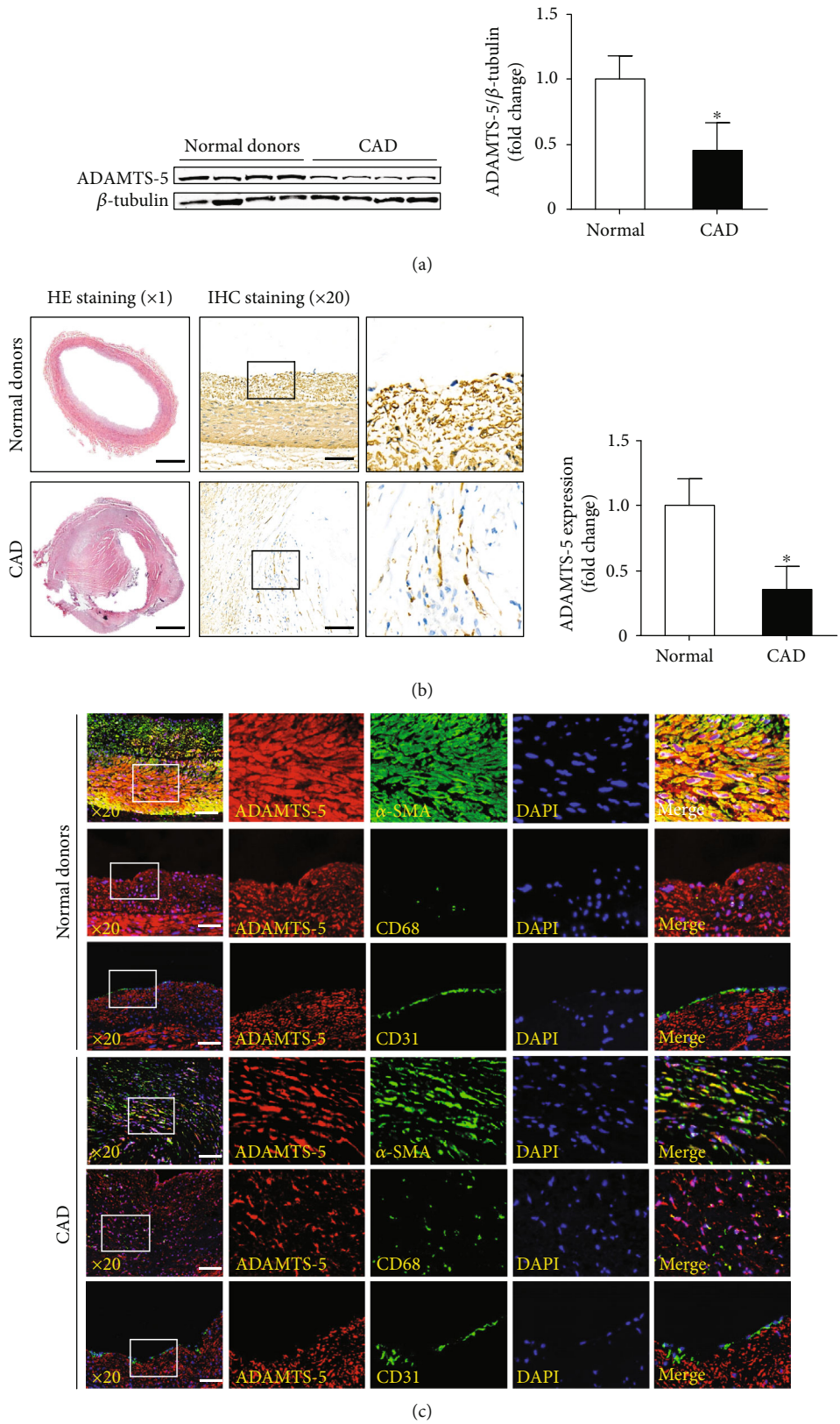

Figure 1: The ADAMTS-5 expression in human atheromatous plaques. (a) Western blot analysis of ADAMTS-5 in atheromatous plaques from normal donors and patients with coronary heart disease (CAD) ($n = 6$/group). $^*P < 0.05$ versus donors. (b) The ADAMTS-5 expression in these two groups were measured by immunohistochemistry staining ($n = 6$/group, scale bar, 1000 μm for the left set of panels and 100 μm for the right panels). (c) The source of ADAMTS-5 was detected by double immunofluorescence staining ($n = 6$/group, scale bar, 100 μm).

Table 2: Information of clinical characteristics and laboratory data in the NCAD group and the CAD group.

Characteristics	NCAD ($n = 36$)	CAD ($n = 123$)	P
Gender (M/F)	20/16	78/45	0.438
Age (years)	63 (51, 74)	64 (57, 73)	0.465
Smoking (n, %)	6 (16.7%)	38 (30.9%)	0.137
Drinking (n, %)	2 (5.56%)	22 (17.9%)	0.110
Hypertension (n, %)	25 (69.4%)	95 (77.2%)	0.380
Hyperlipidemia (n, %)	14 (38.9%)	39 (31.7%)	0.428
Diabetes (n, %)	7 (19.4%)	39 (31.7%)	0.210
Glu (mmol/L)	5.33 (4.84, 5.68)	5.53 (4.93, 6.45)	0.119
SBP (mmHg)	147 (139, 155)	144 (127, 158)	0.627
DBP (mmHg)	80 (74, 93)	82 (74, 90)	0.818
HDL-C (mmol/L)	1.06 (0.87, 1.28)	1.00 (0.83, 1.22)	0.548
LDL-C (mmol/L)	2.20 (1.89, 2.54)	2.03 (1.50, 2.58)	0.174
TC (mmol/L)	4.16 (3.65, 5.04)	4.10 (3.34, 4.83)	0.338
TG (mmol/L)	1.38 (1.18, 1.82)	1.45 (0.97, 2.23)	0.951
CRP (mg/L)	0.94 (0.43, 2.06)	4.04 (1.78, 10.4)	<0.001
ADAMTS-5 (ng/mL)	7.62 (5.60, 10.5)	4.64 (3.02, 6.41)	<0.001
MMP-2 (ng/mL)	7.95 (5.06, 12.4)	13.3 (9.09, 17.6)	<0.001
MMP-9 (ng/mL)	50.1 (34.8, 78.9)	83.4 (59.0, 103.4)	<0.001
Gensini score	—	24 (10, 46)	—
Medications, n (%)			
Aspirin	13 (36.1%)	62 (50.4%)	0.184
Statin	8 (22.2%)	47 (38.2%)	0.110
ACEI/ARB	17 (47.2%)	66 (53.6%)	0.571
CCB	11 (30.5%)	52 (42.3%)	0.247
β-Receptor blockers	6 (16.7%)	35 (28.5%)	0.196
Diuretic	3 (8.33%)	7 (5.69%)	0.696
Insulin	4 (11.1%)	22 (17.8%)	0.446
Oral hypoglycemics	2 (5.56%)	17 (13.8%)	0.248

Data are expressed as median (interquartile range) or the number (percentage) for category variables. Glu: fasting glucose; SBP: systolic blood pressure; DBP: diastolic blood pressure; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TC: total cholesterol; TG: total triglycerides; CRP: C-reactive protein; ADAMTS-5: a disintegrin and metalloproteinase with thrombospondin type 1 motif-5; MMP-2: matrix metalloproteinase-2; MMP-9: matrix metalloproteinase-9; ACEI: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; CCB: calcium channel blockers.

and MMP-9 levels ($r = 0.2633$, $P = 0.0033$) and the Gensini score ($r = 0.3457$, $P < 0.0001$) (Figures 4(b)–4(d)). The plasma ADAMTS-5, MMP-2, and MMP-9 levels in each group are listed in Table 2.

3.5. Simple Linear Regression Analysis and Binary Linear Regression Analysis. To determine the independent predictors of the presence of CAD, simple linear regression analyses and subsequent binary linear regression analyses were performed. Simple linear regression analyses showed that ADAMTS-5, MMP-2, MMP-9, and CRP levels exhibited a trend towards an association with the presence of CAD, whereas gender, age, smoking, SBP, DBP, Glu, TC, TG, HDL-C, and LDL-C showed no obvious trend towards this association. Further, binary linear regression analyses were performed. The results demonstrated that ADAMTS-5 was independently associated with the presence of CAD (as shown in Table 3).

3.6. ROC Curves of ADAMTS-5. To assess the diagnostic accuracy of ADAMTS-5 in the detection of CAD, we performed ROC curve analysis and found that the area under the curve of ADAMTS-5 was 0.801, which was as high as that of MMP-2 (AUC = 0.748) and MMP-9 (AUC = 0.752) (Figure 5). Furthermore, combination of all three cytokines had a higher area under the curve value (AUC = 0.856) (Figure 5), indicating that combination of all three cytokines has a better capability to predict CAD than when either of the parameters is used alone.

4. Discussion

In this study, we found that the expression level of ADAMTS-5 was reduced in the coronary arteries of the patients with CAD, and the VSMCs were the major source of ADAMTS-5 in human CAD atherosclerotic plaques. Furthermore, the results showed that the plasma ADAMTS-5

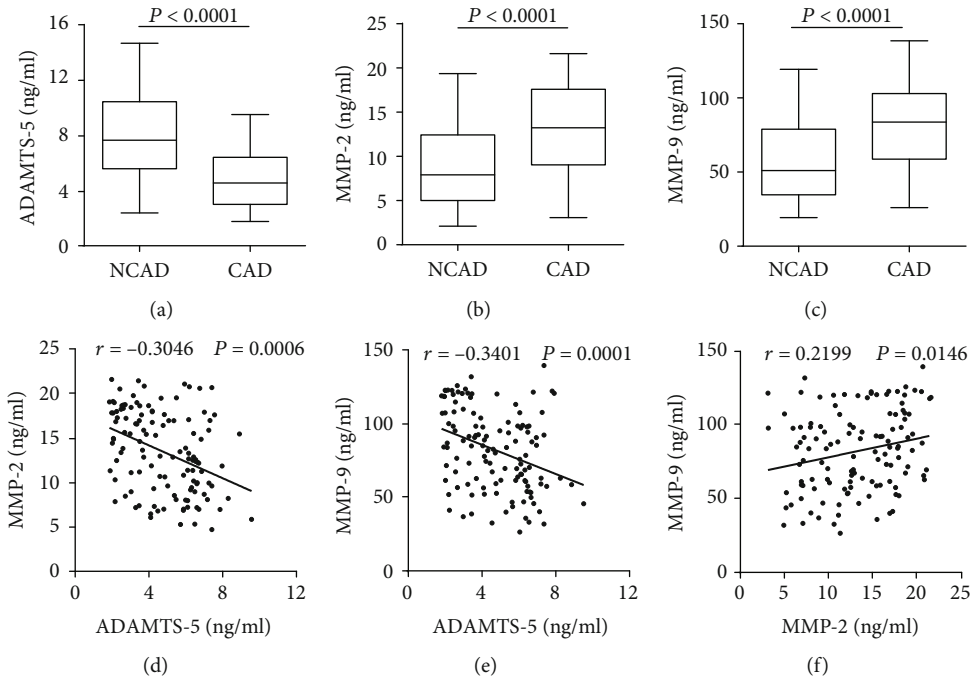

Figure 2: Plasma cytokine levels in each group. The plasma levels of ADAMTS-5 (a), MMP-2 (b), and MMP-9 (c) in NCAD ($n = 36$) and CAD ($n = 123$) groups were measured by ELISA. Spearman's correlation between the plasma levels of MMP-2 (d), MMP-9 (e), and ADAMTS-5 in CAD patients ($n = 123$). (f) Correlation between MMP-2 levels and MMP-9 levels ($n = 123$).

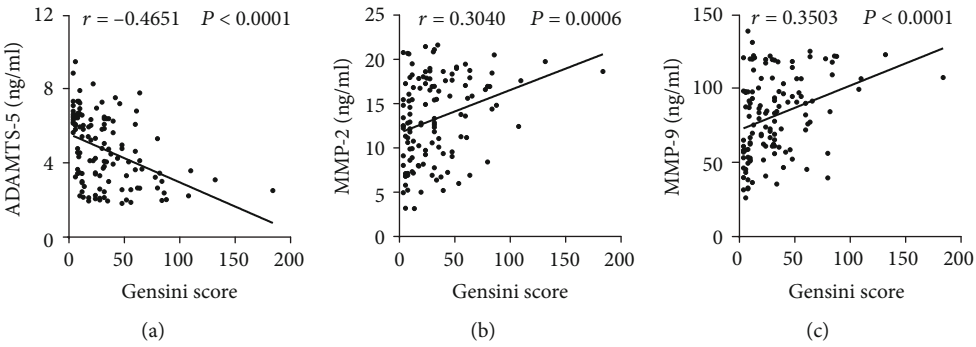

Figure 3: Correlation between plasma cytokine levels and Gensini score. Spearman's correlations between the plasma ADAMTS-5 (a), MMP-2 (b), and MMP-9 levels (c) and the Gensini score in the patients with CAD ($n = 123$).

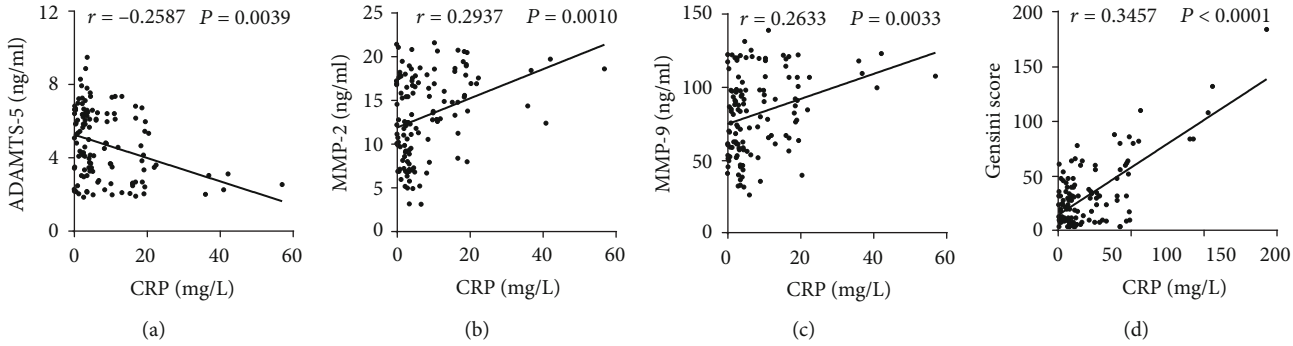

Figure 4: Correlation analysis of ADAMTS-5 (a), MMP-2 (b), and MMP-9 levels (c) and the Gensini score (d) with circulating CRP levels in the patients with CAD ($n = 123$).

Table 3: Association between cytokines, clinical characteristics, and the presence of acute CAD was assessed by simple linear regression analysis and subsequent binary linear regression analysis.

Variables	Simple linear			Binary linear		
	β	95% CI	P value	β	95% CI	P value
ADAMTS-5	-0.518	-0.653 to -0.383	<0.001	-0.352	-0.081 to -0.032	<0.001
MMP-2	0.361	0.214 to 0.508	<0.001	0.147	0.000 to 0.024	0.045
MMP-9	0.365	0.219 to 0.512	<0.001	0.163	0.000 to 0.004	0.028
CRP	0.303	0.153 to 0.454	<0.001	0.144	0.000 to 0.014	0.041
Gender	-0.068	-0.225 to 0.090	0.397			
Age	0.123	-0.034 to 0.279	0.124			
Smoking	-0.133	-0.289 to 0.023	0.094			
SBP	-0.030	-0.187 to 0.128	0.712			
DBP	0.013	-0.145 to 0.171	0.871			
Glu	0.010	-0.100 to 0.121	0.855			
TC	-0.071	-0.228 to 0.086	0.375			
TG	0.024	-0.134 to 0.181	0.766			
HDL-C	-0.095	-0.252 to 0.061	0.231			
LDL-C	-0.082	-0.239 to 0.075	0.304			

ADAMTS-5: a disintegrin and metalloproteinase with thrombospondin type 1 motif-5; MMP-2: matrix metalloproteinase-2; MMP-9: matrix metalloproteinase-9; CRP: C-reactive protein; SBP: systolic blood pressure; DBP: diastolic blood pressure; Glu: fasting glucose; TC: total cholesterol; TG: total triglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

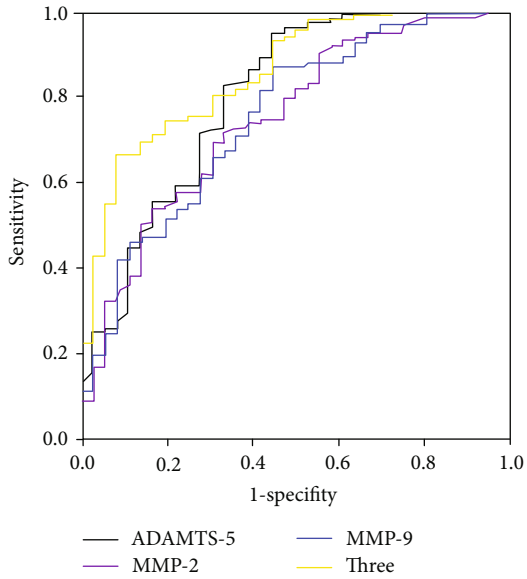

Figure 5: The ROC curve of ADAMTS-5, MMP-2, MMP-9, and combining the three cytokines for the diagnostic value of CAD.

levels also significantly decreased, whereas the plasma MMP-2 and MMP-9 levels increased in the patients with CAD. Meanwhile, the levels of ADAMTS-5 were negatively correlated with the levels of CAD-associated cytokines, such as MMP-2 and MMP-9. More importantly, ROC analysis showed that reduced ADAMTS-5 levels in patients may have a diagnostic value for CAD.

The ECM, known as matrix or stroma, is an essential constituent of the vessel wall and plays a pivotal role in maintaining the structural integrity of the vascular network [24]. ECM remodeling has been reported to be a critical modulator for the pathogenesis of several cardiovascular conditions, including atherosclerosis, restenosis, and heart failure [25–27]. The ECM not only plays an important role in plaque mass by regulating deposition of proteoglycans and collagen but also governs lipid uptake and accumulation of inflammatory cells within the plaque. It is generally accepted that disturbance of the production and degradation of the ECM could result in destabilizing changes in the plaque tissue and thereby increased susceptibility to AMI [28, 29].

ADAMTS-5 is a member of the evolutionary conserved proteoglycanase clade of the ADAMTS superfamily. A previous study has demonstrated that deficiency of ADAMTS-5 contributed to a marked reduction of versikine and resulted in aortic ECM remodeling in angiotensin II-induced thoracic aortic aneurysm [17]. Ozkaramanli et al. reported that serum ADAMTS-5 concentrations were lower in CAD patients with concomitant peripheral artery disease (PAD) when compared with patients with CAD only [30]. Meanwhile, ADAMTS-5 concentrations were a significant predictor of multiple atherosclerotic involvement (CAD+PAD) and had good diagnostic performances to discriminate multiple manifestations of atherosclerosis. This research showed that ADAMTA-5 may be termed as a potential marker for detection of atherosclerotic involvement at multiple vascular territories. In our study, we aimed to detect the expression of ADAMTS-5 in humans with CAD and assayed its predictive power for the severity of coronary stenosis. The results showed that the ADAMTS-5 expression significantly decreased both in coronary arteries and blood samples in CAD patients. Spearman's correlation analysis also showed that the plasma ADAMTS-5 levels were negatively correlated with the Gensini score and CRP. In addition, binary linear regression analyses showed

that ADAMTS-5 was independently associated with the presence of CAD. The results showed that ADAMTS-5 might participate in the onset of CAD and serve as a predictor of CAD.

Components of the ECM, including proteoglycans and collagen, have been identified in atherosclerotic plaques and are synthesized by the VSMCs [31, 32]. It has been demonstrated that the VSMCs play a fundamental role in the pathogenesis of vascular lesions, including lipid retention, ECM production, and plaque integrity maintenance [33, 34]. Previous studies have reported that ADAMTS-5 and proteoglycans were expressed in human coronary arterial VSMCs [16]. Consistent with previous reports, double-immunofluorescence staining indicated that the VSMCs were the major source of ADAMTS-5 in human CAD atherosclerotic plaques. These data also demonstrated that ADAMTS-5 was released from the VSMCs in the arterial wall and ECM turnover was the possible mechanism.

Similar to the ADAMTS family, MMPs are a family of ECM-degrading enzymes and appear to be more active in unstable plaque lesions. Kuzuya et al. reported that MMP-2 deficiency was associated with a decreased incidence of atherosclerotic plaque lesions and accumulation of VSMCs in the ApoE-null mouse [35]. In addition, MMP-9 deficiency in ApoE-null mice also reduced the intimal plaque length, and infiltration of macrophages and migration of the VSMCs [36]. Importantly, accumulating evidence has demonstrated that elevated circulating levels of MMP-2 and MMP-9 were a biomarker for CAD [37–39]. Consistent with previous reports, our results showed that circulating levels of MMP-2 and MMP-9 were remarkably higher in the CAD group than in the control group. In addition, the ROC analysis showed that these three cytokines may have a certain diagnostic value in CAD and that the combination of all three cytokines had a higher diagnostic value. Based on the findings of this analysis, a possible explanation was that decreased ADAMTS-5 levels and increased MMP-2 and MMP-9 levels accelerate ECM remodeling, leading to degradation of the fibrous cap of the vulnerable atherosclerotic plaque and acceleration of plaque rupture.

There are some limitations in our research. First, both the blood sample and coronary artery sample sizes are small, and the patients were not followed up to assess long-term mortality or morbidity. Second, we did not measure the plasma ADAMTS-5 levels in the healthy controls (no chest pain). In conclusion, ADAMTS-5 shows a potential as a novel biomarker for CAD.

Data Availability

Data and material related to this manuscript are available from the corresponding authors on reasonable request.

Conflicts of Interest

All the authors have no conflict of the interest.

Authors' Contributions

Zhen Wang and Di Ye contributed equally to this work and are co-first authors.

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References

[1] E. Falk, M. Nakano, J. F. Bentzon, A. V. Finn, and R. Virmani, "Update on acute coronary syndromes: the pathologists' view," *European Heart Journal*, vol. 34, no. 10, pp. 719–728, 2013.

[2] P. Libby and P. Theroux, "Pathophysiology of coronary artery disease," *Circulation*, vol. 111, no. 25, pp. 3481–3488, 2005.

[3] Y. Kishimoto, S. Ibe, E. Saita et al., "Plasma heme oxygenase-1 levels in patients with coronary and peripheral artery diseases," *Disease Markers*, vol. 2018, Article ID 6138124, 2018.

[4] Z. W. Di Ye, J. Ye, M. Wang et al., "Interleukin-5 levels are decreased in the plasma of coronary artery disease patients and inhibit Th1 and Th17 differentiation in vitro," *Revista Española de Cardiología (English Edition)*, 2019.

[5] M. J. Bom, E. Levin, R. S. Driessen et al., "Predictive value of targeted proteomics for coronary plaque morphology in patients with suspected coronary artery disease," *eBioMedicine*, vol. 39, pp. 109–117, 2019.

[6] C. Frantz, K. M. Stewart, and V. M. Weaver, "The extracellular matrix at a glance," *Journal of Cell Science*, vol. 123, pp. 4195–4200, 2010.

[7] J. Xu and G. P. Shi, "Vascular wall extracellular matrix proteins and vascular diseases," *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, vol. 1842, no. 11, pp. 2106–2119, 2014.

[8] J. E. Wagenseil and R. P. Mecham, "Vascular extracellular matrix and arterial mechanics," *Physiological Reviews*, vol. 89, no. 3, pp. 957–989, 2009.

[9] M. Viola, E. Karousou, M. L. D'Angelo et al., "Extracellular matrix in atherosclerosis: hyaluronan and proteoglycans insights," *Current Medicinal Chemistry*, vol. 23, no. 26, pp. 2958–2971, 2016.

[10] C. Fernandez-Hernando, L. Jozsef, D. Jenkins, A. Di Lorenzo, and W. C. Sessa, "Absence of Akt 1 reduces vascular smooth muscle cell migration and survival and induces features of plaque vulnerability and cardiac dysfunction during atherosclerosis," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 29, no. 12, pp. 2033–2040, 2009.

[11] R. C. Salter, T. G. Ashlin, A. P. L. Kwan, and D. P. Ramji, "ADAMTS proteases: key roles in atherosclerosis?," *Journal of Molecular Medicine (Berlin, Germany)*, vol. 88, no. 12, pp. 1203–1211, 2010.

[12] W. Wu, Y. Zhou, Y. Li et al., "Association between plasma ADAMTS-7 levels and ventricular remodeling in patients with acute myocardial infarction," *European Journal of Medical Research*, vol. 20, no. 1, 2015.

[13] A. Maino, B. Siegerink, L. A. Lotta et al., "Plasma ADAMTS-13 levels and the risk of myocardial infarction: an individual patient data meta-analysis," *Journal of Thrombosis and Haemostasis*, vol. 13, no. 8, pp. 1396–1404, 2015.

[14] W. Wu, H. Wang, C. Yu et al., "Association of ADAMTS-7 levels with cardiac function in a rat model of acute myocardial infarction," *Cellular Physiology and Biochemistry*, vol. 38, no. 3, pp. 950–958, 2016.

[15] H. M. Andersson, B. Siegerink, B. M. Luken et al., "High VWF, low ADAMTS13, and oral contraceptives increase the risk of ischemic stroke and myocardial infarction in young women," *Blood*, vol. 119, no. 6, pp. 1555–1560, 2012.

[16] G. Suna, W. Wojakowski, M. Lynch et al., "Extracellular matrix proteomics reveals interplay of aggrecan and aggrecanases in vascular remodeling of stented coronary arteries," *Circulation*, vol. 137, no. 2, pp. 166–183, 2018.

[17] M. Fava, J. Barallobre-Barreiro, U. Mayr et al., "Role of ADAMTS-5 in aortic dilatation and extracellular matrix remodeling," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 38, no. 7, pp. 1537–1548, 2018.

[18] A. Didangelos, U. Mayr, C. Monaco, and M. Mayr, "Novel role of ADAMTS-5 protein in proteoglycan turnover and lipoprotein retention in atherosclerosis," *The Journal of Biological Chemistry*, vol. 287, no. 23, pp. 19341–19345, 2012.

[19] Z. Wang, M. Wang, J. Liu et al., "Inhibition of TRPA1 attenuates doxorubicin-induced acute cardiotoxicity by suppressing oxidative stress, the inflammatory response, and endoplasmic reticulum stress," *Oxidative Medicine and Cellular Longevity*, vol. 2018, Article ID 5179468, 9 pages, 2018.

[20] Z. Wang, Y. Xu, M. Wang et al., "TRPA1 inhibition ameliorates pressure overload-induced cardiac hypertrophy and fibrosis in mice," *eBioMedicine*, vol. 36, pp. 54–62, 2018.

[21] C. Li, J. W. Chen, Z. H. Liu et al., "CTRP5 promotes transcytosis and oxidative modification of low-density lipoprotein and the development of atherosclerosis," *Atherosclerosis*, vol. 278, pp. 197–209, 2018.

[22] S. Gao, Y. Deng, J. Wu et al., "Eosinophils count in peripheral circulation is associated with coronary artery disease," *Atherosclerosis*, vol. 286, pp. 128–134, 2019.

[23] G. G. Gensini, "A more meaningful scoring system for determining the severity of coronary heart disease," *The American Journal of Cardiology*, vol. 51, no. 3, p. 606, 1983.

[24] J. K. Mouw, G. Ou, and V. M. Weaver, "Extracellular matrix assembly: a multiscale deconstruction," *Nature Reviews. Molecular Cell Biology*, vol. 15, no. 12, pp. 771–785, 2014.

[25] I. M. Chung, H. K. Gold, S. M. Schwartz, Y. Ikari, M. A. Reidy, and T. N. Wight, "Enhanced extracellular matrix accumulation in restenosis of coronary arteries after stent deployment," *Journal of the American College of Cardiology*, vol. 40, no. 12, pp. 2072–2081, 2002.

[26] A. Farb, F. D. Kolodgie, J. Y. Hwang et al., "Extracellular matrix changes in stented human coronary arteries," *Circulation*, vol. 110, no. 8, pp. 940–947, 2004.

[27] M. Lynch, J. Barallobre-Barreiro, M. Jahangiri, and M. Mayr, "Vascular proteomics in metabolic and cardiovascular diseases," *Journal of Internal Medicine*, vol. 280, no. 4, pp. 325–338, 2016.

[28] D. A. Chistiakov, I. A. Sobenin, and A. N. Orekhov, "Vascular extracellular matrix in atherosclerosis," *Cardiology in Review*, vol. 21, no. 6, pp. 270–288, 2013.

[29] J. A. Schwanekamp, A. Lorts, R. J. Vagnozzi, D. Vanhoutte, and J. D. Molkentin, "Deletion of periostin protects against atherosclerosis in mice by altering inflammation and extracellular matrix remodeling," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 36, no. 1, pp. 60–68, 2016.

[30] G. D. Ozkaramanli, S. Guzel, A. Akyuz, S. Alpsoy, and N. Guler, "The role of novel cytokines in inflammation: defining peripheral artery disease among patients with coronary artery disease," *Vascular Medicine*, vol. 23, no. 5, pp. 428–436, 2018.

[31] T. N. Wight and M. J. Merrilees, "Proteoglycans in atherosclerosis and restenosis: key roles for versican," *Circulation Research*, vol. 94, no. 9, pp. 1158–1167, 2004.

[32] T. N. Wight, "Arterial remodeling in vascular disease: a key role for hyaluronan and versican," *Frontiers in Bioscience*, vol. 13, pp. 4933–4937, 2008.

[33] M. R. Bennett, S. Sinha, and G. K. Owens, "Vascular smooth muscle cells in atherosclerosis," *Circulation Research*, vol. 118, no. 4, pp. 692–702, 2016.

[34] M. Grootaert, M. Moulis, L. Roth et al., "Vascular smooth muscle cell death, autophagy and senescence in atherosclerosis," *Cardiovascular Research*, vol. 114, no. 4, pp. 622–634, 2018.

[35] M. Kuzuya, K. Nakamura, T. Sasaki, X. W. Cheng, S. Itohara, and A. Iguchi, "Effect of MMP-2 deficiency on atherosclerotic lesion formation in ApoE-Deficient mice," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 26, no. 5, pp. 1120–1125, 2006.

[36] E. T. Choi, E. T. Collins, L. A. Marine et al., "Matrix metalloproteinase-9 modulation by resident arterial cells is responsible for injury-induced accelerated atherosclerotic plaque development in apolipoprotein E-deficient mice," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, no. 5, pp. 1020–1025, 2005.

[37] M. A. Hlatky, E. Ashley, T. Quertermous et al., "Matrix metalloproteinase circulating levels, genetic polymorphisms, and susceptibility to acute myocardial infarction among patients with coronary artery disease," *American Heart Journal*, vol. 154, no. 6, pp. 1043–1051, 2007.

[38] G. M. Hamed and M. F. Fattah, "Clinical relevance of matrix metalloproteinase 9 in patients with acute coronary syndrome," *Clinical and Applied Thrombosis/Hemostasis*, vol. 21, no. 8, pp. 705–711, 2015.

[39] O. S. Dhillon, S. Q. Khan, H. K. Narayan et al., "Matrix metalloproteinase-2 predicts mortality in patients with acute coronary syndrome," *Clinical Science (London, England)*, vol. 118, pp. 249–257, 2009.

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Research Article

Favorable Response to CD34+ Cell Therapy Is Associated with a Decrease of Galectin-3 Levels in Patients with Chronic Heart Failure

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Background. Galectin-3 plasma levels (gal-3) were shown to correlate with the scar burden in chronic heart failure (CHF) setting. As scar burden predicts response to stem cell therapy, we sought to explore a correlation between gal-3 and response to CD34+ cell transplantation in patients with CHF. *Methods*. We performed a post hoc analysis of patients, enrolled in 2 prospective trials investigating the clinical effects of CD34+ cell therapy in patients with ischemic cardiomyopathy (ICMP) and nonischemic dilated cardiomyopathy (DCMP). CD34+ cells were mobilized by G-CSF, collected via apheresis, and injected transendocardially using NOGA system. Patients were followed for 3 months and demographic, echocardiographic, and biochemical parameters and gal-3 were analyzed at baseline and at follow-up. Response to cell therapy was defined as an LVEF increase of ≥5%. *Results*. 61 patients were included in the analysis. The mean age of patients was 52 years and 83% were male. DCMP and ICMP were present in 69% and 31% of patients, respectively. The average serum creatinine was 86 ± 23 μmol/L, NT-proBNP 1132 (IQR 350-2279) pg/mL, and LVEF 30 ± 6%. Gal-3 at baseline and at 3 months did not differ significantly (13.4 ± 5.5 ng/mL vs. 13.1 ± 5.8 ng/mL; $p = 0.72$), and there were no differences in baseline gal-3 with respect to heart failure etiology (15.1 ± 7.2 ng/mL in ICMP vs. 12.7 ± 4.3 ng/mL in DCMP; $p = 0.12$). Comparing responders ($N = 49$) to nonresponders ($N = 18$), we found no differences in baseline gal-3 (13.6 ± 5.7 ng/mL vs. 13.2 ± 4.9 ng/mL; $p = 0.80$). However, responders had significantly lower gal-3 at 3-month follow-up (12.1 ± 4.0 ng/mL vs. 15.7 ± 8.4 ng/mL; $p < 0.05$). Also, responders demonstrated a significant decrease in gal-3 over 3 months, while in nonresponders, an increase in gal-3 occurred (−1.5 ± 5.4 ng/mL vs. +2.7 ± 4.3 ng/mL; $p = 0.01$). *Conclusions*. In patients with chronic heart failure undergoing CD34+ cell therapy, a decrease in galectin-3 plasma levels is associated with beneficial response to this treatment modality. Further prospective data is warranted to confirm our findings and to deepen our understanding of the role of gal-3 in the field of stem cell therapy.

1. Introduction

In patients with chronic heart failure, stem cell therapy with the use of different stem cell types and different routes of stem cell delivery has recently been shown to improve myocardial performance, decrease neurohumoral activation, and improve exercise capacity and quality of life, and there was even a signal towards improved survival in this patient cohort [1–9].

Although these results are encouraging currently, there is still no consensus regarding the optimal patient selection for this treatment strategy. Published data suggest a

variable response to stem cell therapy with, depending on the definition of response, 30-60% of patients failing to adequately respond to this treatment option [2, 3, 9, 10]. These differences may be related to several patient- and procedure-related factors, such as the underlying cause and the duration of heart failure, heart failure stage and end-organ function, type and quantity of injected stem cells, and the method of stem cell delivery (intracoronary vs. transendocardial vs. transepicardial) amongst others [3, 9, 11].

Additionally, using electroanatomical data, it was demonstrated that scar burden of the failing myocardium may also play a significant role in determining the response to stem cell therapy [10]. However, invasive scar burden assessment is not feasible for routine clinical use and accurate non-invasive determination of myocardial scar burden (with myocardium imaging or serum biomarkers) that could be used to improve patient selection for stem cell therapy remains a challenge.

Recently, our group explored the potential of serum biomarkers to predict response to CD34+ cell therapy in patients with nonischemic dilated cardiomyopathy [12]. Our data failed to establish a correlation between the response to stem cell therapy, defined as improvement of LVEF ≥ 5% at 3 months post stem cell injection, and factors, traditionally related to poor prognosis in heart failure population, such as patient age, gender, exercise capacity, baseline left ventricular ejection fraction (LVEF), and baseline serum levels of NT-proBNP. However, we were able to show that responders on average mobilized higher number of CD34+ cells ($119 \pm 68 \times 106$ vs. $74 \pm 55 \times 106$; $p = 0.03$) and had higher myocardial cell retention rates ($14 \pm 5\%$ vs. $9 \pm 5\%$; $p = 0.01$). Additionally, we were able to identify several immunologic and nonimmunologic biomarkers and metabolic factors, associated with myocardial inflammation, stem cell mobilization and retention, cell growth, and metabolic alterations in the failing myocardium that were positively or negatively independently associated with the response to CD34+ cell therapy [12]. Importantly, biomarkers that may be directly related to myocardial fibrosis were not addressed in this analysis.

In the past decade, galectin-3 has been widely explored in chronic heart failure setting and has been established as a biomarker independently associated with left ventricular remodeling and adverse prognosis in terms of heart failure-related hospitalizations and increased mortality in this patient cohort [13–15]. Galectin-3 is a β-galactoside-binding lectin that is best known for its involvement in tumor growth and metastasis. In chronic heart failure, galectin-3 has been demonstrated to be involved in several pathophysiological processes, including myocardial inflammation and fibrosis, which are both pivotal mechanisms of heart failure development and progression [16].

Currently, the potential underlying mechanisms of the favorable response to stem cell therapy remain undefined. We therefore sought to explore a correlation between changes in galectin-3 plasma levels and response to transendocardial CD34+ cell transplantation in patients with chronic heart failure.

2. Methods

2.1. Patient Population. We performed a post hoc analysis of patients, enrolled in 2 prospective open-label trials investigating the clinical effects of transendocardial CD34+ cell therapy in patients with ischemic cardiomyopathy (ICMP, NCT01350310) and nonischemic dilated cardiomyopathy (DCMP, NCT02445534). The study protocol was approved by the National Medical Ethics Committee. Firstly, 125 patients who underwent stem cell therapy between January 1, 2013, and December 31, 2016, were screened for the study. Inclusion criteria were as follows: age (18-65 years), optimal medical management for ≥6 months, LVEF < 40%, and diagnosis of DCMP according to the European Society of Cardiology position statement [17] or diagnosis of ICM without any option for further percutaneous or surgical myocardial revascularization [18]. Patients with acute coronary syndrome or hospitalization for worsening heart failure requiring inotropic support within 12 months before stem cell therapy, patients with heart failure with preserved ejection fraction, patients who received repetitive stem cell application, and patients with end-stage renal disease or liver cirrhosis were excluded from the study. Chronic kidney disease (CKD) was defined as serum creatinine > 100 mmol/L. Ultimately, 61 patients met the proposed criteria and were included in the study and an informed consent was obtained from all patients before participation in the study.

2.2. Study Design. All patients underwent stem cell mobilization and collection as per institution's protocol as outlined in detail previously by our research group [2, 3, 19]. After 5-day granulocyte colony-stimulating factor therapy (5 μg/kg BID), CD34+ cells were collected through apheresis of peripheral blood. Then, all patients underwent electroanatomical mapping and transendocardial CD34+ cell implantation. In patients who met the inclusion criteria, demographic, echocardiographic, and biochemical parameters and galectin-3 plasma levels were analyzed at the time of stem cell transplantation (baseline) and at 3-month follow-up.

2.3. Peripheral Blood Stem Cell Mobilization and Collection. All patients underwent stem cell mobilization and collection as described previously in detail [2, 3, 19, 20]. In short, following the methods of Vrtovec et al. [2, 3] and Poglajen et al. [19], bone marrow cells were mobilized into peripheral blood by daily subcutaneous injections of G-CSF (5 μg/kg BID). On the fifth day, bone marrow mononuclear cells were collected via cytapheresis with Miltenyi cell separator (Miltenyi Biotec, Germany). Then immunoselection of collected cells was performed with Isolex 300i (Nexell Therapeutics Inc., CA). Selected CD34+ cells were stored in the cell collection bag and additionally concentrated to a final volume of 6 mL.

2.4. Electroanatomical Mapping and Transendocardial 34+ Cell Delivery. Electroanatomical mapping is a procedure performed with the Biosense NOGA system (Biosense Webster, Diamond Bar, CA). This platform allows for point-by-point analysis of left ventricular viability and local contractility [21]. Using this technique, maps of color-coded myocardial

viability (unipolar voltage, UV) and regional myocardial contraction (linear shortening, LLS) and their corresponding bull's-eye maps, consisting of ≥200 sampling points, were generated for each patient prior to stem cell transplantation. Hibernating myocardium was defined as areas with UV ≥ 8.3 mV and LLS < 6%; myocardial scar was defined as segments with UV < 8.3 mV and LLS < 6% [2, 3, 22]. Transendocardial delivery of cell suspension was done using the MyoStar (Biosense Webster) injection catheter. Each patient received 20 injections of stem cell suspension, 0.3 mL of cell suspension per injection; all injections were performed within the areas of myocardial hibernation (UV ≥ 8.3 mV and LLS < 6%).

2.5. Echocardiography, 6-Minute Walk Test, and NT-proBNP Measurements. The echocardiography data were recorded and analyzed at the end of the study by an independent echocardiographer who was blinded to patient's treatment status and the timing of the recordings. Left ventricular end-systolic volume and end-diastolic volume and LVEF were estimated using Simpson's biplane method, and left ventricular end-systolic dimension and end-diastolic dimension were measured in the parasternal long-axis view. All echocardiographic measurements were averaged for 5 cycles [2, 3]. In all patients, a 6-minute walk test was performed by a blinded observer according to the standard protocol [23]. All NT-proBNP assays were performed at a central independent laboratory, blinded to patient's clinical data using a commercially available kit (Roche Diagnostics, Mannheim, Germany).

2.6. Galectin-3 Measurement. Galectin-3 plasma levels were determined in an independent laboratory blinded to patient clinical data on stored specimens (drawn prior to G-CSF stimulation, stored at -80°C, thawed once) using an enzyme-linked immunosorbent assay (BG Medicine, Waltham, MA, USA). This assay quantitatively measures the concentration of human galectin-3 levels in EDTA plasma and has been shown to have high sensitivity (with a lower detection limit of 1.13 ng/mL) and no cross-reactivity with other members of the galectin family, or with other collagens, and no known interference by commonly used heart failure medications.

2.7. Definition of Clinical Response. Response to stem cell therapy was defined as an absolute increase in LVEF of ≥5% at 3 months after CD34+ cell therapy [2, 3]. The threshold of 5% was chosen based on meta-analysis of heart failure trials showing that a 5% increase in the mean EF change corresponded to a relative odds ratio of 0.86 (95% confidence interval (CI) 0.77-0.96) for mortality [24].

2.8. Statistical Methods and Analysis. Continuous variables are presented as mean (±SD) or median (interquartile range) where appropriate and categorical data are given as count (percent). Categorical variables were compared with the chi-squared test (or Fischer exact nonparametric test). All continuous variables were compared using Student's *t*-test and ANOVA (or Mann-Whitney nonparametric test). Statistical significance was assumed for p values of <0.05. All statistical analyses were performed with SPSS software (version 20.0).

3. Results

3.1. Patient Characteristics. Detailed baseline patient characteristics are outlined in Table 1. The majority of included subjects were middle-aged male patients with LVEF around 30%. Neurohumoral activation was significantly elevated with the median NT-proBNP serum levels reaching 1132 pg/mL. End-organ function was not significantly affected in any of patients and no significant electrolyte disturbances or complete blood count deviations were noted. All patients received optimal evidence-based heart failure therapy that was not altered during the study period. Looking at the entire patient cohort, galectin-3 plasma levels did not change significantly between baseline and 3-month follow-up (Figure 1).

3.2. Galectin-3 and Heart Failure Etiology. We further compared patients based on heart failure etiology. No changes were found between patients with ICMP ($N = 19$) and DCMP ($N = 42$) considering age, gender, LVEF, functional capacity, neurohumoral activation, or end-organ function. Additionally, there were no significant differences regarding heart failure medical management observed between the two groups with the exception of antiaggregation therapy which was significantly higher in the ICMP group as expected. Importantly, the two groups did not differ with respect to the galectin-3 plasma levels at the baseline and at 3-month follow-up (Figure 1).

3.3. Changes in Galectin-3 Levels and Response to CD34+ Cell Therapy. We further stratified patients conditional to the response to CD34+ cell therapy (Table 2). The nonresponders ($N = 18$) and responders ($N = 43$) were similar with respect to age, gender, heart failure etiology, LVEF, and exercise capacity. Also, liver function tests, complete blood count, and heart failure medical therapy did not differ between the two groups. However, in comparison to responders, nonresponders had significantly higher serum levels of NT-proBNP and creatinine, higher RDW, and lower baseline LVEF. Considering galectin-3, baseline plasma levels did not differ between the two groups (Table 2). Importantly, galectin-3 plasma levels at 3 months were significantly lower in responders than in nonresponders. Furthermore, a net decrease in plasma galectin-3 levels was significantly higher in responders than in nonresponders in which plasma galectin-3 values actually increased (change in galectin-3: -1.5 ± 5.4 ng/mL in responders vs. $+2.7 \pm 4.3$ ng/mL in nonresponders; $p = 0.01$) (Figure 2).

Univariate correlates of response to CD34+ cell therapy were subsequently analyzed in a multivariate model which showed that a decrease in galectin-3 plasma levels between baseline and 3-month follow-up and baseline serum NT-proBNP > 1000 pg/mL were independent correlates of response to CD34+ cell therapy (Table 3).

Table 1: Baseline patient characteristics. Values are presented as mean ± SD, number of patients (percent), or median (IQR) for NT-proBNP.

	All ($N = 61$)	ICMP ($N = 19$)	DCMP ($N = 42$)	p
Age (y)	52 ± 12	55 ± 8	51 ± 10	0.16
Male gender (%)	51 (83)	15 (79)	36 (85)	0.54
LVEF (%)	30 ± 6	29 ± 6	30 ± 5	0.41
6MWT (m)	482 ± 82	475 ± 82	486 ± 83	0.92
NT-proBNP (pg/mL (IQR))	1132 (350-2279)	1133 (394-2239)	1127 (333-2359)	0.99
Creatinine (mmol/L)	86 ± 23	89 ± 21	84 ± 24	0.52
Bilirubin (μmol/L)	16 ± 8	16 ± 6	17 ± 9	0.75
Sodium (mmol/L)	140 ± 2	140 ± 3	141 ± 2	0.63
AST (μmol/L)	0.5 ± 0.1	0.45 ± 0.14	0.47 ± 0.14	0.62
AF (μmol/L)	1.2 ± 0.4	1.21 ± 0.33	1.13 ± 0.39	0.39
gGT (μmol/L)	1.1 ± 1.4	0.68 ± 0.38	1.2 ± 1.4	0.10
RDW (%)	13.7 ± 1.2	13.7 ± 1.1	13.7 ± 1.2	0.92
Leukocytes (×109)	7.1 ± 1.6	6.9 ± 1.4	7.2 ± 1.6	0.47
Hemoglobin (g/L)	144 ± 11	146 ± 10	143 ± 12	0.32
Platelet count (×109)	208 ± 49	198 ± 34	213 ± 53	0.27
Medical management				
ACEI/ARB	61 (100)	19 (100)	42 (100)	/
Beta blockers	61 (100)	19 (100)	42 (100)	/
MRA	61 (100)	19 (100)	42 (100)	/
Diuretic	33 (54)	12 (63)	21 (51)	0.34
Aspirin	21 (34)	19 (100)	2 (4)	<0.05
Galectin-3 levels (ng/mL)				
Baseline	13.4 ± 5.5	15.1 ± 7.2	12.7 ± 4.3	0.12
3 months	13.1 ± 5.8	13.3 ± 5.8	12.4 ± 5.8	0.83

ICMP: ischemic cardiomyopathy; DCMP: nonischemic cardiomyopathy; LVEF: left ventricular ejection fraction; 6MWT: 6-minute walk test; AST: aspartate transaminase; AF: alkaline phosphatase; gGT: γ-glutamyltranspeptidase; RDW: red cell distribution width; ACEI: angiotensin convertase inhibitor; ARB: angiotensin receptor blocker; MRA: mineralocorticoid receptor antagonist.

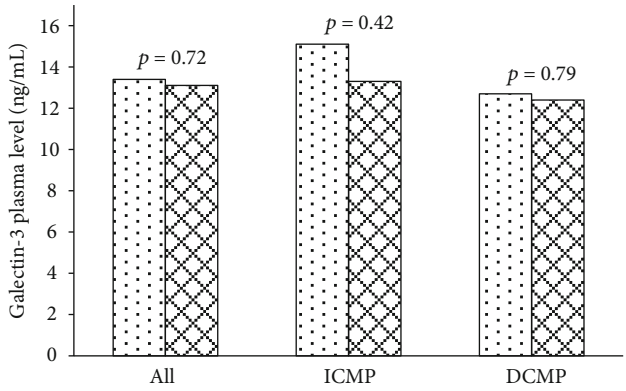

Figure 1: Comparison of plasma galectin-3 baseline (spotted bars) and follow-up (checkered bars) values found no differences when compared in the entire patient cohort and separately in patients with ICMP and DCMP.

4. Discussion

This is the first study evaluating a correlation between changes of galectin-3 plasma levels and a response to CD34+ cell therapy. Despite the lack of association between baseline galectin-3 plasma levels and a response to CD34+ cell therapy, we were able to demonstrate that within 3 months after the procedure galectin-3 plasma decreases significantly more in clinical responders to CD34+ cell therapy.

Galectin-3, a β-galactoside-binding lectin, is a protein that has recently been heavily implicated in the pathophysiology of myocardial fibrosis. While it can be secreted by injured cardiomyocytes, activated M2 macrophages are believed to represent the main source of galectin-3 in the failing myocardium. Galectin-3 has been shown to promote proliferation of fibroblasts and collagen I deposition in extracellular matrix, most likely through a transforming growth factor-β pathway [16, 25]. In preclinical setting, the infusion of galectin-3 in the pericardial sac of normal animals was associated with the development of significant myocardial fibrosis, cardiac remodeling, and subsequent heart failure. The results from the same research group further suggested that levels of galectin-3 correlated significantly with the degree of myocardial fibrosis [26]. Moreover, a significant reduction in cardiac fibrosis occurred with genetic or pharmacological inhibition of galectin-3, further substantiating a central role of galectin-3 in promoting profibrotic processes in the failing

Table 2: Comparison of characteristics of responders and nonresponders to CD34+ cell therapy. Values are presented as mean ± SD, number of patients (percent), or median (IQR) for NT-proBNP.

	Nonresponders ($N = 18$)	Responders ($N = 43$)	p
Age (y)	55 ± 10	51 ± 13	0.32
Male gender (%)	15 (85)	36 (84)	0.96
Nonischemic CMP (%)	13 (72)	29 (67)	0.67
LVEF (%)			
Baseline	27 ± 6	31 ± 5	<0.05
3 months	28 ± 6	39 ± 7	<0.05
6MWT (m)	464 ± 101	490 ± 73	0.28
NT-proBNP (pg/mL (IQR))	3245 (1584-4336)	609 (240-1323)	<0.05
Creatinine (mmol/L)	101 ± 30	79 ± 16	<0.05
Bilirubin (μmol/L)	17 ± 9	16 ± 8	0.48
Sodium (mmol/L)	140 ± 2	141 ± 2	0.84
AST (μmol/L)	0.5 ± 0.1	0.4 ± 0.1	0.10
AF (μmol/L)	1.2 ± 0.4	1.1 ± 0.4	0.60
gGT (μmol/L)	1.2 ± 1.3	1.1 ± 1.4	0.68
RDW (%)	14.3 ± 1.4	13.4 ± 0.9	0.01
Leukocytes (×109)	7.1 ± 1.9	7.1 ± 1.4	0.85
Hemoglobin (g/L)	142 ± 14	144 ± 10	0.44
Platelet count (×109)	207 ± 69	209 ± 37	0.89
Medical management			
ACEI/ARB	18 (100)	42 (100)	/
Beta blockers	18 (100)	42 (100)	/
MRA	18 (100)	42 (100)	/
Diuretic	12 (67)	21 (48)	0.24
Aspirin	7 (39)	26 (60)	0.13
Galectin-3 levels (ng/mL)			
Baseline	13.2 ± 4.9	13.6 ± 5.7	0.80
3 months	15.7 ± 8.4	12.1 ± 4.0	<0.05

ICMP: ischemic cardiomyopathy; DCMP: nonischemic cardiomyopathy; LVEF: left ventricular ejection fraction; 6MWT: 6-minute walk test; AST: aspartate transaminase; AF: alkaline phosphatase; gGT: γ-glutamyltranspeptidase; RDW: red cell distribution width; ACEI: angiotensin convertase inhibitor; ARB: angiotensin receptor blocker; MRA: mineralocorticoid receptor antagonist.

myocardium [27, 28]. In the clinical setting, galectin-3 has been established as a biomarker of adverse left ventricular remodeling and heart failure morbidity and mortality [13–15]. Our data further showed that galectin-3 plasma levels in patients with ischemic and nonischemic heart failure do not differ significantly. This is in line with our previous observations that showed no differences in the myocardial scar burden (assessed by electromechanical mapping) in the two patient cohorts (53 ± 18% in ICMP vs. 55 ± 23% in DCMP; $p = 0.83$), suggesting a common pathophysiological pathway despite different initial injury to the myocardium [10]. Although direct histochemical correlation of scar burden and galectin-3 in humans is lacking, galectin-3 has been shown to correlate with the presence of myocardial replacement fibrosis as assessed by cardiac MRI using late gadolinium enhancement approach [29]. Despite this hypothesis, we failed to demonstrate a correlation between baseline galectin-3 levels and response to CD34+ cell therapy. This is in line with the observation that although levels of galectin measured in myocardial tissue samples correlate well with the degree of myocardial fibrosis the correlation of circulating galectin-3 levels and cardiac fibrosis is not as strong [30].

Cardiac fibrosis is a basic constituent of most cardiac pathologies and represents a universal response of the myocardium to all forms of cardiac injury [31]. Although essential to the healing process, enhanced extracellular matrix synthesis and collagen I deposition have been associated with increased myocardial stiffness, interruption of cardiomyocyte electrical coupling, and diminished oxygen and nutrient flow, aggravating the remodeling of the failing myocardium [32, 33]. Interestingly, despite significant impact of cardiac fibrosis on cardiac structure, function, and patient prognosis, current medical and device-based treatment options have largely failed to inhibit cardiac fibrosis formation and replace the lost cardiomyocyte mass with the new structurally and functionally integrated contractile cells.

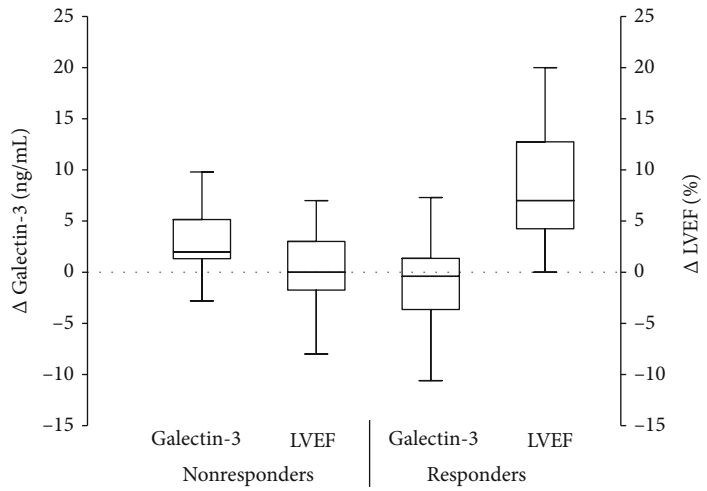

Figure 2: Comparison of plasma galectin-3 level changes between responders and demonstrated that galectin-3 serum levels decreased in responders but increased in nonresponders at 3-month follow-up. Furthermore, LVEF did not change significantly in nonresponders but increased significantly in responders.

Table 3: Multivariate predictors of response to CD34+ cell therapy.

Variable	*B* coefficient	*p* value	95% confidence interval	
			Lower bound	Upper bound
LVEF < 30%	0.122	0.220	-0.075	0.319
CKD	-0.201	0.120	-0.456	0.054
NT-proBNP > 1000 pg/mL	-0.245	0.031	-0.467	-0.023
RDW > 14.5%	-0.121	0.330	-0.367	0.126
Galectin-3 decrease	0.306	0.003	0.113	0.498

LVEF: left ventricular ejection fraction; CKD: chronic kidney disease; RDW: red cell distribution width.

Recently, several preclinical and clinical studies have consistently demonstrated that stem cell therapy improves myocardial perfusion and reduces scar burden in the failing myocardium [6, 7, 34, 35]. Caduceus trial showed that 12 months after intracoronary infusion of cardiosphere-derived cells (Stem Cell Group), myocardial scar burden decreased by 12.3% whereas in the Controls only 2.2% scar reduction was observed ($p = 0.001$). Additionally, regional contractility improved significantly more in the Stem Cell Group than in the Controls ($p = 0.001$) [7]. These data were further corroborated by data from Prometheus trial using transepicardial injections of autologous mesenchymal stem cells. The study showed significant reduction in scar mass ($-48 \pm 8\%$, $p < 0.00001$) and improved global LVEF ($+9 \pm 2\%$, $p = 0.0002$) compared to baseline values, with improved perfusion and contractile properties of the injected segments [36]. These results are in line with our data where responders to CD34+ cell therapy showed a significant decrease in galectin-3 plasma levels and concomitant improvement in LVEF. A decrease in galectin-3 plasma levels thus very likely reflects the reduction of the myocardial scar burden in this patient subgroup. On the other hand, galectin-3 plasma levels increased in nonresponders potentially suggesting that whether or not the patient will respond to stem cell therapy may partially depend on the capacity of the injected stem cells to effectively reduce scar burden in the failing myocardium. However, given the heterogeneous patient population and different stem cell types used in these trials, this speculation should be viewed as hypothesis generating and needs to be validated in prospective settings.

Interestingly, despite nonresponders generally appearing sicker and having lower LVEF, higher serum NT-proBNP, and worse renal function, baseline galectin-3 plasma levels were not significantly different from the levels seen in responders. The most likely explanation for this apparent mismatch is that LVEF, NT-proBNP, and renal function are markers of the patient's hemodynamic and volume status whereas galectin-3 reflects structural changes in the failing myocardium which only inconsistently correlate with patient's hemodynamics [16].

This study has several limitations. For one, we have performed a post hoc analysis of the patients included in two randomized clinical trials. However, all patients received the same CD34+ cell type and were treated using the same cell mobilization protocols and transendocardial cell injection procedure using NOGA system; thus, we believe that patient population included in our study was sufficiently homogeneous for the analysis. Additionally, only 13% of our patients had galectin-3 levels higher than 17.9 ng/mL which is currently accepted as the cut-off value for worse patient prognosis which means that prognostic significance

of baseline galectin-3 plasma levels on patient response to CD34+ cell therapy may have been underestimated. Also, our analysis was performed on fairly small number of patients. Finally, due to the retrospective design of the study, we were not able to perform any noninvasive imaging to quantify the degree of fibrosis in our patient cohort.

5. Conclusions

To date, this is the first study to explore an association between changes in galectin-3 plasma levels and response to stem cell therapy in patients with chronic heart failure. Although in the analyzed patient cohort baseline galectin-3 plasma levels did not predict the response to stem cell therapy, our data suggests that a decrease in galectin-3 plasma levels at 3-month follow-up correlates with a response to CD34+ cell therapy. This suggests that the effects of stem cells on scar burden may play a significant role in patients' response to stem cell therapy. Further prospective trials are warranted to confirm our initial findings and to deepen our understanding of galectin-3 in the field of stem cell therapy.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

None of the authors has any conflicts of interest pertinent to this publication.

Acknowledgments

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References

[1] E. C. Perin, H. F. Dohmann, R. Borojevic et al., "Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure," *Circulation*, vol. 107, no. 18, pp. 2294–2302, 2003.

[2] B. Vrtovec, G. Poglajen, M. Sever et al., "Effects of intracoronary stem cell transplantation in patients with dilated cardiomyopathy," *Journal of Cardiac Failure*, vol. 17, no. 4, pp. 272–281, 2011.

[3] B. Vrtovec, G. Poglajen, L. Lezaic et al., "Effects of intracoronary CD34+ stem cell transplantation in nonischemic dilated cardiomyopathy patients: 5-year follow-up," *Circulation Research*, vol. 112, no. 1, pp. 165–173, 2013.

[4] G. Poglajen, M. Sever, M. Cukjati et al., "Effects of transendocardial $CD34^+$ cell transplantation in patients with ischemic cardiomyopathy," *Circulation Cardiovascular Interventions*, vol. 7, no. 4, pp. 552–559, 2014.

[5] S. Frljak, M. Jaklic, G. Zemljic, A. Cerar, G. Poglajen, and B. Vrtovec, "$CD34^+$ cell transplantation improves right ventricular function in patients with nonischemic dilated cardiomyopathy," *Stem Cells Translational Medicine*, vol. 7, no. 2, pp. 168–172, 2018.

[6] J. M. Hare, D. L. DiFede, A. C. Rieger et al., "Randomized comparison of allogeneic versus autologous mesenchymal stem cells for nonischemic dilated cardiomyopathy: POSEIDON-DCM trial," *Journal of the American College of Cardiology*, vol. 69, no. 5, pp. 526–537, 2017.

[7] K. Malliaras, R. R. Makkar, R. R. Smith et al., "Intracoronary cardiosphere-derived cells after myocardial infarction: evidence of therapeutic regeneration in the final 1-year results of the CADUCEUS trial (CArdiosphere-Derived aUtologous stem CElls to reverse ventricUlar dySfunction)," *Journal of the American College of Cardiology*, vol. 63, no. 2, pp. 110–122, 2014.

[8] A. B. Mathiasen, A. A. Qayyum, E. Jørgensen et al., "Bone marrow-derived mesenchymal stromal cell treatment in patients with severe ischaemic heart failure: a randomized placebo-controlled trial (MSC-HF trial)," *European Heart Journal*, vol. 36, no. 27, pp. 1744–1753, 2015.

[9] B. Assmus, U. Fischer-Rasokat, J. Honold et al., "Transcoronary transplantation of functionally competent BMCs is associated with a decrease in natriuretic peptide serum levels and improved survival of patients with chronic postinfarction heart failure: results of the TOPCARE-CHD Registry," *Circulation Research*, vol. 100, no. 8, pp. 1234–1241, 2007.

[10] G. Zemljic, G. Poglajen, M. Sever et al., "Electroanatomic properties of the myocardium predict response to CD34+ cell therapy in patients with ischemic and nonischemic heart failure," *Journal of Cardiac Failure*, vol. 23, no. 2, pp. 153–160, 2017.

[11] B. Vrtovec, G. Poglajen, L. Lezaic et al., "Comparison of transendocardial and intracoronary CD34+ cell transplantation in patients with nonischemic dilated cardiomyopathy," *Circulation*, vol. 128, no. 11, Supplement 1, pp. S42–S49, 2013.

[12] F. Haddad, M. Sever, G. Poglajen et al., "Immunologic network and response to intramyocardial $CD34^+$ stem cell therapy in patients with dilated cardiomyopathy," *Journal of Cardiac Failure*, vol. 21, no. 7, pp. 572–582, 2015.

[13] A. R. van der Velde, L. Gullestad, T. Ueland et al., "Prognostic value of changes in galectin-3 levels over time in patients with heart failure: data from CORONA and COACH," *Circulation Heart Failure*, vol. 6, no. 2, pp. 219–226, 2013.

[14] R. R. van Kimmenade, J. L. Januzzi Jr., P. T. Ellinor et al., "Utility of amino-terminal pro-brain natriuretic peptide, galectin-3, and apelin for the evaluation of patients with acute heart failure," *Journal of the American College of Cardiology*, vol. 48, no. 6, pp. 1217–1224, 2006.

[15] D. J. Lok, S. I. Lok, P. W. Bruggink-André de la Porte et al., "Galectin-3 is an independent marker for ventricular remodeling and mortality in patients with chronic heart failure," *Clinical Research in Cardiology*, vol. 102, no. 2, article 500, pp. 103–110, 2013.

[16] G. Suarez and G. Meyerrose, "Heart failure and galectin 3," *Annals of Translational Medicine*, vol. 2, no. 9, p. 86, 2014.

[17] P. Elliott, B. Andersson, E. Arbustini et al., "Classification of the cardiomyopathies: a position statement from the European Society Of Cardiology Working Group on Myocardial and Pericardial Diseases," *European Heart Journal*, vol. 29, no. 2, pp. 270–276, 2008.

[18] P. Ponikowski, A. A. Voors, S. D. Anker et al., "2016 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure: the task force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of

the Heart Failure Association (HFA) of the ESC," *European Journal of Heart Failure*, vol. 18, no. 8, pp. 891–975, 2016.

[19] G. Poglajen, G. Zemljič, A. Cerar et al., "Transendocardial CD34+ cell therapy does not increase the risk of ventricular arrhythmias in patients with chronic heart failure," *Cell Transplantation*, vol. 28, no. 7, pp. 856–863, 2019.

[20] P. Dreger, T. Haferlach, V. Eckstein et al., "G-CSF-mobilized peripheral blood progenitor cells for allogeneic transplantation: safety, kinetics of mobilization, and composition of the graft," *British Journal of Haematology*, vol. 87, no. 3, pp. 609–613, 1994.

[21] M. Gyöngyösi and N. Dib, "Diagnostic and prognostic value of 3D NOGA mapping in ischemic heart disease," *Nature Reviews Cardiology*, vol. 8, no. 7, pp. 393–404, 2011.

[22] B. Campos, M. E. Jauregui, K. M. Park et al., "New unipolar electrogram criteria to identify irreversibility of nonischemic left ventricular cardiomyopathy," *Journal of the American College of Cardiology*, vol. 60, no. 21, pp. 2194–2204, 2012.

[23] L. G. Olsson, K. Swedberg, A. L. Clark, K. K. Witte, and J. G. Cleland, "Six minute corridor walk test as an outcome measure for the assessment of treatment in randomized, blinded intervention trials of chronic heart failure: a sys-tematic review," *European Heart Journal*, vol. 26, no. 8, pp. 778–793, 2005.

[24] D. G. Kramer, T. A. Trikalinos, D. M. Kent, G. V. Antonopoulos, M. A. Konstam, and J. E. Udelson, "Quantitative evaluation of drug or device effects on ventricular remodeling as predictors of therapeutic effects on mortality in patients with heart failure and reduced ejection fraction: a meta-analytic approach," *Journal of the American College of Cardiology*, vol. 56, no. 5, pp. 392–406, 2010.

[25] N. Suthahar, W. C. Meijers, H. H. W. Silljé, J. E. Ho, F. T. Liu, and R. A. de Boer, "Galectin-3 activation and inhibition in heart failure and cardiovascular disease: an update," *Theranostics*, vol. 8, no. 3, pp. 593–609, 2018.

[26] U. C. Sharma, S. Pokharel, T. J. van Brakel et al., "Galectin-3 marks activated macrophages in failure-prone hypertrophied hearts and contributes to cardiac dysfunction," *Circulation*, vol. 110, no. 19, pp. 3121–3128, 2004.

[27] L. Yu, W. P. T. Ruifrok, M. Meissner et al., "Genetic and pharmacological inhibition of galectin-3 prevents cardiac remodeling by interfering with myocardial fibrogenesis," *Circulation Heart Failure*, vol. 6, no. 1, pp. 107–117, 2013.

[28] X. Song, X. Qian, M. Shen et al., "Protein kinase C promotes cardiac fibrosis and heart failure by modulating galectin-3 expression," *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, vol. 1853, no. 2, pp. 513–521, 2015.

[29] G. Vergaro, A. del Franco, C. Prontera et al., "Galectin-3 and myocardial fibrosis at cardiac magnetic resonance in nonischemic dilated cardiomyopathy," *Journal of the American College of Cardiology*, vol. 63, no. 12, article A800, 2014.

[30] C. Besler, D. Lang, D. Urban et al., "Plasma and cardiac galectin-3 in patients with heart failure reflects both inflammation and fibrosis: implications for its use as a biomarker," *Circulation Heart Failure*, vol. 10, no. 3, article e003804, 2017.

[31] M. Dobaczewski and N. G. Frangogiannis, "Chemokines and cardiac fibrosis," *Frontiers in Bioscience*, vol. S1, no. 2, pp. 391–405, 2009.

[32] C. A. Beltrami, N. Finato, M. Rocco et al., "Structural basis of endstage failure in ischemic cardiomyopathy in humans," *Circulation*, vol. 89, no. 1, pp. 151–163, 1994.

[33] M. T. Elnakish, P. Kuppusamy, and M. Khan, "Stem cell transplantation as a therapy for cardiac fibrosis," *The Journal of Pathology*, vol. 229, no. 2, pp. 347–354, 2013.

[34] N. Nagaya, K. Kangawa, T. Itoh et al., "Transplantation of mesenchymal stem cells improves cardiac function in a rat model of dilated cardiomyopathy," *Circulation*, vol. 112, no. 8, pp. 1128–1135, 2005.

[35] L. Lezaic, A. Socan, G. Poglajen et al., "Intracoronary transplantation of $CD34^+$ cells is associated with improved myocardial perfusion in patients with nonischemic dilated cardiomyopathy," *Journal of Cardiac Failure*, vol. 21, no. 2, pp. 145–152, 2015.

[36] V. Karantalis, D. L. DiFede, G. Gerstenblith et al., "Autologous mesenchymal stem cells produce concordant improvements in regional function, tissue perfusion, and fibrotic burden when administered to patients undergoing coronary artery bypass grafting: the Prospective Randomized Study of Mesenchymal Stem Cell Therapy in Patients Undergoing Cardiac Surgery (PROMETHEUS) trial," *Circulation Research*, vol. 114, no. 8, pp. 1302–1310, 2014.

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Research Article

Circulating MicroRNAs as Novel Potential Biomarkers for Left Ventricular Remodeling in Postinfarction Heart Failure

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Circulating microRNAs (miRNAs) have been proposed as potential biomarkers for left ventricular remodeling in postinfarction heart failure (HF). However, the diagnostic reproducibility of the use of circulating miRNAs may be affected by the temporal expression of miRNAs following myocardial infarction (MI). In the current study, using a MI-induced HF rat cohort (4-, 8-, and 12-week post-MI groups), we investigated the temporal expression of plasma miRNAs during the development of left ventricular remodeling. The plasma miRNA expression profile was obtained using miRNA sequencing. The expression of candidate miRNAs in plasma and tissues was examined with real-time PCR. Target genes of candidate miRNAs were predicted using a parallel miRNA-messenger RNA expression profiling approach. The value of plasma miRNAs as biomarkers for left ventricular remodeling was evaluated in patients with postinfarction HF ($n = 32$) and control patients with stable angina and without significant coronary lesions and HF ($n = 16$) with real-time PCR. Although the expression levels of miR-20a-5p, miR-340-5p, and let-7i-5p were temporally regulated in plasma, myocardium, and peripheral blood mononuclear cells, the expression levels of plasma miRNAs, especially miR-20a-5p, were associated with the development of left ventricular remodeling in the postinfarction HF rat cohort. The target genes of these 3 miRNAs were associated with the mechanistic target of rapamycin, nuclear factor-κB, tumour necrosis factor, apoptosis, and p53 signaling pathways. Additionally, the plasma levels of miR-20a-5p, miR-340-5p, and let-7i-5p were significantly increased in patients with postinfarction HF. However, only the expression levels of miR-20a-5p presented significant positive correlations with left ventricular internal end diastolic dimension and left ventricular end diastolic volume. In conclusion, the expression levels of plasma miR-20a-5p were significantly associated with the degree of left ventricular dilatation, and plasma miR-20a-5p may be a potential biomarker for postinfarction left ventricular remodeling.

1. Introduction

Heart failure (HF) is one of the leading causes of hospital admission and mortality worldwide [1]. Myocardial infarction (MI) is a common predisposing cause of HF [1]. Following MI, progressive left ventricular remodeling occurs in the noninfarcted myocardium and plays a central role in the pathophysiology of HF [2, 3]. Left ventricular remodeling is characterized by left ventricular dilatation, hypertrophy, distortion of contour, activation of interstitial fibrosis, and deterioration in cardiac dysfunction [2–4]. In clinical practice, circulating biomarkers such as natriuretic peptides associated with cardiac overload facilitate the clinical management of HF [1]. Nevertheless, there is a need to find reliable circulating biomarkers for tracking the process of left ventricular remodeling to help clinicians recognize high-risk patients for more aggressive prevention as early as possible.

Recently, circulating microRNAs (miRNAs) have become an emerging class of biomarkers. miRNAs are a large class of small noncoding RNAs that are powerful posttranscriptional regulators of gene expression [5]. Gene expression reprogramming is the base of ventricular remodeling [6]. Several studies have demonstrated that miRNAs are involved in the development of HF by silencing the expression of genes [7–9]. As miRNAs can be packaged in microparticles (exosomes, microvesicles, and apoptotic bodies) or bound to transport

proteins, they are protected from degradation and can be detected by quantitative methods in the plasma samples [10]. Recent clinical studies have indicated that circulating miRNA may be valuable biomarkers for postinfarction left ventricular remodeling [11–18]. However, the diagnostic reproducibility of using circulating miRNAs as biomarkers for postinfarction cardiac remodeling remains imperfect, which may be due to their time-dependent expression during the development of HF, the complex mechanisms underlying the progression of cardiac remodeling, and the use of antiremodeling medical therapy [11–18].

The rat coronary artery ligation model exhibits many pathophysiologic and clinical characteristics that are similar to MI-induced HF in humans [19]. Progressive left ventricular dilatation can be continuously observed for up to 3-4 months following coronary artery ligation [19]. In the present study, using a rat cohort (4-, 8-, and 12-week postcoronary artery ligation groups), we assessed the temporal expression of plasma miRNAs during the development of left ventricular remodeling and conducted a preliminary study to explore the possible origins and target genes of candidate miRNAs. The value of candidate plasma miRNAs as biomarkers for postinfarction left ventricular remodeling was subsequently examined in a case control study between patients with postinfarction HF and patients with stable angina (without significant coronary lesions and HF).

2. Materials and Methods

2.1. Animals. All animal procedures were conducted in accordance with the institutional guidelines for the care and use of laboratory animals at Jilin University, Jilin, China. All experimental procedures were approved by the Ethical Review Board of China-Japan Union Hospital of Jilin University. Male Wistar rats (8 weeks, weighing 250~280 g) were obtained from the Center for Laboratory Animals, Medical College, Jilin University, Changchun, China. The animals were subjected to sham surgery or surgery involving ligation of the left anterior descending artery. Briefly, the rats were anaesthetized with oxygen containing 3% isoflurane supplied by a rodent respirator. Following anaesthetization, the thorax was opened in the left parasternal area, and MI was induced by ligating the left anterior descending coronary artery using 3-0 suture between the pulmonary cone and left atrium. After 4 weeks, successful induction of HF was confirmed by echocardiography, and the animals were randomly divided into 4-week ($n = 16$), 8-week ($n = 20$), and 12-week ($n = 16$) post-MI groups and corresponding sham groups ($n = 12$, $n = 16$, and $n = 12$). At 4-week, 8-week, and 12-week post-MI induction, echocardiography was recorded. Animals were then euthanized, and the samples (heart, liver, spleen, lung, kidney, thymus, and blood) were quickly harvested. Tissue samples were conserved in liquid nitrogen until use. Blood was collected in ethylenediaminetetraacetic acid-treated tubes. Following preliminary centrifugation (2,000 × g, 10 minutes, 4°C), the plasma samples were further centrifuged (16,000 × g, 10 minutes, 4°C) to pellet platelets and cellular debris, and the aliquots of supernatant were stored at -80°C in a freezer. Peripheral blood mononuclear cells (PBMCs) were purified with Ficoll separating solution (TBDscience, Tianjin, China).

2.2. Patient Population. Patients with postinfarction HF admitted to China-Japan Union Hospital of Jilin University between December 2018 and June 2019 were eligible for the current study. Prior MI and HF were defined according to the current definitions [1, 20, 21]. Patients who met the criteria "pathological Q waves, in the absence of nonischemic causes and/or imaging evidence of the loss of viable myocardium in a pattern consistent with ischemic etiology" were diagnosed as prior MI [20]. Patients with symptoms and/or signs of HF, N-terminal pro-B type natriuretic peptide (NT – proBNP) > 125 pg/ml, and other cardiac functional and structural alterations underlying HF were diagnosed as HF [1, 21]. Considering that the expression levels of plasma miRNAs may be affected by atherosclerosis, hypertension, diabetes, and hyperlipaemia [10], patients with stable angina and without significant coronary lesions requiring percutaneous coronary intervention (>50% stenosis determined by angiography) [22] and HF, who often have similar risk factor baselines for coronary artery disease as patients with prior MI, were enrolled as controls. Demographic, clinical, and echocardiographic characteristics were abstracted from the electronic medical records. Patients were excluded from the current study when they were known to have a recent acute MI, autoimmune disease, infection, cardiogenic shock, valvular disease, severe renal insufficiency (eGFR < 30 ml/min/1.73 m^2), malignancy, or pregnancy. Baseline characteristics were compared via chi-squared test (categorical variables), two-independent-sample t-test (normally distributed variables), or Mann-Whitney U test (nonnormally distributed variables). The present study was approved by the Ethical Review Board of the China-Japan Union Hospital of Jilin University, China. Plasma samples were obtained with informed consent for the proper secondary use of human samples at Jilin Provincial Molecular Biology Research Center for Precision Medicine of Major Cardiovascular Disease. All procedures, including blood storage and data collection were performed in accordance with the institutional guidelines for the use of human samples by China-Japan Union Hospital of Jilin University.

2.3. Echocardiography. Rats were mildly anaesthetized using 3% isoflurane, and transthoracic echocardiography was performed using a Vivid-i echocardiography machine (General Electric Company, Fairfield, CT, USA) equipped with an 11.5 MHz transducer. Electrocardiographic assessments for patients were performed by three cardiologists who were blinded to the clinical data.

2.4. Pathology. Histologic studies were conducted with 4% paraformaldehyde-fixed and paraffin-embedded left ventricular samples from rats of all groups. Haematoxylin/eosin staining and Masson's trichrome staining were performed on cross sections of the left ventricle. Images were acquired with a microscope (Olympus Corporation, Tokyo, Japan) equipped with a digital camera and cellSens Dimension software (version 1.16; Olympus Corporation, Tokyo, Japan).

2.5. ELISA Assay for BNP. The plasma levels of BNP in rats were measured by an ELISA assay (R&D Systems, Inc., Minneapolis, MN, USA) following the manufacturer's instructions. The optical density values of samples were detected by a spectrophotometer with a wavelength of 450 nm. The normalization curve was obtained from a series of BNP standards provided in the kit.

2.6. RNA Extraction. Total plasma RNA extraction was performed using TRIzol LS solution (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with a *Caenorhabditis elegans* (*C. elegans*) RNA template as a spike-in control miRNA for normalization. Total RNA from tissues and PBMCs of rats was extracted using a TRIzol solution (Invitrogen; Thermo Fisher Scientific, Inc.).

2.7. RNA Sequencing and Bioinformatic Analysis. Following the RNA integrity and concentration examination, 2.5 ng of RNA per plasma sample and 1 μg of RNA per myocardial sample were used to prepare the library for small RNAs and messenger RNAs (mRNAs), respectively. Differential expression analysis for miRNAs or mRNAs between the 8-week post-MI group and the sham group was conducted using the DESeq package (version 1.10.1). miRNAs or mRNAs with a fold change (FC) < 0.5 or >2 were determined to be differentially expressed. The target genes of miRNAs were predicted using miRWalk (version 3.0), miRanda (version 3.3a), RNAhybrid (version 2.1.1), and TargetScan (version 6.2) analyses. The functions of the target genes were annotated using KOBAS (version 3.0) based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) and the Gene Ontology (GO) databases.

2.8. Real-Time PCR. An aliquot of 3.8 μl of RNA was reverse-transcribed to cDNA in a 10 μl reaction using a Mir-X First-Strand Synthesis Kit (Takara Bio; Clontech Laboratories, Inc., Mountain View, CA, USA). Both miRNAs and mRNAs were polyadenylated, reverse-transcribed, and quantified using a Mir-X RT-qPCR kit (Takara Bio; Clontech Laboratories, Inc.). The expression levels of miRNAs were normalized to the expression levels of *C. elegans* miRNA-39 for plasma samples and U6 for tissue samples. The expression levels of mRNAs were normalized to the expression levels of α-actin. Specific primers for quantitative PCR were used as follows: DNA-damage-inducible transcript 4 (*Ddit4*) forward primer, 5′-GCTCTGGACCCCAG TCTAGT-3′; *Ddit4* reverse primer, 5′-GGGACAGTCCT TCAGTCCTT-3′; sestrin 1 (*Sesn1*) forward primer, 5′-AA GTGAGGTGGGAGGGTCTGTG-3′; *Sesn1* reverse primer, 5′-TTTGAAAGCCCGTCCGAAGGTC-3′; NF-κB inhibitor α (*Nfkbia*) forward primer, 5′-GTCTGAACTCGCCACC CAACTG-3′; *Nfkbia* reverse primer, 5′-GTCCACCAACC GCTCCTTCTTG-3′; α-actin forward primer, 5′-GGGTAT GGGTCAGAAGGACT-3′; and α-actin reverse primer, 5′-GAGGCATACAGGGACAACAC-3′. The entire sequence of mature miRNA with all U's converted to T's was used as a forward primer for each miRNA. The reverse primer was provided in the kit and was complementary to the adapter sequence of the reverse-transcribed primer.

2.9. Exosome Isolation. The isolation of plasma exosomes was supported by a Total Exosome Isolation Kit (Invitrogen; Thermo Fisher Scientific, Inc.) following the manufacturer's instructions. Proteinase K was used to remove the bulk of protein from the plasma. The exosome-containing pellet was resuspended in PBS. The morphology of the exosomes was observed by transmission electron microscopy. The exosomal markers were examined by western blot analysis. Exosome samples were homogenized in ice-cold RIPA buffer (consisting of 50 mM Tris (pH 7.4), 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate) supplemented with protease inhibitors (Beyotime Biotechnology, Shanghai, China). The total protein was loaded for resolution by sodium dodecyl sulfate-polyacrylamide gels under reducing conditions. The primary antibodies against apoptosis-linked gene 2-interacting protein X (ALIX; 1 : 2000; cat. no. ab186429), tumour susceptibility gene 101 protein (TSG101; 1 : 2000; cat. no. ab125011), and cluster of differentiation 63 (CD63; 1 : 1000; cat. no. ab108950) were purchased from Abcam (Cambridge, MA, USA). The HRP-conjugated secondary antibody (1 : 5000; cat. no. BS13278) was purchased from Bioworld Technology, Inc. (Louis Park, MN, USA).

2.10. Statistical Analysis. All measurement data are expressed as the means ± S.E.M. Statistical analysis was performed with PASW Statistics 18 software (version 18.0.0; IBM, Inc., Armonk, NY, USA). Differences between groups were tested by a two-independent-sample t-test. Statistical graphs were generated using GraphPad Prism software (version 7.04; GraphPad Software, Inc., San Diego, CA, USA). Receiver operator characteristic (ROC) curves and the area under the curve (AUC) were calculated using MedCalc software (version 18.11; MEDCALC, Inc., Ostend, Belgium). For all analyses, a P value < 0.05 was considered significantly different.

3. Results

3.1. General Characteristics of Postinfarction HF Rats. At 4 weeks post-MI, a reduction in the echocardiographic ejection fraction and fractional shortening measurements and an increase in the plasma levels of BNP were observed in post-MI rats (4-week post-MI group) compared with sham rats, indicating damage to cardiac function and the initiation of chronic HF (Figure 1(a)). Over time, cardiac function progressively deteriorated at 8 weeks post-MI (8-week post-MI group) and was dramatically damaged at 12 weeks post-MI (12-week post-MI group) (Figure 1(a)).

Echocardiographic left ventricular internal end diastolic dimension (LVIDd) and left ventricular internal end systolic dimension (LVIDs) values were significantly increased from 4 weeks post-MI to 8 weeks post-MI and mildly increased from 8 weeks post-MI to 12 weeks post-MI (Figure 1(a)). Progressively increased extracellular matrix synthesis and cardiac fibrosis, as determined by histopathology, were seen

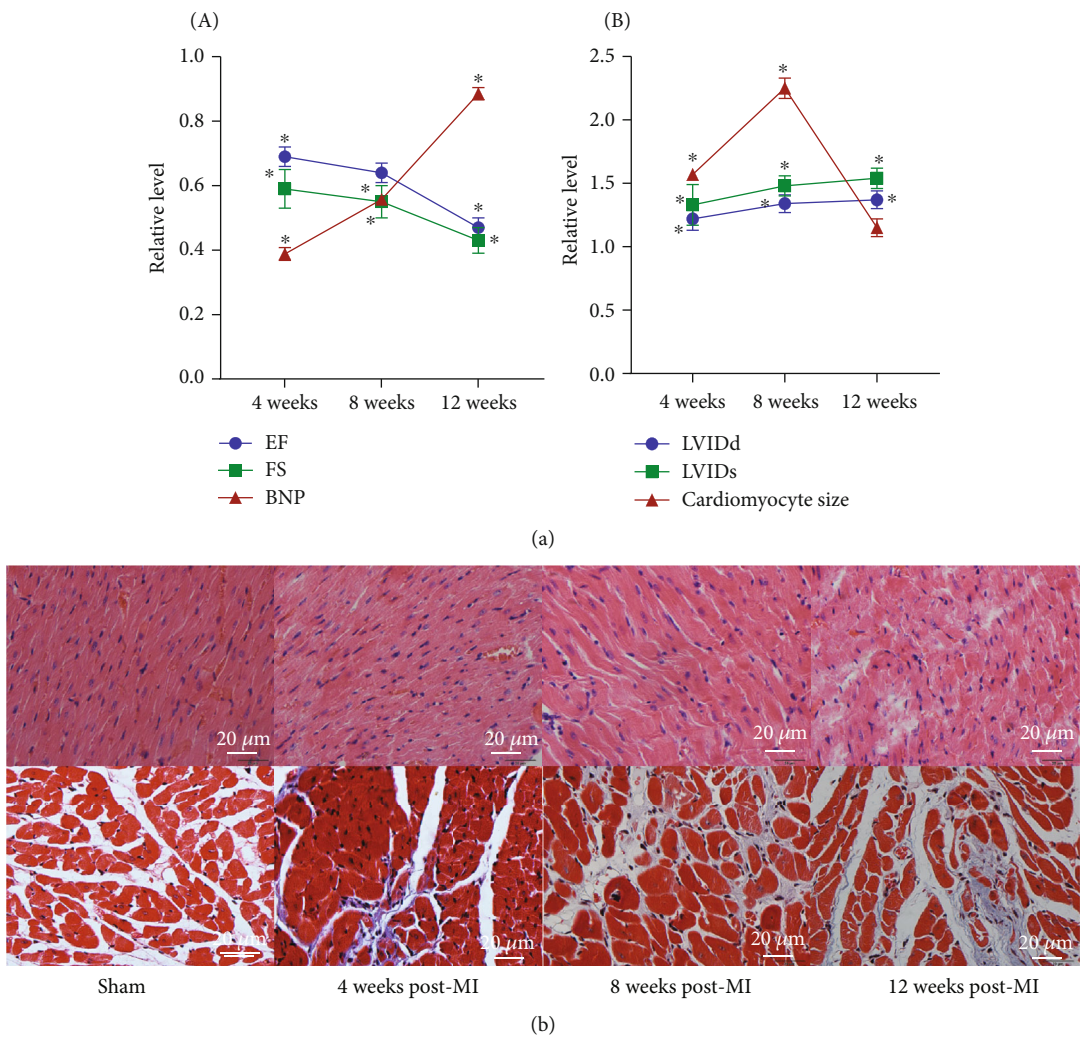

Figure 1: General characteristics of sham and post-MI rats. (a) Echocardiographic EF and FS measurements and plasma BNP levels of sham and post-MI rats at 4 weeks, 8 weeks, and 12 weeks post-MI (A). LVIDd, LVIDs, and cardiomyocyte size values of sham and post-MI rats at 4 weeks, 8 weeks, and 12 weeks post-MI (B). (b) Haematoxylin/eosin staining (upper) and Masson's trichrome staining (lower) of the left ventricle of sham and post-MI rats at 4 weeks, 8 weeks, and 12 weeks post-MI. $^{*}P < 0.05$ versus the corresponding sham group. EF: ejection fraction; FS: fractional shortening; BNP: brain natriuretic peptide; MI: myocardial infarction; LVIDd: left ventricular internal end diastolic dimension; LVIDs: left ventricular internal end systolic dimension.

in the left ventricle of post-MI rats over time, which indicated the development of cardiac remodeling (Figure 1(b)). Furthermore, we found that the cardiomyocyte size in an area distant from the site of infarction was larger in the 4-week post-MI and 8-week post-MI groups than in the corresponding sham groups, and this cardiomyocyte hypertrophy was attenuated at 12 weeks post-MI (Figure 1(b)). The dilatation of the left ventricle, the activation of cardiac fibrosis, and cardiomyocyte hypertrophy may be compensatory mechanisms triggered to maintain cardiac function at 4 and 8 weeks post-MI. However, these mechanisms became maladaptive at 12 weeks post-MI and led to further left ventricular dilatation and thinning and significant deterioration in cardiac function.

3.2. Discovery of Candidate Plasma miRNAs. We first analysed the global plasma miRNA expression in the 8-week post-MI group and corresponding sham group. We detected 619 known miRNAs in the plasma samples. Differential expression analysis indicated that 143 miRNAs were differentially expressed in the 8-week post-MI group compared with the sham group (FC < 0.5 or >2; $P < 0.05$). Of the 143 differentially expressed miRNAs, 138 were upregulated and 5 were downregulated in the 8-week post-MI group compared with the sham group. Hierarchical clustering analysis indicated that differentially expressed miRNAs could clearly distinguish the 8-week post-MI group from the sham group (Figure 2). From the 143 differentially expressed miRNAs, we selected 13 miRNAs based on their FCs and probability values for further study.

3.3. Validation of Candidate Plasma miRNAs. We validated the expression levels of 13 candidate miRNAs in the 8-week post-MI group and sham group. In the 8-week post-MI

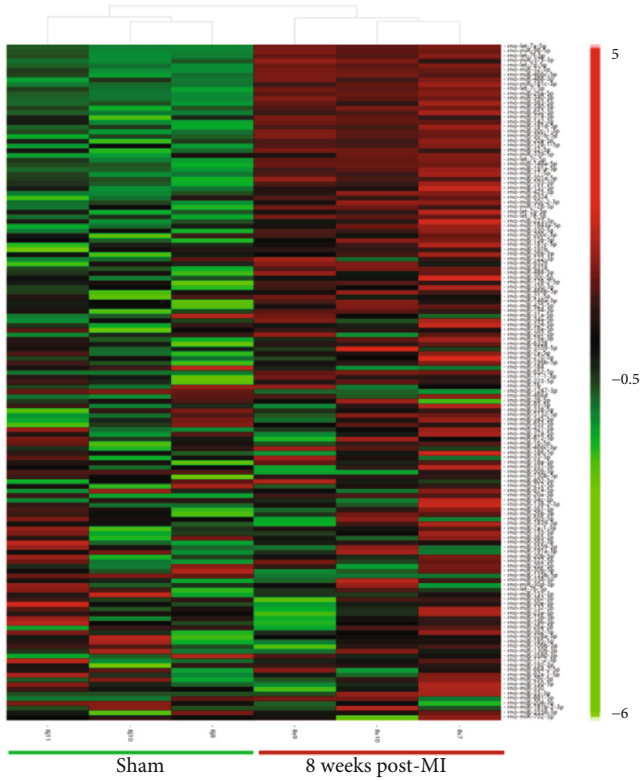

Figure 2: Hierarchical clustering of 143 DE circulating miRNAs (FC < 0.5 or >2; $P < 0.05$) in the 8-week post-MI group ($n = 3$) compared with the sham group ($n = 3$). DE: differentially expressed; FC: fold change.

group, rats had an ejection fraction lower than 50%. In the sham group, rats had an ejection fraction higher than 70%. The FCs of the plasma miRNA levels in the 8-week post-MI group versus the sham group are shown in Figure 3(a). Of the 13 miRNAs that were significantly upregulated in miRNA sequencing, 5 miRNAs were also significantly upregulated in the validated 8-week post-MI group compared with the sham group. The expression levels of miR-20a-5p, miR-340-5p, and let-7i-5p were increased more than threefold in the 8-week post-MI group compared with the sham group. In addition, we evaluated the accuracy and sensitivity of miR-20a-5p, miR-340-5p, and let-7i-5p for predicting HF in the 8-week post-MI group and the sham group using a ROC curve and the AUC. The AUCs of miR-20a-5p, miR-340-5p and let-7i-5p were 0.863 ($P < 0.05$), 0.883 ($P < 0.05$), and 0.892 ($P < 0.05$), respectively (Figure 3(b)). The expression levels of miR-20a-5p, miR-340-5p, and let-7i-5p were closely associated with HF.

3.4. Temporal Expression of miRNAs in Plasma and Myocardium from 4 Weeks Post-MI to 12 Weeks Post-MI. We evaluated the expression levels of miR-20a-5p, miR-340-5p, and let-7i-5p in plasma and myocardium of the post-MI rat cohort. The expression levels of miR-20a-5p, miR-340-5p, and let-7i-5p showed an upward trend from 4 weeks to 8 weeks post-MI, whereas their expression dramatically decreased to baseline levels at 12 weeks post-MI, and the expression levels of miR-20a-5p were significantly decreased in the 12-week post-MI group compared with the sham group (Figure 4). As shown in Table 1, there were significant positive correlations between the expression levels of miR-20a-5p, miR-340-5p, and let-7i-5p and LVIDd, LVIDs, and cardiomyocyte size at 4 weeks and 8 weeks post-MI. Additionally, inverse correlations were identified between the expression levels of miR-20a-5p and LVIDd and LVIDs at 12 weeks post-MI.

The expression levels of miR-20a-5p and let-7i-5p were elevated in the myocardium of the 4-week post-MI group compared with the sham group. No significant difference in the expression levels of miR-20a-5p, miR-340-5p, and let-7i-5p in the myocardium was identified between the post-MI groups and the sham groups at 8 and 12 weeks post-MI (Figure 4).

3.5. Possible Origins of Plasma miRNAs. We quantified the expression levels of miR-20a-5p, miR-340-5p, and let-7i-5p in the HF-related tissues, including the liver, spleen, lung, kidney, thymus, and PBMCs at 8 weeks post-MI. The expression levels of miR-20a-5p, miR-340-5p, and let-7i-5p were upregulated in PBMCs at 8 weeks post-MI (Figure 5(a)), and there was no significant difference in the miR-20a-5p, miR-340-5p, and let-7i-5p levels in the liver, spleen, lung, kidney, and thymus tissues between the 8-week post-MI group and the sham group (see Figure S1 in the Supplementary Material for quantitative results). We subsequently investigated the temporal expression of miR-20a-5p, miR-340-5p, and let-7i-5p in PBMCs at 4 weeks and 12 weeks post-MI. The expression levels of miR-20a-5p and miR-340-5p in PBMCs showed an upward trend that was similar to the trend in plasma from 4 weeks to 8 weeks post-MI (Figure 5(a)). These results indicated that PBMCs may be a potential origin of plasma miR-20a-5p and miR-340-5p. Furthermore, exosomes were isolated from plasma (Figure 5(b)) and used to evaluate the expression levels of exosome-original miR-20a-5p, miR-340-5p, and let-7i-5p. Only the expression levels of miR-20a-5p showed a significant 1.7-fold increase in the 8-week post-MI group compared with the sham group, indicating that plasma miR-20a-5p may be partly transported by exosomes (Figure 5(c)).

3.6. Target mRNAs of miR-20a-5p, miR-340-5p, and let-7i-5p. Plasma miR-20a-5p, miR-340-5p, and let-7i-5p may play important roles in the development of left ventricular remodeling. Since miRNAs are known to regulate gene expression via the degradation or translational repression of their target mRNAs [5], a parallel miRNA-mRNA expression profiling approach [23, 24] was used to identify potential mRNA targets of miR-20a-5p, miR-340-5p, and let-7i-5p. The global mRNA expression in the myocardium (an area distant from the site of infarction) of rats in the 8-week post-MI group and the corresponding sham group was analysed using mRNA sequencing. Differential expression analysis indicated that 36 mRNAs were differentially expressed in the 8-week post-MI group compared with the sham group (FC < 0.5 or >2; $P < 0.05$). Of the 36 differentially expressed mRNAs, 10 were upregulated and 26 were downregulated in the

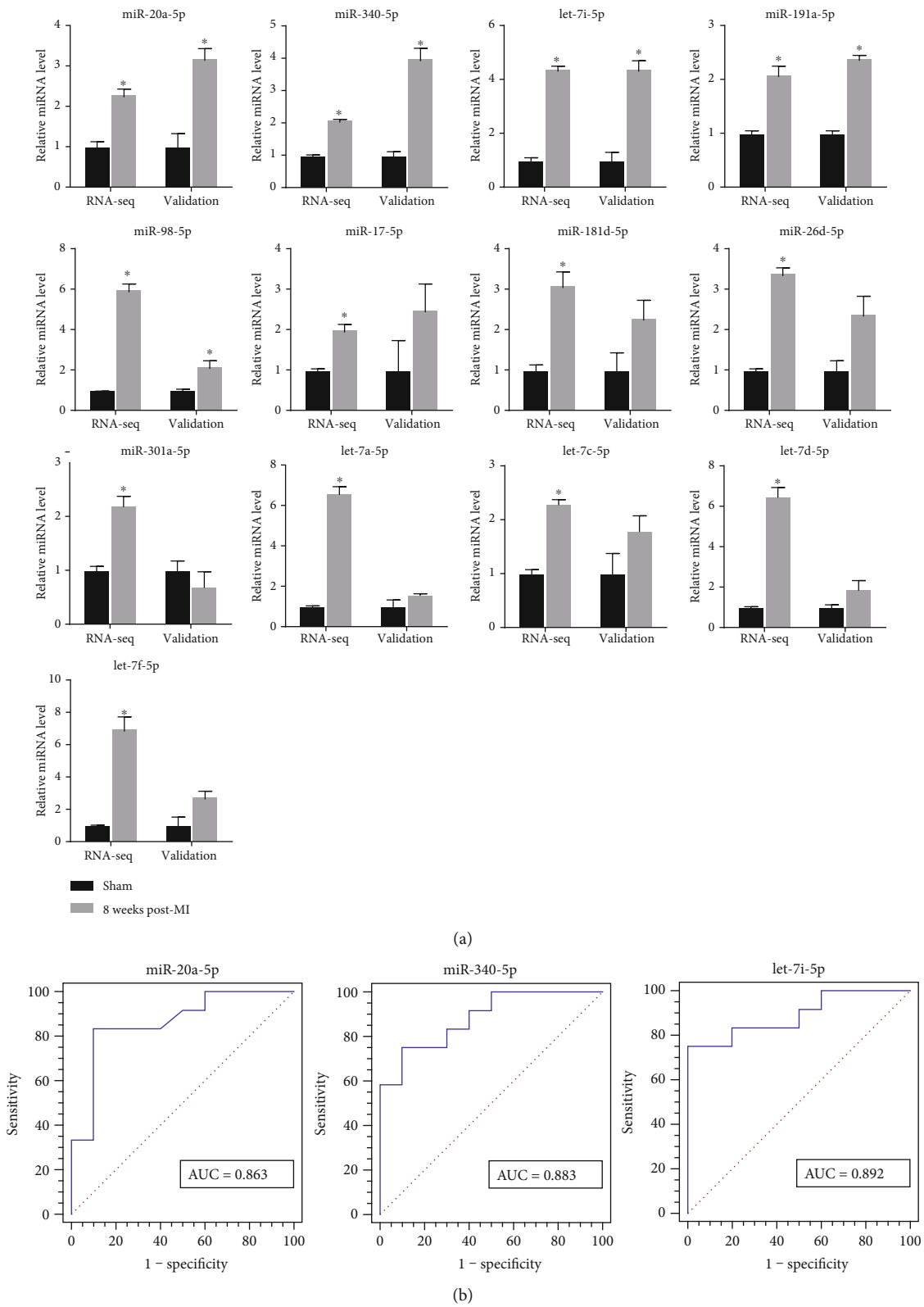

Figure 3: Validation of candidate plasma miRNAs. (a) Expression levels of 13 candidate plasma miRNAs in the 8-week post-MI group and the sham group. The left 2 bars of each panel show miRNA levels detected by miRNA sequencing in the 8-week post-MI group ($n = 3$) and the sham group ($n = 3$). The right 2 bars of each panel show the same miRNA levels validated using real-time PCR in the 8-week post-MI group ($n = 16$) and the sham group ($n = 12$). (b) ROC curves and the AUCs of miR-20a-5p, miR-340-5p, and let-7i-5p for predicting HF at 8 weeks post-MI. $^*P < 0.05$ versus the corresponding sham group. RNA-seq: RNA sequencing; MI: myocardial infarction; HF: heart failure; ROC: receiver operator characteristic; AUC: area under the curve.

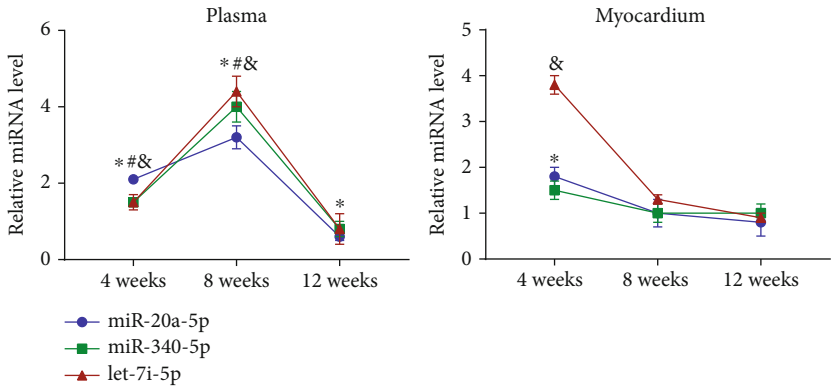

Figure 4: Temporal expression of miR-20a-5p, miR-340-5p, and let-7i-5p in the plasma and myocardium of post-MI rats from 4 weeks post-MI to 12 weeks post-MI. $^{*}P < 0.05$ versus the corresponding sham group (miR-20a-5p); $^{\#}P < 0.05$ versus the corresponding sham group (miR-340-5p); $^{\&}P < 0.05$ versus the corresponding sham group (let-7i-5p). MI: myocardial infarction.

Table 1: Correlations between the expression levels of plasma miRNAs and LVIDd, LVIDs, and cardiomyocyte size at 4 weeks, 8 weeks, and 12 weeks post-MI.

	LVIDd			LVIDs			Cardiomyocyte size		
	4 weeks	8 weeks	12 weeks	4 weeks	8 weeks	12 weeks	4 weeks	8 weeks	12 weeks
miR-20a-5p	$r = 0.793^{*}$ $P = 0.019$	$r = 0.889^{*}$ $P = 0.003$	$r = -0.816^{*}$ $P = 0.013$	$r = 0.783^{*}$ $P = 0.022$	$r = 0.861^{*}$ $P = 0.006$	$r = -0.735^{*}$ $P = 0.038$	$r = 0.977^{*}$ $P = 0$	$r = 0.932^{*}$ $P = 0.01$	$r = -0.609$ $P = 0.109$
miR-340-5p	$r = 0.863^{*}$ $P = 0.006$	$r = 0.733^{*}$ $P = 0.039$	$r = -0.393$ $P = 0.336$	$r = 0.855^{*}$ $P = 0.007$	$r = 0.707^{*}$ $P = 0.049$	$r = -0.434$ $P = 0.283$	$r = 0.848^{*}$ $P = 0.008$	$r = 0.74^{*}$ $P = 0.036$	$r = -0.386$ $P = 0.345$
let-7i-5p	$r = 0.754^{*}$ $P = 0.031$	$r = 0.829^{*}$ $P = 0.011$	$r = -0.061$ $P = 0.887$	$r = 0696^{*}$ $P = 0.019$	$r = 0.799^{*}$ $P = 0.017$	$r = -0.302$ $P = 0.468$	$r = 0.719^{*}$ $P = 0.044$	$r = 0.868^{*}$ $P = 0.005$	$r = -0.009$ $P = 0.984$

MI: myocardial infarction; r: Pearson correlation coefficient; LVIDd: left ventricular internal end diastolic dimension; LVIDs: left ventricular internal end systolic dimension; MI: myocardial infarction. $^{*}P < 0.05$ versus the corresponding sham group.

8-week post-MI group compared with the sham group. Differentially expressed mRNAs clearly distinguished the 8-week post-MI group from the sham group (Figure 6(a)). The target genes of miR-20a-5p, miR-340-5p, and let-7i-5p were predicted using miRWalk, miRanda, RNAhybrid, and TargetScan analyses and subsequently paired to the downregulated mRNAs generated from mRNA sequencing (Figure 6(b)). The symbols of paired genes are presented in Figure 6(c) (19 genes in total). There was no paired gene between the targets of miR-340-5p and the downregulated myocardial mRNAs. However, there were 671 target genes of miR-340-5p in common with the target genes of miR-20a-5p and/or let-7i-5p. Additionally, several pathways mechanistically associated with cardiac remodeling were presented in the KEGG annotation results of the 19 common target genes, including the mechanistic target of rapamycin (mTOR), nuclear factor- (NF-) κB, tumour necrosis factor (TNF), apoptosis, p53, and other signaling pathways (see Table S1 in Supplementary Materials for comprehensive KEGG analysis). From the 19 common genes, *Ddit4*, *Sesn1*, and *Nfkbia* were selected for further validation with real-time PCR in the 8-week post-MI group and the sham group. These 3 genes play roles in the cellular processes, including cellular growth, autophagy, apoptosis, inflammation, and fibrosis [25–29], which are associated with the progression of cardiac remodeling [30]. As presented in Figure 6(d), the expression levels of *Ddit4*, *Sesn1*, and *Nfkbia* mRNAs were also significantly reduced in the validated 8-week post-MI group compared with the corresponding sham group. *Ddit4*, *Sesn1*, and *Nfkbia* may be potential target genes of miR-20a-5p, and *Ddit4* may be a potential target gene of let-7i-5p.

3.7. Evaluation of Candidate Plasma miRNAs as Biomarkers for Postinfarction Left Ventricular Remodeling in Patient Population. The sequences of miR-20a-5p, miR-340-5p, and let-7i-5p share significant homology between rats and humans. The expression levels of these 3 miRNAs were subsequently investigated in 32 patients with postinfarction HF and 16 control patients with stable angina and without significant coronary lesions and HF. The baseline characteristics of the subjects are presented in Table 2. The echocardiographic ejection fraction measurements of patients with postinfarction HF were lower than 60% and higher than 21%. The levels of NT-proBNP in patients with postinfarction HF were higher than 125 pg/ml. Additionally, 56.3% and 62.5% patients with postinfarction HF used β-receptor blockers and angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, respectively, to treat postinfarction left ventricular remodeling and/or hypertension. No significant difference in the echocardiographic measurements of the interventricular septum and left ventricular posterior wall was identified between patients with postinfarction HF and

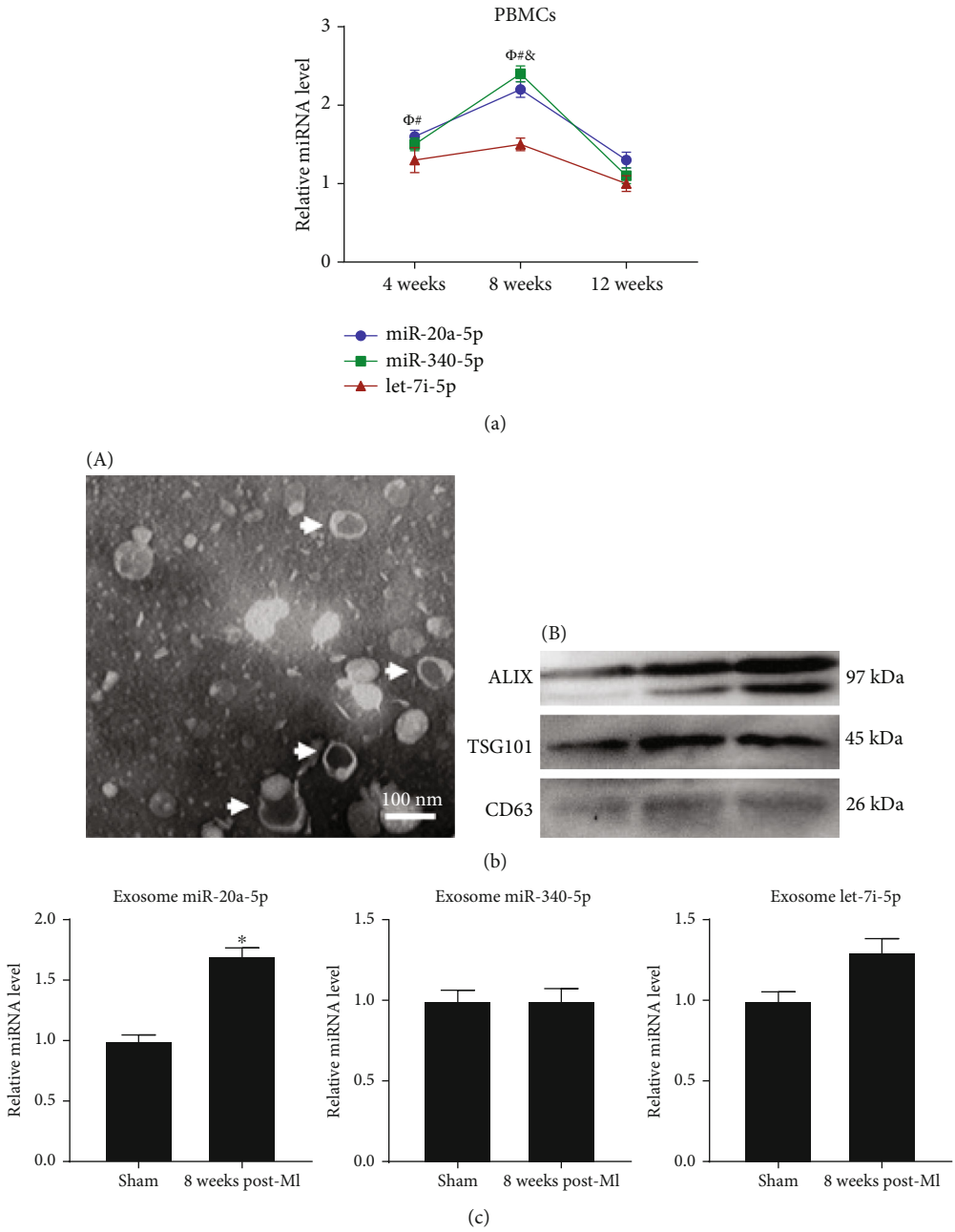

Figure 5: Possible origins of plasma miR-20a-5p, miR-340-5p, and let-7i-5p. (a) The temporal expression of miR-20a-5p, miR-340-5p, and let-7i-5p in the PBMCs of post-MI rats and sham controls at 4, 8, and 12 weeks post-MI. (b) Transmission electron microscope image of exosomes isolated from plasma (A; white arrow) and western blot of exosomal markers ALIX, TSG101, and CD63 (B). (c) Expression levels of exosome-original miR-20a-5p, miR-340-5p, and let-7i-5p at 8 weeks post-MI. $^{*}P < 0.05$ versus the corresponding sham group; $^{\Phi}P < 0.05$ versus the corresponding sham group (miR-20a-5p); $^{\#}P < 0.05$ versus the corresponding sham group (miR-340-5p); $^{\&}P < 0.05$ versus the corresponding sham group (let-7i-5p). MI: myocardial infarction; PBMCs: peripheral blood mononuclear cells; ALIX: apoptosis-linked gene 2-interacting protein X; TSG101: tumour susceptibility gene 101 protein; CD63: cluster of differentiation 63.

controls. The plasma levels of miR-20a-5p, miR-340-5p, and let-7i-5p were significantly increased in patients with postinfarction HF compared with controls (Figure 7(a)). Additionally, the AUCs of miR-20a-5p, miR-340-5p, and let-7i-5p were 0.867 ($P < 0.05$), 0.825 ($P < 0.05$), and 0.779 ($P < 0.05$), respectively (Figure 7(b)). However, only the expression levels of miR-20a-5p presented significant positive correlations with LVIDd (Pearson correlation coefficient: 0.435; P value: 0.002) and left ventricular end diastolic volume (LVEDV; Pearson correlation coefficient: 0.323; P value: 0.025) (Table 3).

4. Discussion

Following MI, progressive cardiac remodeling is associated with cardiac dysfunction and poor prognosis for survival [31]. During the development of left ventricular remodeling,

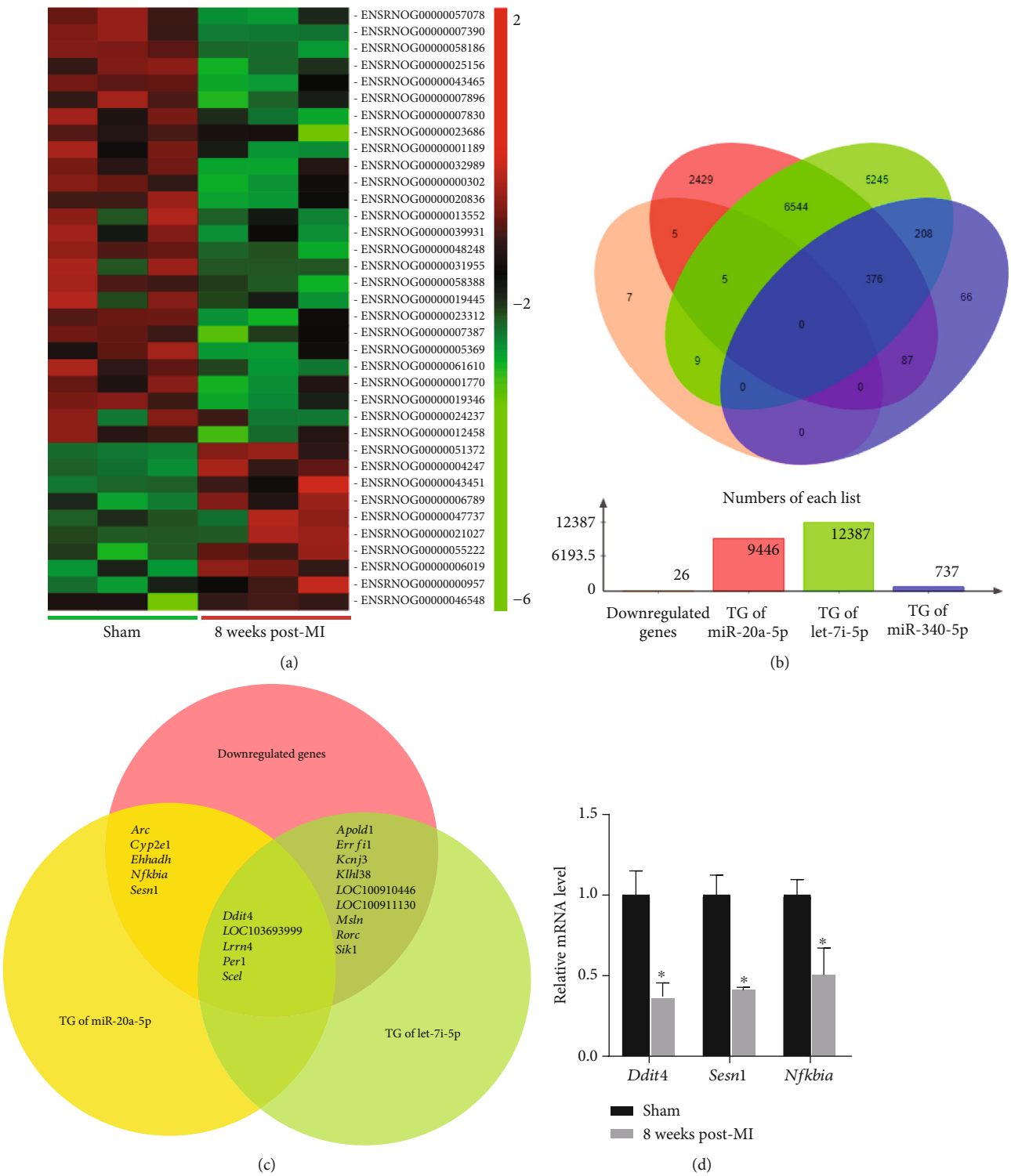

Figure 6: Target genes of plasma miR-20a-5p, miR-340-5p, and let-7i-5p. (a) Hierarchical clustering of 36 DE mRNAs (FC < 0.5 or >2; $P < 0.05$) in the 8-week post-MI group ($n = 3$) compared with the sham group ($n = 3$). (b) The number and overlap among the downregulated mRNAs generated from mRNA sequencing and the predicted target genes of miR-20a-5p, miR-340-5p, and let-7i-5p. (c) The common genes among the downregulated genes, the target genes of miR-20a-5p, and the target genes of let-7i-5p. (d) The expression levels of *Ddit4*, *Sesn1*, and *Nfkbia* mRNAs in the validated 8-week post-MI group ($n = 4$) compared with the corresponding sham group ($n = 4$), which were examined with real-time PCR. $^*P < 0.05$ versus the sham group. DE: differentially expressed; mRNA: messenger RNA; MI: myocardial infarction; FC: fold change; TG: target genes; *Ddit4*: DNA-damage-inducible transcript 4; *Sesn1*: sestrin 1; *Nfkbia*: NF-κB inhibitor α.

Table 2: Baseline characteristics of the subjects.

Variable	Control ($n = 16$)	Postinfarction HF ($n = 32$)
Age (years)[I]	61.1 (12.4)	62.4 (10.2)
Female[¶]	9 (56.3)	17 (53.1)
Diabetes[¶]	4 (25.0)	10 (31.3)
Hypertension[¶]	8 (50.0)	15 (46.9)
Current smoker[¶]	3 (18.8)	5 (15.6)
Triglycerides (mmol/l)[I]	1.92 (1.62)	1.58 (1.02)
Total cholesterol (mmol/l)[I]	4.47 (1.81)	4.00 (1.00)
LDL (mmol/l)[I]	2.65 (1.13)	2.54 (0.89)
HDL (mmol/l)[I]	1.20 (0.26)	1.05 (0.21)
Creatinine (μmol/l)[I]	73.47 (11.20)	84.47 (14.82)
β-Receptor blockers[¶]	4 (25.0)	18 (56.3)*
ACE inhibitors or AR blockers[¶]	5 (31.3)	20 (62.5)*
NT-proBNP (pg/ml)[§]	99.1 (71.5, 132.8)	831.0 (152.5, 1605.0)*
Echocardiography		
LVIDd (mm)[I]	42.6 (2.3)	54.1 (7.7)*
LVEDV (ml)[I]	105.4 (34.6)	159.9 (65.4)*
Ejection fraction (%)[I]	73.2 (3.7)	44.5 (9.4)*
Fractional shortening (%)[I]	42.0 (2.8)	21.3 (5.6)*
IVS (mm)[I]	9.5 (1.9)	10.3 (1.6)
LVPW (mm)[I]	9.5 (1.8)	9.8 (1.2)

[I]Mean (SEM), no. (%), and [§]median (25% percentile, 75% percentile) of nonnormally distributed variables. HF: heart failure; LDL: low-density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol; ACE: angiotensin-converting enzyme; AR: angiotensin receptor; NT-proBNP: N-terminal pro-B type natriuretic peptide; LVIDd: left ventricular internal end diastolic dimension; LVEDV: left ventricular end diastolic volume; IVS: interventricular septum; LVPW: left ventricular posterior wall. *$P < 0.05$ versus controls.

gross pathologic changes in the left ventricular configuration and volume evaluated by echocardiography have been widely used to predict the clinical outcomes of individuals [2]. However, on a molecular level, biomarkers for left ventricular remodeling are limited to proteins that reflect haemodynamic stress (such as natriuretic peptides) or necrosis (such as cardiac troponins) [12]. Recently, miRNAs, a class of evolutionarily conserved small noncoding RNAs, have been discovered to be stably transported in the blood [10]. Since miRNAs are known to function as gene expression repressors at the posttranscriptional level by targeting the corresponding mRNAs and play important roles in the development of HF, circulating miRNAs have been proposed to be promising biomarkers for left ventricular remodeling [5, 32, 33]. However, the temporal and tissue-specific expression of miRNAs following MI may affect the diagnostic specificity and reproducibility of the use of circulating miRNAs as biomarkers for left ventricular remodeling [11, 34]. In the current study, using a postinfarction HF rat cohort, our data revealed that the expression levels of plasma miR-20a-5p, miR-340-5p, and let-7i-5p gradually increased and were closely associated with left ventricular hypertrophy and dilatation from 4 weeks to 8 weeks post-MI. At 12 weeks post-MI, the mechanisms underlying left ventricular remodeling became maladaptive and led to significant deterioration in cardiac function and further left ventricular dilatation and thinning. The expression levels of plasma miR-340-5p and let-7i-5p were restored to baseline levels, and the expression levels of miR-20a-5p presented significant inverse correlations with the dilatation of the left ventricle at 12 weeks post-MI. The target genes of miR-20a-5p and let-7i-5p were involved in several pathways associated with the development of left ventricular remodeling. The expression levels of miR-20a-5p, miR-340-5p, and let-7i-5p were also temporally regulated in the myocardium and PBMCs. It may be suggested that these 3 miRNAs played functional roles in the pathophysiology of left ventricular remodeling, and the loss of increased miR-20a-5p, miR-340-5p, and let-7i-5p was associated with the process of maladaptive left ventricular remodeling. In the clinical study, patients with postinfarction HF presented a wide range of echocardiographic left ventricular phenotypes, and a lack of extreme phenotypes associated with severe left ventricular remodeling, which may be due to the development of left ventricular remodeling, can be affected by different infarct sizes and time points, antiremodeling medical therapy, and myocardial revascularization strategies. Similar to the expression pattern in postinfarction HF rats at 4 and 8 weeks post-MI, the expression levels of plasma miR-20a-5p, miR-340-5p, and let-7i-5p were increased in patients with postinfarction HF. Additionally, the expression levels of miR-20a-5p presented significant correlations with LVIDd and LVEDV. In accordance with our findings, in a community-based observational cohort, the expression levels of miR-20a-5p were demonstrated to be associated with the development of left

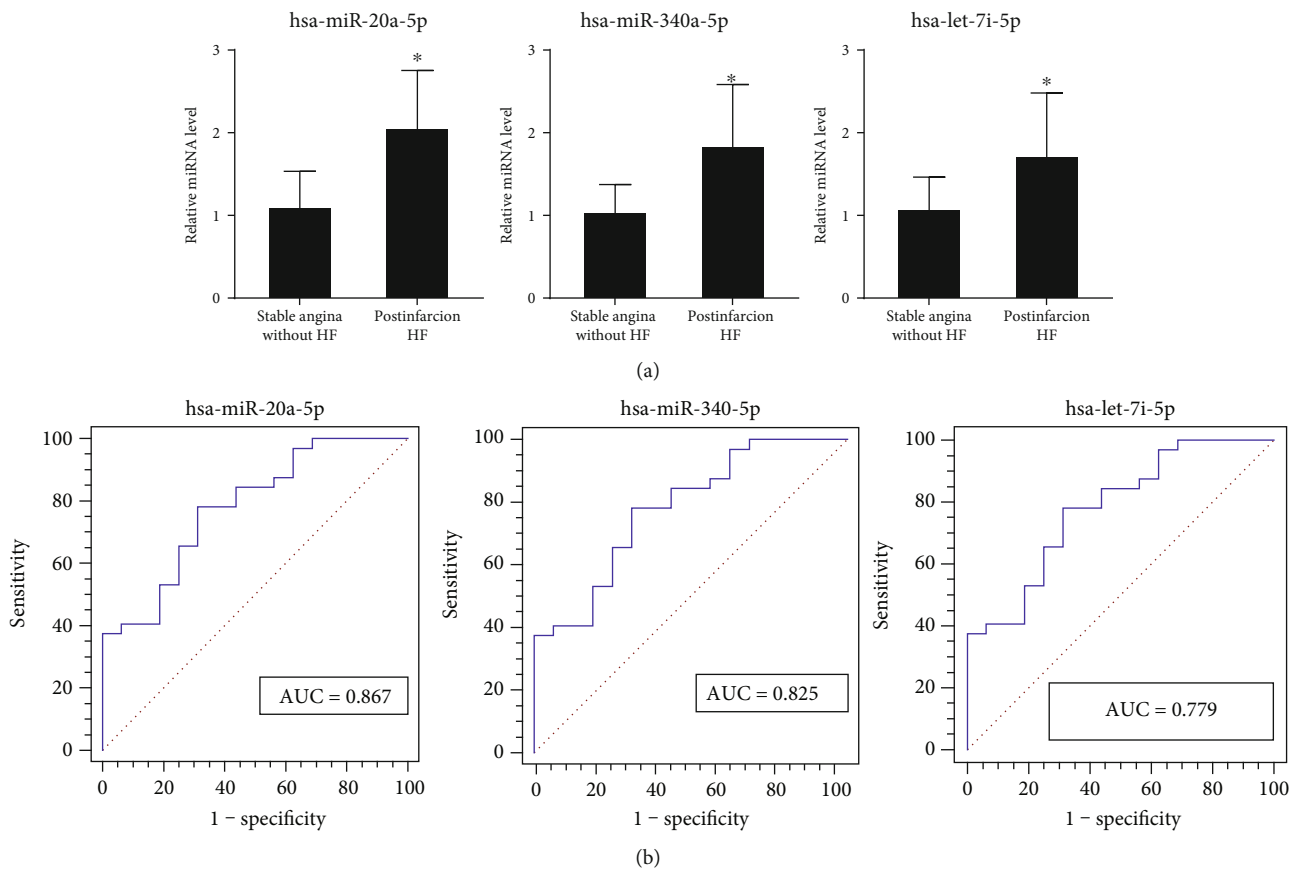

Figure 7: Expression of candidate plasma miRNAs in the patient population. (a) The expression levels of plasma miR-20a-5p, miR-340-5p, and let-7i-5p in patients with postinfarction HF ($n = 32$) and control patients with stable angina and without significant coronary lesions and HF ($n = 16$). (b) ROC curves and the AUCs of miR-20a-5p, miR-340-5p, and let-7i-5p. $^*P < 0.05$ versus patients with stable angina and without significant coronary lesions and HF. HF: heart failure; ROC: receiver operator characteristic; AUC: area under the curve.

Table 3: Correlations between the expression levels of plasma miRNAs and LVIDd, LVEDV, IVS, and LVPW in the patient population.

	LVIDd	LVEDV	IVS	LVPW
hsa-miR-20a-5p	$r = 0.435^*$, $P = 0.002$	$r = 0.323^*$, $P = 0.025$	$r = 0.218$, $P = 0.137$	$r = 0.199$, $P = 0.174$
hsa-miR-340-5p	$r = 0.219$, $P = 0.134$	$r = 0.225$, $P = 0.124$	$r = 0.180$, $P = 0.221$	$r = 0.018$, $P = 0.904$
hsa-let-7i-5p	$r = 0.200$, $P = 0.173$	$r = 0.223$, $P = 0.128$	$r = 0.062$, $P = 0.675$	$r = 0.090$, $P = 0.544$

HF: heart failure; r: Pearson correlation coefficient; LVIDd: left ventricular internal end diastolic dimension; LVEDV: left ventricular end diastolic volume; IVS: interventricular septum; LVPW: left ventricular posterior wall. $^*P < 0.05$ versus stable angina patients without significant coronary lesions and HF.

ventricular remodeling and HF incidence during the follow-up period [35]. Therefore, miR-20a-5p may be a novel potential biomarker for postinfarction left ventricular remodeling.

The results in the present study indicated that miR-20a-5p and let-7i-5p targeted a set of genes involved in the pathways potentially associated with the pathophysiology of left ventricular remodeling, including the mTOR, NF-κB, TNF, apoptosis, and p53 signaling pathways. Among these target genes, the expression levels of *Ddit4*, *Sesn1*, and *Nfkbia* mRNAs, which are closely associated with cellular growth, inflammation, and cell survival, were verified to be reduced in the myocardium of rats at 8 weeks post-MI. DDIT4 is a stress-induced protein and functions as an inhibitor of mTOR kinase [25, 36, 37]. mTOR kinase is an important regulator that promotes cellular growth and proliferation [38, 39]. *Sesn1* is a target gene of the tumour suppressor p53, a stress-activated transcription factor that inhibits cellular growth and proliferation [40]. The protein of *Nfkbia* is an inhibitor of NF-κB transcription factor, which plays multifaceted roles in the regulation of inflammation and cell survival during the development of cardiac remodeling [41]. Additionally, previous studies supported the progrowth and antiapoptosis effects of miR-20a-5p and let-7i-5p on cardiomyocytes or myoblasts [42–45]. Therefore, *Ddit4*, *Sesn1*, and *Nfkbia* may be potential target genes of miR-20a-5p and/or let-7i-5p.

In patients with acute decompensated HF, the expression levels of plasma let-7i-5p were lower in patients with worsening of renal function compared with patients without worsening of renal function [46]. In a cohort of HF patients

with and without atherosclerotic disease, the lower levels of plasma let-7i-5p were associated with the risk of rehospitalizations due to cardiovascular causes during 18 months of follow-up [45]. In the current study, the loss of increased let-7i-5p was associated with the phenotypes of adverse left ventricular remodeling. It may be suggested that let-7i-5p plays protective roles in the development of HF and the lower circulating let-7i-5p levels are associated with the poor prognosis of HF.

In the present study, our data indicated that the expression of miRNAs in plasma, myocardium, and PBMCs may be temporally interconnected. Similarly, in patients with dilated cardiomyopathy, the miRNA expression profile was specifically modulated in PBMCs, and many differentially expressed miRNAs were deregulated in the myocardium in previous studies [47]. Additionally, in an ischemia reperfusion mouse cohort, the expression levels of the majority of candidate miRNAs associated with left ventricular remodeling were upregulated in the myocardium at acute time points and in plasma at chronic time points [11]. However, these findings are exploratory and require further investigation in the *in vivo* and *in vitro* mechanistic studies.

5. Conclusions

Left ventricular remodeling is identified as a central pathophysiologic mechanism involved in the development of HF. Finding reliable biomarkers for left ventricular remodeling may facilitate the risk stratification and prognosis assessment of patients with HF. The results of the present study suggested that the expression levels of plasma miR-20a-5p, miR-340-5p, and let-7i-5p were associated with the development of left ventricular remodeling. The expression levels of plasma miR-20a-5p presented significant correlations with left ventricular dilatation in rats and patients with postinfarction HF. Therefore, miR-20a-5p may serve as a novel potential biomarker for cardiac remodeling during the development of postinfarction HF.

Data Availability

The database used to support the findings of this study is available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

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Supplementary Materials

Supplementary Figure 1: expression levels of miR-20a-5p, miR-340-5p, and let-7i-5p in the liver, spleen, lung, kidney, and thymus tissues in the 8-week post-MI group and the sham group. Supplementary Table 1: the KEGG annotation results of the 19 common target genes. *(Supplementary Materials)*

References

[1] P. Ponikowski, A. A. Voors, S. D. Anker et al., "2016 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure: the task force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) developed with the special contribution of the Heart Failure Association (HFA) of the ESC," *European Heart Journal*, vol. 37, no. 27, pp. 2129–2200, 2016.

[2] M. A. Konstam, D. G. Kramer, A. R. Patel, M. S. Maron, and J. E. Udelson, "Left ventricular remodeling in heart failure: current concepts in clinical significance and assessment," *JACC: Cardiovascular Imaging*, vol. 4, no. 1, pp. 98–108, 2011.

[3] G. Heusch, P. Libby, B. Gersh et al., "Cardiovascular remodelling in coronary artery disease and heart failure," *Lancet*, vol. 383, no. 9932, pp. 1933–1943, 2014.

[4] M. A. Pfeffer and E. Braunwald, "Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications," *Circulation*, vol. 81, no. 4, pp. 1161–1172, 1990.

[5] E. A. van Vliet, N. Puhakka, J. D. Mills et al., "Standardization procedure for plasma biomarker analysis in rat models of epileptogenesis: focus on circulating microRNAs," *Epilepsia*, vol. 58, no. 12, pp. 2013–2024, 2017.

[6] T. Thum and G. Condorelli, "Long noncoding RNAs and microRNAs in cardiovascular pathophysiology," *Circulation Research*, vol. 116, no. 4, pp. 751–762, 2015.

[7] A. Castaldi, T. Zaglia, V. Di Mauro et al., "MicroRNA-133 modulates the β1-adrenergic receptor transduction cascade," *Circulation Research*, vol. 115, no. 2, pp. 273–283, 2014.

[8] I. Karakikes, A. H. Chaanine, S. Kang et al., "Therapeutic cardiac-targeted delivery of miR-1 reverses pressure overload-induced cardiac hypertrophy and attenuates pathological remodeling," *Journal of the American Heart Association*, vol. 2, no. 2, article e000078, 2013.

[9] R. L. Montgomery, T. G. Hullinger, H. M. Semus et al., "Therapeutic inhibition of miR-208a improves cardiac function and survival during heart failure," *Circulation*, vol. 124, no. 14, pp. 1537–1547, 2011.

[10] E. E. Creemers, A. J. Tijsen, and Y. M. Pinto, "Circulating microRNAs: novel biomarkers and extracellular communicators in cardiovascular disease?," *Circulation Research*, vol. 110, no. 3, pp. 483–495, 2012.

[11] K. M. Danielson, R. Shah, A. Yeri et al., "Plasma circulating extracellular RNAs in left ventricular remodeling post-myocardial infarction," *eBioMedicine*, vol. 32, pp. 172–181, 2018.

[12] R. Shah, O. Ziegler, A. Yeri et al., "MicroRNAs associated with reverse left ventricular remodeling in humans identify pathways of heart failure progression," *Circulation. Heart Failure*, vol. 11, no. 2, article e004278, 2018.

[13] P. Lv, M. Zhou, J. He et al., "Circulating miR-208b and miR-34a are associated with left ventricular remodeling after acute myocardial infarction," *International Journal of Molecular Sciences*, vol. 15, no. 4, pp. 5774–5788, 2014.

[14] D. de Gonzalo-Calvo, G. Cediel, C. Bar et al., "Circulating miR-1254 predicts ventricular remodeling in patients with ST-segment-elevation myocardial infarction: a cardiovascular magnetic resonance study," *Scientific Reports*, vol. 8, no. 1, p. 15115, 2018.

[15] C. Bauters, R. Kumarswamy, A. Holzmann et al., "Circulating miR-133a and miR-423-5p fail as biomarkers for left

ventricular remodeling after myocardial infarction," *International Journal of Cardiology*, vol. 168, no. 3, pp. 1837–1840, 2013.

[16] S. C. Latet, P. L. Van Herck, M. J. Claeys et al., "Failed downregulation of circulating microRNA-155 in the early phase after ST elevation myocardial infarction is associated with adverse left ventricular remodeling," *Cardiology*, vol. 138, no. 2, pp. 91–96, 2017.

[17] U. Grabmaier, S. Clauss, L. Gross et al., "Diagnostic and prognostic value of miR-1 and miR-29b on adverse ventricular remodeling after acute myocardial infarction - the SITAGRAMI-miR analysis," *International Journal of Cardiology*, vol. 244, pp. 30–36, 2017.

[18] Y. Devaux, M. Vausort, G. P. McCann et al., "MicroRNA-150: a novel marker of left ventricular remodeling after acute myocardial infarction," *Circulation. Cardiovascular Genetics*, vol. 6, no. 3, pp. 290–298, 2013.

[19] S. Goldman and T. E. Raya, "Rat infarct model of myocardial infarction and heart failure," *Journal of Cardiac Failure*, vol. 1, no. 2, pp. 169–177, 1995.

[20] K. Thygesen, J. S. Alpert, A. S. Jaffe et al., "Fourth universal definition of myocardial infarction (2018)," *Journal of the American College of Cardiology*, vol. 72, no. 18, pp. 2231–2264, 2018.

[21] M. Writing Committee, C. W. Yancy, M. Jessup et al., "2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association task force on practice guidelines," *Circulation*, vol. 128, no. 16, pp. e240–e327, 2013.

[22] Authors/Task Force members, S. Windecker, P. Kolh et al., "2014 ESC/EACTS guidelines on myocardial revascularization," *European Heart Journal*, vol. 35, no. 37, pp. 2541–2619, 2014.

[23] J. C. Huang, T. Babak, T. W. Corson et al., "Using expression profiling data to identify human microRNA targets," *Nature Methods*, vol. 4, no. 12, pp. 1045–1049, 2007.

[24] Y. P. Wang and K. B. Li, "Correlation of expression profiles between microRNAs and mRNA targets using NCI-60 data," *BMC Genomics*, vol. 10, p. 218, 2009.

[25] M. D. Dennis, N. K. McGhee, L. S. Jefferson, and S. R. Kimball, "Regulated in DNA damage and development 1 (REDD1) promotes cell survival during serum deprivation by sustaining repression of signaling through the mechanistic target of rapamycin in complex 1 (mTORC1)," *Cellular Signalling*, vol. 25, no. 12, pp. 2709–2716, 2013.

[26] L. C. Caetano, M. L. Bonfleur, R. A. Ribeiro et al., "Taurine supplementation regulates Iκ-Bα protein expression in adipose tissue and serum IL-4 and TNF-α concentrations in MSG obesity," *European Journal of Nutrition*, vol. 56, no. 2, pp. 705–713, 2017.

[27] E. M. Seymour, M. R. Bennink, S. W. Watts, and S. F. Bolling, "Whole grape intake impacts cardiac peroxisome proliferator-activated receptor and nuclear factor kappaB activity and cytokine expression in rats with diastolic dysfunction," *Hypertension*, vol. 55, no. 5, pp. 1179–1185, 2010.

[28] G. Sun, R. Xue, F. Yao et al., "The critical role of sestrin 1 in regulating the proliferation of cardiac fibroblasts," *Archives of Biochemistry and Biophysics*, vol. 542, pp. 1–6, 2014.

[29] J. H. Lee, A. V. Budanov, E. J. Park et al., "Sestrin as a feedback inhibitor of TOR that prevents age-related pathologies," *Science*, vol. 327, no. 5970, pp. 1223–1228, 2010.

[30] B. Swynghedauw, "Molecular mechanisms of myocardial remodeling," *Physiological Reviews*, vol. 79, no. 1, pp. 215–262, 1999.

[31] E. Braunwald and M. A. Pfeffer, "Ventricular enlargement and remodeling following acute myocardial infarction: mechanisms and management," *The American Journal of Cardiology*, vol. 68, no. 14, pp. 1D–6D, 1991.

[32] E. M. Small and E. N. Olson, "Pervasive roles of microRNAs in cardiovascular biology," *Nature*, vol. 469, no. 7330, pp. 336–342, 2011.

[33] S. P. Romaine, M. Tomaszewski, G. Condorelli, and N. J. Samani, "MicroRNAs in cardiovascular disease: an introduction for clinicians," *Heart*, vol. 101, no. 12, pp. 921–928, 2015.

[34] K. W. Witwer, "Circulating microRNA biomarker studies: pitfalls and potential solutions," *Clinical Chemistry*, vol. 61, no. 1, pp. 56–63, 2015.

[35] R. V. Shah, J. Rong, M. G. Larson et al., "Associations of circulating extracellular RNAs with myocardial remodeling and heart failure," *JAMA Cardiology*, vol. 3, no. 9, pp. 871–876, 2018.

[36] J. A. Park and C. H. Lee, "Time-course change of Redd1 expressions in the hippocampal CA1 region following chronic cerebral hypoperfusion," *Cellular and Molecular Neurobiology*, vol. 37, no. 3, pp. 563–569, 2017.

[37] C. Liu, R. Xue, D. Wu et al., "REDD1 attenuates cardiac hypertrophy via enhancing autophagy," *Biochemical and Biophysical Research Communications*, vol. 454, no. 1, pp. 215–220, 2014.

[38] M. Laplante and D. M. Sabatini, "mTOR signaling in growth control and disease," *Cell*, vol. 149, no. 2, pp. 274–293, 2012.

[39] S. Sciarretta, M. Forte, G. Frati, and J. Sadoshima, "New insights into the role of mTOR signaling in the cardiovascular system," *Circulation Research*, vol. 122, no. 3, pp. 489–505, 2018.

[40] A. V. Budanov and M. Karin, "p53 target genes sestrin1 and sestrin2 connect genotoxic stress and mTOR signaling," *Cell*, vol. 134, no. 3, pp. 451–460, 2008.

[41] J. W. Gordon, J. A. Shaw, and L. A. Kirshenbaum, "Multiple facets of NF-κB in the heart: to be or not to NF-κB," *Circulation Research*, vol. 108, no. 9, pp. 1122–1132, 2011.

[42] W. Luo, G. Li, Z. Yi, Q. Nie, and X. Zhang, "E2F1-miR-20a-5p/20b-5p auto-regulatory feedback loop involved in myoblast proliferation and differentiation," *Scientific Reports*, vol. 6, p. 27904, 2016.

[43] Y. Hu, G. Jin, B. Li et al., "Suppression of miRNA let-7i-5p promotes cardiomyocyte proliferation and repairs heart function post injury by targetting CCND2 and E2F2," *Clinical Science*, vol. 133, no. 3, pp. 425–441, 2019.

[44] J. Zhang, J. Ma, K. Long et al., "Overexpression of exosomal cardioprotective miRNAs mitigates hypoxia-induced H9c2 cells apoptosis," *International Journal of Molecular Sciences*, vol. 18, no. 4, 2017.

[45] E. L. Vegter, E. S. Ovchinnikova, D. J. van Veldhuisen et al., "Low circulating microRNA levels in heart failure patients are associated with atherosclerotic disease and cardiovascular-related rehospitalizations," *Clinical Research in Cardiology*, vol. 106, no. 8, pp. 598–609, 2017.

[46] N. Bruno, J. M. ter Maaten, E. S. Ovchinnikova et al., "MicroRNAs relate to early worsening of renal function in patients with acute heart failure," *International Journal of Cardiology*, vol. 203, pp. 564–569, 2016.

[47] C. Voellenkle, J. van Rooij, C. Cappuzzello et al., "MicroRNA signatures in peripheral blood mononuclear cells of chronic heart failure patients," *Physiological Genomics*, vol. 42, no. 3, pp. 420–426, 2010.

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Research Article

Prognostic Value of MicroRNAs in Patients after Myocardial Infarction: A Substudy of PRAGUE-18

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Background. The evaluation of the long-term risk of major adverse cardiovascular events and cardiac death in patients after acute myocardial infarction (AMI) is an established clinical process. Laboratory markers may significantly help with the risk stratification of these patients. Our objective was to find the relation of selected microRNAs to the standard markers of AMI and determine if these microRNAs can be used to identify patients at increased risk. *Methods*. Selected microRNAs (miR-1, miR-133a, and miR-499) were measured in a cohort of 122 patients from the PRAGUE-18 study (ticagrelor vs. prasugrel in AMI treated with primary percutaneous coronary intervention (pPCI)). The cohort was split into two subgroups: 116 patients who did not die (survivors) and 6 patients who died (nonsurvivors) during the 365-day period after AMI. Plasma levels of selected circulating miRNAs were then assessed in combination with high-sensitivity cardiac troponin T (hsTnT) and N-terminal probrain natriuretic peptide (NT-proBNP). *Results*. miR-1, miR-133a, and miR-499 correlated positively with NT-proBNP and hsTnT 24 hours after admission and negatively with left ventricular ejection fraction (LVEF). Both miR-1 and miR-133a positively correlated with hsTnT at admission. Median relative levels of all selected miRNAs were higher in the subgroup of nonsurvivors ($N = 6$) in comparison with survivors ($N = 116$), but the difference did not reach statistical significance. All patients in the nonsurvivor subgroup had miR-499 and NT-proBNP levels above the cut-off values (891.5 ng/L for NT-proBNP and 0.088 for miR-499), whereas in the survivor subgroup, only 28.4% of patients were above the cut-off values ($p = 0.001$). *Conclusions*. Statistically significant correlation was found between miR-1, miR-133a, and miR-499 and hsTnT, NT-proBNP, and LVEF. In addition, this analysis suggests that plasma levels of circulating miR-499 could contribute to the identification of patients at increased risk of death during the first year after AMI, especially when combined with NT-proBNP levels.

1. Introduction

The in-hospital mortality rate for acute myocardial infarction is low, due to efficient antiplatelet treatment and primary percutaneous coronary intervention (pPCI); unfortunately, the risk of cardiac death increases during the chronic phase of ischemic heart disease that follows.

Decreased left ventricular systolic function with left ventricular ejection fraction (LVEF) ≤ 35% and recurrent ventricular tachycardia or ventricular fibrillation, beyond the early phase of myocardial infarction, are connected with a poor prognosis and are a potential indication for cardioverter implantation [1].

Despite the clear benefit of these widely used predictors, they seem to be inadequate for identifying all patients at risk of sudden death, since it fails to identify about 50% of patients who die suddenly [2] after acute myocardial infarction (AMI). Some of the standard laboratory markers associated

with the risk of sudden death can be used in combination with LVEF to improve the risk assessment process, but unfortunately, well-defined cut-off values are still not known.

Among factors that can be used for risk stratification after AMI, the following play an important role: elevated levels of troponin T or I (TnT or TnI) [3, 4] and a combination of (A) increased TnT and CRP plasma levels, (B) increased levels of N-terminal prohormone of brain natriuretic peptide (NT-proBNP) with LVEF < 40% [4–7], and (C) decreased clearance of creatinine (with a reduced LVEF) [8].

A promising group of new biomarkers, released from cells into circulation, is microRNAs (miRNAs), which are small noncoding RNA molecules, 20–22 nucleotides in length, involved in posttranscriptional regulation of gene expression. Mature miRNAs and Ago proteins (Argonaute proteins) form in the cytoplasm RISC complexes (RNA-induced silencing complexes) that interact with protein-coding mRNA molecules. This interaction usually leads to the inhibition of translation or directly to the degradation of mRNA molecules. One particular microRNA can regulate many genes (i.e., interacting with a variety of different protein-coding mRNAs), and one particular gene can be regulated by several different microRNAs. MicroRNAs can act directly within the cells where they are synthesized, or they can be exported, in complexes with proteins or in membrane-bound vesicles (exosomes or microvesicles), to other cells where they can also regulate gene expression. MicroRNAs are involved in the control of many processes in both healthy and infarcted myocardia, including proliferation, differentiation, apoptosis, repair, and revascularization [9]. Additionally, miRNA dysregulation has been strongly implicated in the destabilization and rupture of atherosclerotic plaques [10] as well as being involved in the process of myocardial recovery.

In cardiovascular diseases (CVD), the use of miRNAs as biomarkers for specific disease entities has been successfully investigated in numerous studies [11]. Nonetheless, it is not yet possible to use them in clinical practice [12]. miRNAs also have the potential for clinical use in CVD where protein biomarkers are not available.

More than 2500 mature miRNAs have been identified in humans. Four of them, miR-1, miR-133, miR-208a, and miR-499 have been found to be specific for the myocardium (or the myocardium and skeletal muscle) and are sometimes called "myomiRs" [13].

Many authors have shown that levels of circulating myomiRs increase significantly during the first few hours after the onset of myocardial infarction symptoms. After reaching a peak, myomiRs return to normal after a few hours or a few days [14, 15].

We decided to retrospectively measure the relative levels of circulating miR-1, miR-133a, miR-208a, and miR-499 in a well-described cohort of 122 patients with known one-year mortality, previously involved in the PRAGUE-18 study [16, 17]. The listed miRNAs were assessed alone and in combination with several standard markers in an effort to better characterize the nonsurvivor subgroup, with the goal of finding additional predictors of patients at increased risk of one-year cardiovascular death.

2. Material and Methods

2.1. Patients. The whole cohort of 122 patients was treated in the Department of Cardiology, University Hospital and Faculty of Medicine of Charles University, Pilsen, Czech Republic, which was one of the centers involved in phase IV of a multicenter, open-label, randomized, controlled clinical trial called the PRAGUE-18 study [16, 17].

The PRAGUE-18 study, which compared prasugrel and ticagrelor in the treatment of acute myocardial infarction, was the first randomized head-to-head comparison of these two active substances, with regard to efficacy and safety in patients after AMI undergoing pPCI. One of the outcomes was the combined endpoint of cardiovascular death, MI, or stroke within the first year. Prasugrel and ticagrelor had been similarly effective during the first year after AMI [16, 17]. Plasma samples from 122 patients in the study were used for this retrospective data analysis, where (I) levels of selected circulating microRNAs, (II) standard AMI biomarkers, and (III) LVEF were used to (A) look for correlations between miRNAs and standard AMI markers, (B) identify differences in biomarkers between survivors and nonsurvivors during the first year after AMI, and (C) better characterize the nonsurvivor subgroup relative to measures I, II, and III mentioned above.

2.2. Echocardiography. Two-dimensional, M-mode, and Doppler echocardiograms were acquired using an ultrasound system (Vivid 7, GE Medical Systems, Horton, Norway) using a 3.4 MHz multifrequency transducer. The systolic function of the left ventricle was determined according to the Simpson method from the apical 4-chamber view and the apical 2-chamber view (the biplane Simpson method).

2.3. Levels of Biomarkers. Data for the basic characteristics of all patients involved in the analysis were available from the PRAGUE-18 study. Levels of standard AMI biomarkers were known, including hsTnT, NT-proBNP, cystatin C, myoglobin, growth/differentiation factor 15 (GDF-15), and creatine kinase (CK) at patient admission and hsTnT also after 24 hours.

NT-proBNP was determined using the original analytical kits from Roche on a cobas® 8000 analyzer. NT-proBNP and high-sensitivity cardiac troponin were determined using the original analytical kits from Roche with the electrochemiluminiscence (ECLIA) principle on a cobas e602 analyzer. Imprecission of the hsTnT method on the 99th percentile was below 10% which is the required analytical performance specification. Growth/differentiation factor 15 (GDF-15) (RayBiotech, Norcross, USA) was determined using ELISA kits on a NEXgen Four ELISA reader (Adaltis, Rome, Italy).

Since hsTnT is the most frequently used standard biomarker of AMI and NT-proBNP is a sensitive marker of left ventricular dysfunction, we used them in combination with the potential new microRNA biomarkers, in subsequent analyses.

2.4. MicroRNA Analysis

2.4.1. RNA Isolation. MicroRNA was isolated from plasma samples taken 24 hours after admission (all patients were

already after pPCI at that time) and stored at −80°C. Total cell-free RNA was isolated from 200 μL of plasma using miRNeasy Serum/Plasma Kits (miRNeasy Serum/Plasma Kit (50), Cat no./ID 217184; Qiagen, Hilden, Germany) according to the manufacturer's instructions. Total RNA was eluted in 14 μL of ribonuclease-free water and stored at −80°C until further analyses. MicroRNA-39 (*C. elegans* miR-39) was used as the RNA spike-in control. A fixed volume of 1 μL of this RNA eluate was used for each reverse transcription reaction.

2.4.2. Quantitative Estimation of MicroRNA Expression. For reverse transcriptions and quantitative estimations of selected microRNAs using real-time PCR reactions, TaqMan® MicroRNA Assays and master mixes were used (catalogue number 4440887: hsa-miR-133a-3p—Assay ID 002246, hsa-miR-1-3p—Assay ID 002222, hsa-miR-499a-5p—Assay ID 001352, hsa-miR-208-3p—Assay ID 000511, and cel-miR-39-3p—Assay ID 000200; TaqMan Universal MMIX II: catalogue number 4440049; and TaqMan® MicroRNA RT Kit: catalogue number 4366597). A T100TM thermal cycler (Bio-Rad, California, United States) was used for reverse transcription. The reaction volume was 15 μL. A fixed volume of 2.5 μL from this RT reaction was used into each real-time PCR reaction. Due to either too high or absent Ct values, levels of miR-208a could not be quantified and evaluated.

2.4.3. Processing of Real-Time PCR Data. Samples were assessed in technical duplicate. The Ct values were corrected using calibrators to eliminate differences between individual runs of the Stratagene Mx3000P Real-Time PCR apparatus (Agilent Technologies, CA, United States). In cases where a disagreement between results obtained from both technical duplicates was found, the sample assessment was repeated. Plasma levels for each miRNA were calculated in the form of a relative expression. This relative expression was calculated using the ΔCt method (i.e., the $2^{-\Delta Ct}$ algorithm was $\Delta Ct = Ct_{miR-x} - Ct_{miR-39}$).

2.5. Objectives. Our objectives were to find relationships between selected miRNAs and the standard biomarkers of AMI as well as to find a panel of standard and potential biomarkers that might contribute to the identification of high-risk patients after acute myocardial infarction and post-pPCI treatment. The whole cohort was split according to the primary outcome (death within 365 days after AMI) into two subgroups (survivors and nonsurvivors), and both subgroups were characterized according to their biomarker levels.

2.6. Statistical Analysis. In this analysis, standard descriptive statistics were applied; absolute and relative frequencies were used for categorical variables and medians (supplemented with the 5th and 95th percentiles) were used for continuous variables (mean, SD, and CV were also used for the description of miRs). The statistical significance of differences among groups of patients was tested using Fisher's exact test for categorical variables and the Mann-Whitney test for continuous variables. The Spearman correlation coefficient was used for the analysis of the statistical relationship between miRNAs and the standard markers. Cut-off points (cut-off values) of predictors of all-cause death during 365 days were established by ROC analysis. The point that guarantees the greatest sum of sensitivity and specificity was chosen as the best point. Risk factors for all-cause death during 365 days were analyzed by a Cox regression model of proportional hazards. Analysis was performed in IBM SPSS Statistics 24.0 with 5% level of significance.

3. Results

3.1. Baseline Characteristics. The analysis involved 122 adult patients (78.7% men and 21.3% women) with AMI followed by pPCI; the median age was 61.1 years. All patients used either prasugrel (53.3%) or ticagrelor (46.7%) for antiplatelet therapy. The cohort of patients was split into two subgroups: nonsurvivors ($N = 6$) and survivors ($N = 116$). Only six patients died within one year after AMI (three patients from the prasugrel and three from the ticagrelor group): five died suddenly and one died while in the hospital from an unconfirmed diagnosis of pulmonary embolism. All patients in this subgroup had an LVEF ≥ 40% at their control visit, which was 2–3 months after discharge from the hospital. The baseline characteristics of all patients, and both subgroups, including their comparison, are shown in Table 1.

3.2. Correlation of miRNAs with Standard Biomarkers. The relative levels of all three miRNAs were related to the levels of standard biomarkers: hsTnT (at admission), hsTnT (24 hours after admission), NT-proBNP, GDF-15, cystatin C, and LVEF.

miR-133a and miR-1 weakly positively correlated with hsTnT at admission and strongly positively correlated with hsTnT 24 hours after admission (Figure 1). miR-499 moderately correlated with hsTnT 24 hours after admission. A strong negative correlation was found between all three miRNAs and the LVEF (Figure 1). A strong positive correlation was identified between both miR-133a and miR-499 and NT-proBNP, and a moderate positive correlation was found between miR-1 and NT-proBNP (Figure 1).

No correlation was found between any of the miRNAs and GDP-15 or cystatin C.

3.3. The Relationship between miRNAs and One-Year Mortality. The assessment of the prognostic potential of the selected biomarkers, for the identification of patients at increased risk of death, was based on their peripheral plasma levels and one-year survival.

Median relative levels of miRNAs were higher in the nonsurvivor subgroup. But the total number of patients in this subgroup was small in comparison with that in the group of survivors (six vs. one hundred and sixteen), and the differences found did not reach statistical significance for any of the tested microRNAs (Figure 2).

The calculated cut-off values for miR-1, miR-133a, and miR-499 were 0.031, 0.330, and 0.088, respectively. Relative miRNA concentrations below these cut-off values were described as "low," and those above the value were described as "high."

Table 1: Baseline characteristics.

	All patients	Survivors	Nonsurvivors	p values
		Median (5th-95th percentile)		
Number of patients	122	116	6	
Age (years)	61.1 (40.4–76.8)	61.1 (40.1–76.7)	65.7 (56.1–81.0)	0.166
Men (number, %)	96 (78.7%)	91 (78.4%)	5 (83.3%)	0.999
BMI	27.6 (22.2–34.3)	27.6 (22.1–34.3)	26.7 (24.7–44.1)	0.929
Drug used: prasugrel (number, %)	65 (53.3%)	62 (53.4%)	3 (50.0%)	0.999
Drug used: ticagrelor (number, %)	57 (46.7%)	54 (46.6%)	3 (50.0%)	
STEMI (number, %)	121 (99.2%)	115 (99.1%)	6 (100.0%)	0.999
Left bundle branch block (LBBB) (number, %)	1 (0.8%)	1 (0.9%)	0 (0.0%)	0.999
Right bundle branch block (RBBB) (number, %)	1 (0.8%)	1 (0.9%)	0 (0.0%)	0.999
Hyperlipidaemia (number, %)	36 (29.5%)	35 (30.2%)	1 (16.7%)	0.669
Obesity (number, %)	23 (18.9%)	22 (19.0%)	1 (16.7%)	0.999
Arterial hypertension (number, %)	56 (45.9%)	52 (44.8%)	4 (66.7%)	0.412
Smoking (number, %)	84 (68.9%)	80 (69.0%)	4 (66.7%)	0.999
Diabetes mellitus (number, %)	17 (13.9%)	16 (13.8%)	1 (16.7%)	0.999
Time since the first symptoms to admission (hours)	3.0 (0.5–36.0)	3.0 (0.5–12.0)	6.0 (3.0–72.0)	0.061
Left ventricular ejection fraction (%)	50.0 (30.0–60.0)	55.0 (30.0–60.0)	45.0 (30.0–50.0)	0.054
Laboratory values (median (5th-95th percentile))				
hsTnT (at admission) (ng/L)	86.0 (12.0–1325.0)	84.0 (12.0–1325.0)	201.5 (27.0–4978.0)	0.257
hsTnT (24 hours after admission) (ng/L)	2432.0 (377.0–9651.0)	2324.0 (368.0–9651.0)	4306.5 (1526.0–15114.0)	0.201
Myoglobin (at admission) (μg/L)	198.0 (30.0–1385.0)	176.0 (30.0–1547.0)	652.0 (161.0–1317.0)	0.066
Creatine kinase (at admission) (μkat/L)	3.9 (1.4–23.7)	3.8 (1.3–23.7)	6.4 (2.6–26.8)	0.097
NT-proBNP (at admission) (ng/L)	757.0 (105.0–4142.0)	666.5 (104.0–4285.0)	1373.5 (904.0–3096.0)	0.074
Cystatin C (at admission) (mg/L)	121; 0.99 (0.80–1.47)	1.00 (0.79–1.49)	0.92 (0.85–1.09)	0.417
GDF-15 (at admission) (ng/L)	807.1 (372.8–1827.7)	796.3 (372.8–1827.7)	1044.9 (357.3–1848.8)	0.305

GDF-15 = growth/differentiation factor 15; hsTnT = high-sensitivity troponin T; NT-proBNP = N-terminal prohormone of brain natriuretic peptide; STEMI = acute myocardial infarction with ST-segment elevation.

Comparisons of the number of patients with low and high concentrations of particular miRNAs were made in both subgroups; in the nonsurvivor group, the relative frequency of high concentrations was higher, and in the case of miR-133a and miR-499, this difference reached statistical significance (Table 2, microRNAs). All 6 nonsurvivors had a high concentration of miR-499, whereas, in the survivor subgroup, only 46% of patients had a high concentration.

3.4. Relationship between Standard Biomarkers and One-Year Mortality. For the standard markers hsTnT and NT-proBNP, cut-off values were found in the same way as for miRNAs, and values were then described as either "low" or "high." The cut-off value for hsTnT was 154.5 ng/L, and for NT-proBNP, it was 891.5 ng/L.

The number of patients with low and high concentrations of these two biomarkers was compared in both subgroups, and in the nonsurvivor group, the frequency of high marker levels was higher; in the case of NT-proBNP, this difference was statistically significant (Table 3, standard biomarkers). All patients who died within one year had a high concentration of NT-proBNP, whereas in the survivor group, only 43% had a high concentration of NT-proBNP.

3.5. Combinations of Biomarkers. Using the estimated cut-off values, two or three biomarkers were combined, in an effort to better describe the nonsurvivor subgroup and identify patients at risk of death. Combinations included (A) combinations of different microRNAs, (B) combinations of standard markers, and (C) combinations of microRNAs and standard markers. All tested combinations are shown in Table 3.

Based on a combination of NT-proBNP and miR-499 levels, a test group of 39 "at-risk" patients was created, which was 32% of the entire (survivor+nonsurvivor) cohort. The NT-proBNP and miR-499 combination criteria put all six nonsurvivors in the "at-risk" group, where they represented 15% of the "at-risk" group.

4. Discussion

In patients with a proven increased risk of death based on cardiovascular risk stratification during hospitalization, treatment with ACE inhibitors (or angiotensin AT1 blockers), beta-blocker therapy, and aldosterone antagonists are indicated when EF LK is ≤40% and/or there is heart failure [1]. Implantation of cardioverter-defibrillator (ICD) in a

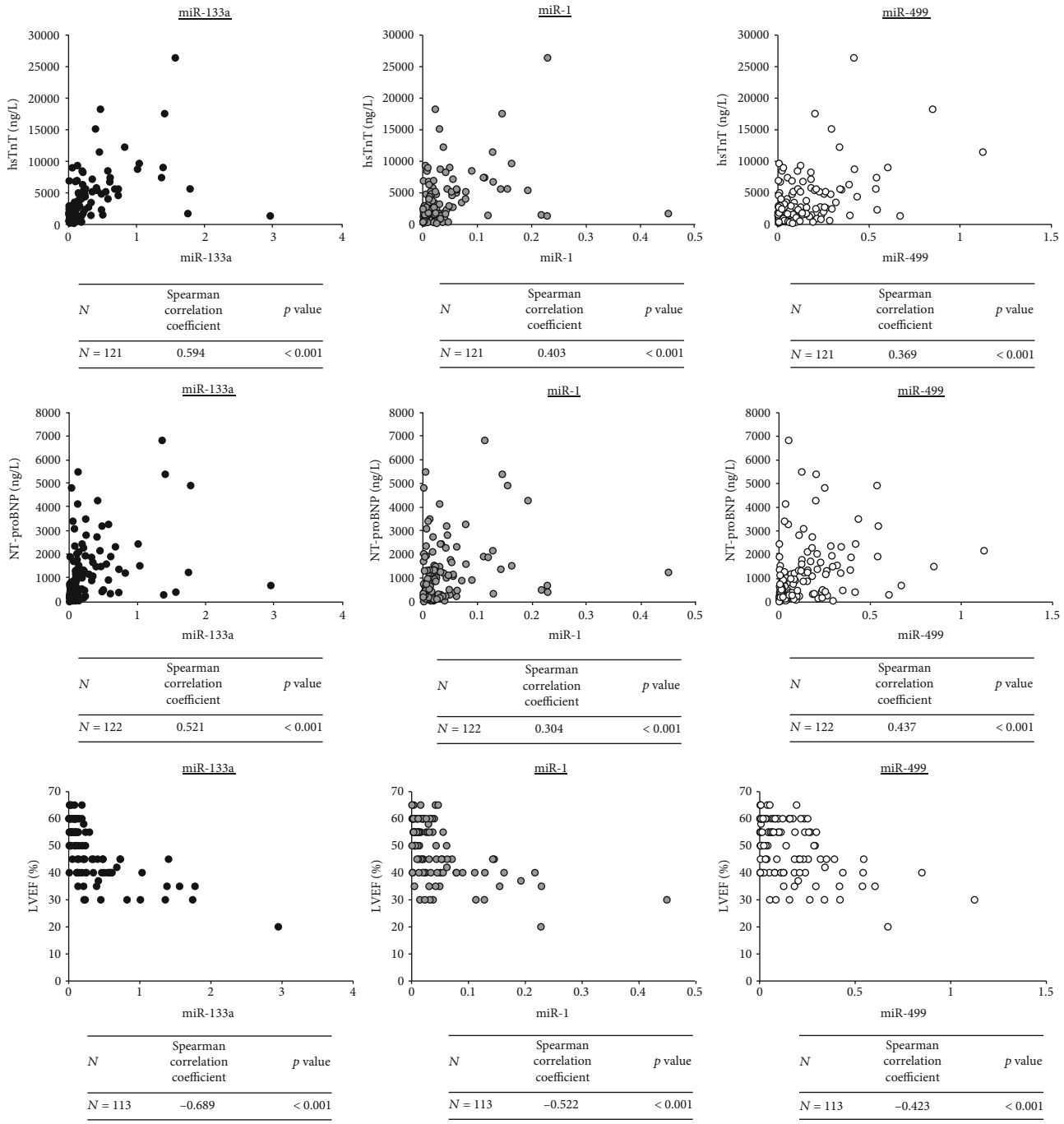
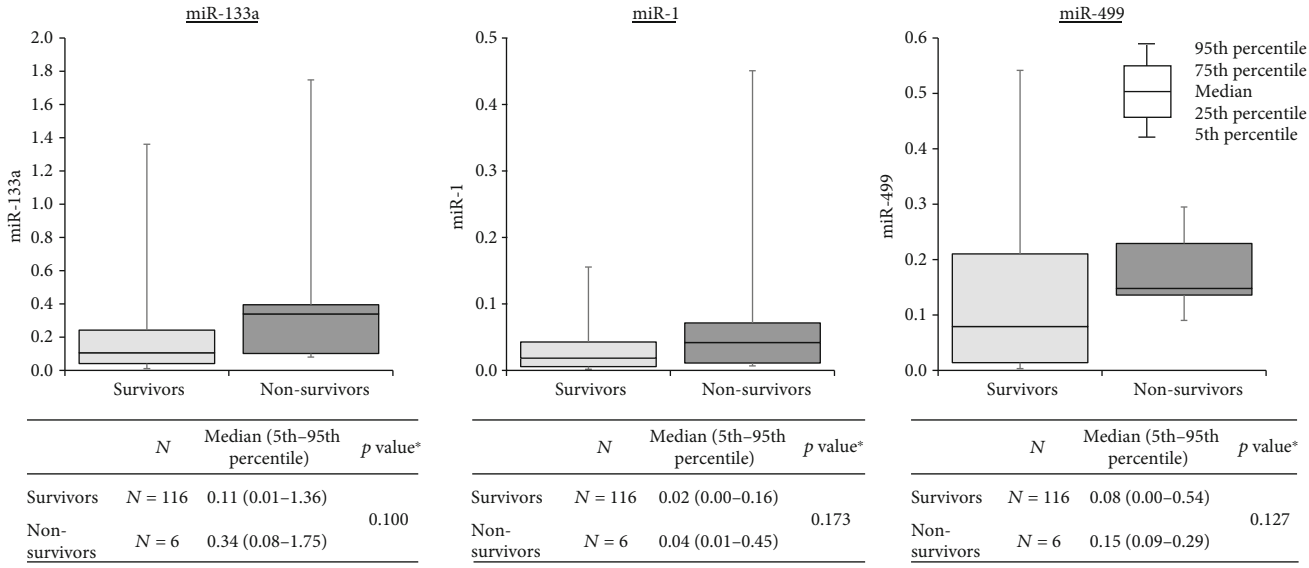


Figure 1: Correlations between particular miRNAs (relative expression) and hsTnT levels 24 hours after admission, NT-proBNP, and LVEF. hsTnT = high-sensitivity troponin T; LVEF = left ventricular ejection fraction; NT-proBNP = N-terminal prohormone of brain natriuretic peptide.

selected patient population is indicated when the indication criteria are met [1].

Despite the risk stratification of patients after myocardial infarction, ischemic complications recur even at low calculated risk, and these events can be fatal. miRNAs, as a group of the potential new markers, could help in the stratification of these patients. Then, if an increased miRNA value and usual risk parameters including LVEF are found without significant pathology, supplementation of the Holter ECG to exclude ventricular arrhythmias and careful follow-up of these patients should be considered.

For this reason, we used a well-defined and very homogeneous cohort of AMI patients after pPCI and tested the prognostic value of three cardiomyo-specific miRNAs (miR-1, miR-133, and miR-499) in one-year cardiovascular mortality and their relation to standard laboratory markers. We proved correlations between levels of miR-1, miR-133, and miR-499 with hsTnT, NT-proBNP, and LVEF in this cohort of



	N	Median (5th–95th percentile)	p value*
Survivors	N = 116	0.11 (0.01–1.36)	0.100
Non-survivors	N = 6	0.34 (0.08–1.75)	

	N	Median (5th–95th percentile)	p value*
Survivors	N = 116	0.02 (0.00–0.16)	0.173
Non-survivors	N = 6	0.04 (0.01–0.45)	

	N	Median (5th–95th percentile)	p value*
Survivors	N = 116	0.08 (0.00–0.54)	0.127
Non-survivors	N = 6	0.15 (0.09–0.29)	

Figure 2: Relationship between miRNAs (relative expression) and one-year mortality. *Mann-Whitney test.

Table 2: Relationship between individual marker levels and one-year mortality.

Marker	Gene Locus (OMIM)	Concentration	Patients, number (%)	Survivors, number (%)	Nonsurvivors, number (%)	p value*
MicroRNAs (relative concentration)						
miR-133a	MIR133A1/MIR133A2	<0.330	93 (76.2)	91 (78.4)	2 (33.3)	**0.028**
	18q11.2/20q13.33	≥0.330	29 (23.8)	25 (21.6)	4 (66.7)	
miR-1	MIR1-1/MIR1-2	<0.031	78 (63.9)	76 (65.5)	2 (33.3)	0.187
	20q13.33/18q11.2	≥0.031	44 (36.1)	40 (34.5)	4 (66.7)	
miR-499	MIR499	<0.088	63 (51.6)	63 (54.3)	0 (0.0)	**0.011**
	20q11.22	≥0.088	59 (48.4)	53 (45.7)	6 (100.0)	
Standard biomarkers (concentration in ng/L)						
hsTnT	TNN2	<154.5	77 (63.6)	75 (65.2)	2 (33.3)	0.189
	1q32.1	≥154.5	44 (36.4)	40 (34.8)	4 (66.7)	
NT-proBNP	NPPB	<891.5	66 (54.1)	66 (56.9)	0 (0.0)	**0.008**
	1p36.22	≥891.5	56 (45.9)	50 (43.1)	6 (100.0)	

*Fisher exact test. NT-proBNP = N-terminal prohormone of brain natriuretic peptide; hsTnT = high-sensitivity troponin.

patients. In addition, we found a possible relationship between combined levels of miR-499 with NT-proBNP and increased one-year mortality risk in these patients on dual antiplatelet therapy that has not been published yet.

4.1. MyomiR Levels after Myocardial Infarction. Many authors focus on miRNA levels during cardiovascular events and their possible contribution to the diagnostics or differential diagnostics [14, 18]. Published papers found that levels of miR-1 and miR-133a/b increase soon after AMI, reaching a peak shortly before TnI and returning to baseline within five days, while miR-499 peaks later, about 12 hours after the onset of the first symptoms [14]. miR-499 levels are naturally very low in healthy people and increase after AMI, with levels being higher in acute myocardial infarction with ST-segment elevation (STEMI) compared to non-STEMI patients [18], and provide a comparable diagnostic value to that of hsTnT [18]. Concentrations of miR-499 are higher in patients after AMI compared to patients with unstable angina [19]. miR-499 remains increased 24 hours after MI and then slowly decreases to original levels over 7 days [19]. Increased levels of circulating miR-499 and miR-208 after AMI reflect the cardiac damage caused by the AMI [19]. miR-208 levels are usually under the limits of detection in healthy individuals but rapidly increase after AMI. The peak is observed 3 hours after reperfusion, which is then followed by a rapid fall in concentration back to initial levels within 24 hours [20]. Since our samples were taken 24 hours after admission to

Table 3: Relationship between various combinations of marker levels and one-year mortality.

Markers and their levels	Patients, number (%)	Survivors, number (%)	Nonsurvivors, number (%)	*p* value*
MicroRNAs				
miR-133a+miR-1				
Both low	75 (61.5)	73 (62.9)	2 (33.3)	
One low and one high	21 (17.2)	21 (18.1)	0 (0.0)	**0.045**
Both high	26 (21.3)	22 (19.0)	4 (66.7)	
miR-133a+miR-499				
Both low	57 (46.7)	57 (49.1)	0 (0.0)	
One low and one high	42 (34.4)	40 (34.5)	2 (33.3)	**0.004**
Both high	23 (18.9)	19 (16.4)	4 (66.7)	
miR-1+miR-499				
Both low	47 (38.5)	47 (40.5)	0 (0.0)	
One low and one high	47 (38.5)	45 (38.8)	2 (33.3)	**0.019**
Both high	28 (23.0)	24 (20.7)	4 (66.7)	
miR-133a+miR-1+miR-499				
All low	47 (38.5)	47 (40.5)	0 (0.0)	
Minimum one low, minimum one high	55 (45.1)	53 (45.7)	2 (33.3)	**0.003**
All high	20 (16.4)	16 (13.8)	4 (66.7)	
Standard biomarkers				
hsTnT+NT-proBNP				
Both low	42 (34.7)	42 (36.5)	0 (0.0)	
One low and one high	58 (47.9)	56 (48.7)	2 (33.3)	**0.006**
Both high	21 (17.4)	17 (14.8)	4 (66.7)	
MicroRNAs and standard biomarkers				
hsTnT+miR-133a				
Both low	63 (52.1)	62 (53.9)	1 (16.7)	
One low and one high	43 (35.5)	41 (35.7)	2 (33.3)	**0.024**
Both high	15 (12.4)	12 (10.4)	3 (50.0)	
hsTnT+miR-1				
Both low	53 (43.8)	52 (45.2)	1 (16.7)	
One low and one high	48 (39.7)	46 (40.0)	2 (33.3)	0.094
Both high	20 (16.5)	17 (14.8)	3 (50.0)	
hsTnT+miR-499				
Both low	39 (32.2)	39 (33.9)	0 (0.0)	
One low and one high	61 (50.4)	59 (51.3)	2 (33.3)	**0.005**
Both high	21 (17.4)	17 (14.8)	4 (66.7)	
NT-proBNP+miR-133a				
Both low	59 (48.4)	59 (50.9)	0 (0.0)	
One low and one high	41 (33.6)	39 (33.6)	2 (33.3)	**0.003**
Both high	22 (18.0)	18 (15.5)	4 (66.7)	
NT-proBNP+miR-1				
Both low	51 (41.8)	51 (44.0)	0 (0.0)	
One low and one high	42 (34.4)	40 (34.5)	2 (33.3)	**0.014**
Both high	29 (23.8)	25 (21.6)	4 (66.7)	
NT-proBNP+miR-499				
Both low	46 (37.7)	46 (39.7)	0 (0.0)	
One low and one high	37 (30.3)	37 (31.9)	0 (0.0)	**0.001**
Both high	39 (32.0)	33 (28.4)	6 (100.0)	

*Fisher exact test: difference between both subgroups. hsTnT = high-sensitivity troponin T; NT-proBNP = N-terminal prohormone of brain natriuretic peptide.

the hospital, the concentration of miR-208 was either under the detection limit or too low to be quantified, so this miRNA was not included in our analysis, and only levels of miR-1, miR-133a, and miR-499 were measured.

4.2. Correlations of miRNAs with Standard Markers. We focused on the correlation with selected standard biomarkers and found a significant positive correlation of the three microRNAs with hsTnT and NT-proBNP. Our findings agree with other published papers, where levels of miR-499 were found to be positively correlated with levels of troponin T and I [14, 19, 21], despite minor differences in methods, the cohort of MI patients, and time of sampling. A positive correlation ($r = 0.596$, $p < 0.001$) between miR-133a and cTnI was previously published [19, 22] and reported a similar trend in levels of both markers in the early phase of AMI [22]; another work described an early miR-1, miRNA-133a, and miR-133b peak that occurred at a similar time as the TnI peak, whereas miR-499-5p exhibited a slower time course [14]. A correlation was also found between miR-499 and creatinine kinase (CK) [18, 19].

All the three analyzed miRNAs were found to have a moderate or strong positive correlation with NT-proBNP, which was published to be an important independent predictor of poor outcomes [23]. Furthermore, we found a strong negative correlation between all the three miRNAs and LVEF, which is in line with several other authors who found a similar negative correlation of miR-499 with LVEF ($r = -0.36$, $p = 0.008$) [16] or a weak negative correlation of miR-499-5p with LVEF (-0.11, $p = 0.037$) and miR-1 with LVEF ($r = -0.16$, $p = 0.003$) [21].

4.3. miRNAs in One-Year Prognosis. Finally, we looked for differences in the levels of laboratory markers between patients at an increased risk of death (nonsurvivors) and survivors. We found that all nonsurvivors had high levels of NT-proBNP and high levels of miR-499. Levels of NT-proBNP were measured in all AMI patients shortly after admission to the hospital before pPCI; in addition, the levels of microRNAs were also measured as potential new biomarkers. The choice of microRNAs was based on promising assessments for diagnostics or prognostics in recently published literature [14, 21, 22, 24].

Current risk stratification is based primarily on left ventricular dysfunction, measured as left ventricular ejection fraction [1, 2]. Many studies have found a clear relationship between reduced LVEF and mortality, which increases when LVEF falls under 50% and progressively increases when LVEF declines under 40% [2]. Despite this important predictor, about 50% of patients who die suddenly do not meet the abovementioned LVEF criteria [2]. Also, in our cohort, only 2 patients out of 6 in the nonsurvivor subgroup had an LVEF ≤ 35% during hospitalization and none at the time of follow-up. Our goal was to find a combination of laboratory markers that could contribute to the better identification of patients at increased risk of death after myocardial infarction and thus decrease the relatively high post-AMI mortality that reaches 7–20% at one year, 24–38% at five years, and 40–56% at ten years [2].

In our work, we analyzed cardio-enriched microRNAs, measurable 24 hours after patient admission to the hospital, to see if some of them could potentially fit into such a panel of biomarkers. Our results found that miR-499 in combination with NT-proBNP was best able to characterize the nonsurvivor subgroup. The number of papers dealing with myomiRs and AMI patients' prognosis is relatively limited. A recently published work confirms increased levels of cardio-enriched miRNAs (miR-499 and miR-208) in the blood of AMI patients and establishes an association of increased miRNA levels with reduced systolic function after AMI and risk of death or heart failure within 30 days [21]. Another work found that circulating levels of miR-133a and miR-208b were associated with all-cause mortality at 6 months, but this did not add prognostic information to hsTnT, the standard marker of AMI [25]. miR-133 was also studied in the high-risk STEMI patient cohort, where its levels provided prognostic information but do not add independent prognostic information to traditional markers of AMI [26].

In spite of the undeniable advantages of a well-defined and very homogeneous cohort of patients, this analysis was limited by the low number of patients in the nonsurvivor subgroup and by its retrospective character.

5. Conclusion

One-year mortality in patients after AMI treated with pPCI was very low (4.9%). A positive correlation was found between miRNA-1, miR-133a, and miR-499 and hsTnT (24 hours after admission) and NT-proBNP, and a negative correlation with LVEF. Further, this work suggests that plasma levels of circulating miR-499 might contribute to the identification of patients at increased risk of death, especially when combined with NT-proBNP levels. Further analyses are needed to determine if miR-499 or some other miRNAs can be effectively used in practice to better identify at-risk patients, to better understand the roles of these miRNAs in AMI, and to thus improve the clinical management of patients after AMI.

Data Availability

The data (miRNA Ct values and values of hsTnT and NT-proBNP) used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

No potential conflict of interest was reported by the authors.

Acknowledgments

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References

[1] B. Ibanez, S. James, S. Agewall et al., "2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation," *European Heart Journal*, vol. 39, no. 2, pp. 119–177, 2017.

[2] J. W. Waks and A. E. Buxton, "Risk stratification for sudden cardiac death after myocardial infarction," *Annual Review of Medicine*, vol. 69, no. 1, pp. 147–164, 2018.

[3] G. Ndrepepa, S. Kufner, M. Hoyos et al., "High sensitivity cardiac troponin T and prognosis in patients with ST-segment elevation myocardial infarction," *Journal of Cardiology*, vol. 72, no. 3, pp. 220–226, 2018.

[4] S. Gupta, G. S. Pressman, and V. M. Figueredo, "Incidence of, predictors for, and mortality associated with malignant ventricular arrhythmias in non-ST elevation myocardial infarction patients," *Coronary Artery Disease*, vol. 21, no. 8, pp. 460–465, 2010.

[5] J. M. Tapanainen, K. S. Lindgren, T. H. Mäkikallio, O. Vuolteenaho, J. Leppäluoto, and H. V. Huikuri, "Natriuretic peptides as predictors of non-sudden and sudden cardiac death after acute myocardial infarction in the beta-blocking era," *Journal of the American College of Cardiology*, vol. 43, no. 5, pp. 757–763, 2004.

[6] M. Grabowski, K. J. Filipiak, L. A. Malek et al., "Admission B-type natriuretic peptide assessment improves early risk stratification by Killip classes and TIMI risk score in patients with acute ST elevation myocardial infarction treated with primary angioplasty," *International Journal of Cardiology*, vol. 115, no. 3, pp. 386–390, 2007.

[7] H. V. Huikuri, J. M. Tapanainen, K. Lindgren et al., "Prediction of sudden cardiac death after myocardial infarction in the beta-blocking era," *Journal of the American College of Cardiology*, vol. 42, no. 4, pp. 652–658, 2003.

[8] L. Savic, I. Mrdovic, J. Perunicic et al., "Impact of the combined left ventricular systolic and renal dysfunction on one-year outcomes after primary percutaneous coronary intervention," *Journal of Interventional Cardiology*, vol. 25, no. 2, pp. 132–139, 2012.

[9] T. Sun, Y. H. Dong, W. Du et al., "The role of microRNAs in myocardial infarction: from molecular mechanism to clinical application," *International Journal of Molecular Sciences*, vol. 18, no. 4, p. 745, 2017.

[10] S. M. J. Welten, E. A. C. Goossens, P. H. A. Quax, and A. Y. Nossent, "The multifactorial nature of microRNAs in vascular remodelling," *Cardiovascular Research*, vol. 110, no. 1, pp. 6–22, 2016.

[11] C. Schulte, M. Karakas, and T. Zeller, "MicroRNAs in cardiovascular disease - clinical application," *Clinical Chemistry and Laboratory Medicine*, vol. 55, no. 5, pp. 687–704, 2017.

[12] E. Cavarretta and G. Frati, "MicroRNAs in coronary heart disease: ready to enter the clinical arena?," *BioMed Research International*, vol. 2016, Article ID 2150763, 10 pages, 2016.

[13] J. J. McCarthy, "The myomiR network in skeletal muscle plasticity," *Exercise and Sport Sciences Reviews*, vol. 39, no. 3, pp. 150–154, 2011.

[14] Y. D'Alessandra, P. Devanna, F. Limana et al., "Circulating microRNAs are new and sensitive biomarkers of myocardial infarction," *European Heart Journal*, vol. 31, no. 22, pp. 2765–2773, 2010.

[15] M. F. Corsten, R. Dennert, S. Jochems et al., "Circulating microRNA-208b and microRNA-499 reflect myocardial damage in cardiovascular disease," *Circulation. Cardiovascular Genetics*, vol. 3, no. 6, pp. 499–506, 2010.

[16] Z. Motovska, O. Hlinomaz, P. Kala et al., "1-year outcomes of patients undergoing primary angioplasty for myocardial infarction treated with prasugrel versus ticagrelor," *Journal of the American College of Cardiology*, vol. 71, no. 4, pp. 371–381, 2018.

[17] Z. Motovska, O. Hlinomaz, R. Miklik et al., "Prasugrel versus ticagrelor in patients with acute myocardial infarction treated with primary percutaneous coronary intervention: multicenter randomized PRAGUE-18 study," *Circulation*, vol. 134, no. 21, pp. 1603–1612, 2016.

[18] Y. Devaux, M. Vausort, E. Goretti et al., "Use of circulating microRNAs to diagnose acute myocardial infarction," *Clinical Chemistry*, vol. 58, no. 3, pp. 559–567, 2012.

[19] X. Chen, L. Zhang, T. Su et al., "Kinetics of plasma microRNA-499 expression in acute myocardial infarction," *Journal of Thoracic Disease*, vol. 7, no. 5, pp. 890–896, 2015.

[20] S. Białek, D. Górko, A. Zajkowska et al., "Release kinetics of circulating miRNA-208a in the early phase of myocardial infarction," *Kardiologia Polska*, vol. 73, no. 8, pp. 613–619, 2015.

[21] O. Gidlöf, J. G. Smith, K. Miyazu et al., "Circulating cardio-enriched microRNAs are associated with long-term prognosis following myocardial infarction," *BMC Cardiovascular Disorders*, vol. 13, no. 1, p. 12, 2013.

[22] F. Wang, G. Long, Z. C et al., "Plasma microRNA-133a is a new marker for both acute myocardial infarction and underlying coronary artery stenosis," *Journal of Translational Medicine*, vol. 11, no. 1, p. 222, 2013.

[23] J. A. Ezekowitz, P. Théroux, W. Chang et al., "N-terminal pro-brain natriuretic peptide and the timing, extent and mortality in ST elevation myocardial infarction," *The Canadian Journal of Cardiology*, vol. 22, no. 5, pp. 393–397, 2006.

[24] L. Zhang, X. Chen, T. Su et al., "Circulating miR-499 are novel and sensitive biomarker of acute myocardial infarction," *Journal of Thoracic Disease*, vol. 7, no. 3, pp. 303–308, 2015.

[25] C. Widera, S. K. Gupta, J. M. Lorenzen et al., "Diagnostic and prognostic impact of six circulating microRNAs in acute coronary syndrome," *Journal of Molecular and Cellular Cardiology*, vol. 51, no. 5, pp. 872–875, 2011.

[26] I. Eitel, V. Adams, P. Dieterich et al., "Relation of circulating microRNA-133a concentrations with myocardial damage and clinical prognosis in ST-elevation myocardial infarction," *American Heart Journal*, vol. 164, no. 5, pp. 706–714, 2012.

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Research Article

Association of Plasma Renalase and Left Ventricle Mass Index in Heart Failure Patients Stratified to the Category of the Ejection Fraction: A Pilot Study

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Heart failure represents a growing health problem, with increasing morbidity and mortality globally. According to the mechanisms involved in the pathogenesis of heart failure, many biomarkers have been proposed for the timely diagnosis and prognostication of patients with heart failure, but other than natriuretic peptides, none of them has gained enough clinical significance. Renalase, a new protein derived from kidneys was demonstrated to metabolize catecholamines and to have a cardioprotective role. The aim of the study was to determine whether renalase and brain natriuretic peptide (BNP) concentration could be used to differentiate heart failure patients stratified to the category of the ejection fraction and whether plasma renalase could be used as a biomarker for left ventricle hypertrophy in all subgroups of heart failure patients. We included patients diagnosed with heart failure and stratified them to the three subgroups according to the ejection fraction. Regarding echocardiographic parameters, HFmrEF had an intermediate profile in between HFrEF and HFpEF, with statistical significance in most evaluated parameters. BNP concentration was significantly different in all three subgroups ($p < 0.001$), and renalase was statistically higher in HFrEF ($p = 0.007$) compared to the HFmrEF and HFpEF, where its results were similar, without statistical significance. Renalase plasma concentration was demonstrated to be highly and positively associated with left ventricle mass index in HFrEF ($p = 0.029$), as well as increased plasma concentration of BNP ($p = 0.006$). In the HFmrEF group of patients, body mass index was positively associated with LVMI ($p = 0.05$), while in the patients with HFpEF, diabetes mellitus was demonstrated to have a positive association with LVMI ($p = 0.043$). These findings suggest that renalase concentration may be measured in order to differentiate patients with reduced ejection fraction. Plasma renalase concentrations positively correlated with left ventricle hypertrophy in patients with reduced ejection fraction, being strongly associated with increased left ventricular mass index.

1. Background

Heart failure (HF) represents a response to a previous cardiovascular injury presented with an abnormal cardiac structure or function, leading to increased intracardiac pressures or a decreased cardiac output [1], with increasing morbidity and mortality globally [2]. For the first time, the 2016 European Society of Cardiology (ESC) heart failure (HF) guidelines

created three separate clinical entities, according to the left ventricle ejection fraction (LVEF), called heart failure with reduced EF (HFrEF), heart failure with mid-range ejection fraction (HFmrEF), and heart failure with preserved EF (HFpEF) [3].

In order to maintain cardiovascular and homeostasis in general, during the early stages of heart failure development, many physiological compensatory mechanisms are initiated, including the activation of the sympathetic nervous system (SNS) and cytokine secretion [4]. The activation of the SNS is able to preserve organ perfusion for a short time, but in the long term, it may lead to catecholamine-induced cardiotoxicity, resulting in reduced EF [4], while cytokine overproduction makes this a predictor of a worse outcome [5]. Therefore, the final result is ventricular remodeling, thus promoting heart failure progression [6].

In order to test the hypothesis that the kidney has an important role in preserving cardiovascular health, a new protein, subsequently called renalase, was discovered in 2005 [7], predominantly expressed in proximal tubules and to a lesser extent in the heart, liver, and skeletal muscles [7], after subtotal nephrectomy experiments in neonatal and adult rats showed that they develop left ventricular hypertrophy [7]. It was also demonstrated that soluble renalase degrades plasma catecholamines; therefore, it may have a significant role in blood pressure regulation [7–9]. Furthermore, two single nucleotide polymorphisms within the renalase gene were demonstrated to be risk factors for essential hypertension development [10]. Nevertheless, it was demonstrated that high plasma concentration of renalase may be a risk factor for the cardiovascular disease development and that it may predict all-cause mortality in patients with advanced kidney disease [11]. The most recent data, however, pointed to some other very important renalase roles, expanding its function beyond only being catalytic [12]. It was concluded that extracellular renalase may have cytokine properties [13], thus promoting cell survival.

Hypothesizing that renalase may reflect sympathetic activity, thus exerting cytokine properties, the aims of the study were to determine firstly whether renalase and brain natriuretic peptide (BNP) concentration could be used to differentiate heart failure patients stratified to the category of the ejection fraction and secondly whether plasma renalase could be used as a biomarker for left ventricle hypertrophy in all subgroups of heart failure patients.

2. Patients and Methods

The investigation was designed as a single-center, cross-sectional study and was conducted between May and October 2018 in the Institute for Treatment and Rehabilitation Niska Banja, in Nis, Serbia. All study participants were over 18 years old and gave written informed consent prior to the investigation. The study was approved by the institutional ethics committees. The study cohort represented 75 patients with a current history of HF, independent of etiology, and 35 healthy volunteers. All heart failure patients were clinically stable or in compensated heart failure status and had been admitted to the institute for the purpose of rehabilitation. The patients received the standard recommended pharmacological therapy [3]. The biochemical and clinical measurements were obtained within 24 hours of the hospital admission. The participants in the control group were community-based, without history of a coronary artery disease or heart failure and were age- and gender-matched with the participants in the clinical group.

The diagnosis of heart failure was established according to the presence of signs and symptoms of heart failure, BNP plasma concentration (over 35 pg/mL), relevant structural heart changes (LV mass index ≥ 115 g for males and ≥95 g for females or left atrial dilatation ≥ 40 mm), and/or diastolic abnormality (E/A ratio < 0.75 or ≥1.5 or deceleration time of E-wave < 140 ms) [3].

Two-dimensional echocardiography was performed on all participants, using a commercially available system (Acuson Sequoia 256, New York) and was analyzed according to the current guidelines [14]. Left ventricular ejection fraction (LVEF) and LV volumes were obtained with biplane apical views (Simpson's biplane), and the dimensions of the left ventricle, left atrium, and LV mass were provided by M-mode imaging. The existence of mitral regurgitation was assessed systematically. Diastolic function was assessed by the E/A ratio, deceleration time, isovolumetric relaxation time, and E/E′ ratio. The structure and the function of the right heart were assessed by the dimensions of the right ventricle (RV), systolic pulmonary artery pressure, and tricuspid annular plane systolic excursion (TAPSE) in an apical 4-chamber view. The maximum systolic excursion of the lateral tricuspid annulus is measured by M-mode, with TAPSE of <17 mm indicating RV dysfunction [15]. Consequently, patients were divided into three subgroups according to the LVEF: HFrEF (LVEF < 40%, $n = 27$), HFmrEF (LVEF 40–49%, $n = 23$) and HFpEF (LVEF ≥ 50%, $n = 25$). Afterwards, their baseline characteristics and possible risk factors were compared.

All biochemical measurements were routinely obtained on the day of enrolment by using the apparatus Sysmex XS 1000, Europe GmbH. The serum concentrations of CRP were determined quantitatively, with a nephelometric test (Orion Diagnostica Turbox), and afterwards, plasma was stored and frozen at -80°C until all the samples were collected and prepared for biomarker quantification. Estimated glomerular filtration rate was assessed by the MDRD (Modification of Diet in Renal Disease) equation, while the subjects' most recent height and weight measurements were used to calculate body mass index.

The concentrations of evaluated biomarkers were measured in plasma samples using commercially available ELISA kits, according to the manufacturer's instructions. For renalase (USCN Life Science Inc., China) the range of detection was 3.12-200 ng/mL, and for BNP (Abcam, ab193694, United Kingdom), the minimum detectable dose was 14 pg/mL.

2.1. Statistical Analyses. Data are presented as mean ± standard deviation or as absolute and relative numbers. Data distribution was tested using a Shapiro-Wilk test, and normally distributed data were analyzed with one-way ANOVA and Tukey test. Nonparametric analysis of data was carried

Table 1: Baseline demographic and clinical characteristics of heart failure patients stratified to the category of the ejection fraction.

Parameter	HFrEF $n = 27$		HFmrEF $n = 23$		HFpEF $n = 25$		p^1
Clinical							
Age (years)	59.44 ± 10.88		62.00 ± 9.54		63.96 ± 9.31		0.267[2]
Females (*n*, %)	6	22.2	7	30.4	6	24.0	0.791
HF etiology							
CAD (*n*, %)	21	80.8	16	69.6	12	48.0	0.042
CMP (*n*, %)	19	73.1	15	65.2	18	72.0	0.815
MI (*n*, %)	18	69.2	14	60.9	15	60.0	0.750
STEMI (*n*, %)	14	53.8	13	56.5	11	44.0	0.691
NSTEMI (*n*, %)	3	11.5	1	4.3	4	16.0	
VHD (*n*, %)	12	44.4	5	21.7	9	36.0	0.229
NYHA class (*n*, %)							
I	3	11.1	6	26.1	21	84.0	<0.001
II	7	25.9	16	69.6	4	16.0	
III	10	37.0	1	4.3	0	0.0	
IV	7	25.9	0	0.0	0	0.0	
Clinical history							
Hypertension (*n*, %)	24	88.9	23	100.0	22	88.0	0.099
Smoking (*n*, %)	14	51.9	10	43.5	12	48.0	0.840
Hyperlipidemia (*n*, %)	19	70.4	22	95.7	25	100.0	0.001
Family history (*n*, %)	19	70.4	9	39.1	18	72.0	0.033
Diabetes mellitus	7	25.9	7	30.4	10	40.0	0.546
BMI (kg/m^2)	27.27 ± 4.22		29.58 ± 4.38		27.57 ± 3.48		0.126[2]
Obesity (*n*, %)	15	53.6	16	69.6	17	65.4	0.466
Anemia (*n*, %)	2	7.4	3	13.0	2	8.0	0.772
Depression (*n*, %)	5	18.5	2	8.7	4	16.0	0.582
Hemodynamics							
Systolic BP (mmHg)	122.22 ± 12.88		130.43 ± 14.21		127.60 ± 20.16		0.188[2]
Diastolic BP (mmHg)	75.74 ± 8.05		80.43 ± 8.78		78.40 ± 7.87		0.136[2]
MAP (mmHg)	91.22 ± 9.05		97.17 ± 9.50		94.72 ± 11.40		0.114[2]
PP (mmHg)	46.48 ± 8.86		50.00 ± 11.48		49.25 ± 14.63		0.480[3]

Continuous variables are expressed as mean ± standard deviation. [1]Chi-squared test, [2]ANOVA, and [3]Kruskal-Wallis test. HFrEF: heart failure with reduced ejection fraction; HFmrEF: heart failure with mid-range ejection fraction; HFpEF: heart failure with preserved ejection fraction; HF: heart failure; CAD: coronary artery disease; CMP: cardiomyopathy; MI: myocardial infarction; STEMI: ST-elevation myocardial infarction; NSTEMI: non-ST segment elevation myocardial infarction; VHD: valvular heart disease; NYHA: New York Heart Association; BMI: body mass index; BP: blood pressure; MAP: mean arterial pressure; PP: pulse pressure.

out with the Kruskal-Wallis and the Mann-Whitney test. Linear regression was used to establish the relationship between variables. The level of significance was set at $p < 0.05$, and all statistical analyses were performed using R software, version 3.0.3. (R Foundation for Statistical Computing, Vienna, Austria) [16].

3. Results

The total cohort of the study consisted of 110 participants, where 75 patients diagnosed with heart failure represented the clinical group and 35 community-based healthy volunteers were included as controls. The baseline characteristics of the study patients, stratified to the category of the ejection fraction, are presented in Table 1. Out of 75 patients, 27 (36.0%) were classified as patients with reduced heart failure (HFrEF), 23 patients (30.7%) had mid-range heart failure (HFmrEF), and 25 patients (33.3%) were classified as HFpEF. The mean age of the clinical group was 61.73 ± 10.02 (min 37, max 83 years), with no statistical significance between either the subgroups ($p = 0.445$) or the gender ($p = 0.744$). With regard to the etiology of the heart failure, coronary artery disease was most prevalent in the HFrEF patients (80.8%), compared to the HFmrEF (69.6%) and HFpEF (48%), with statistical significance between all three subgroups ($p = 0.042$). However, there were no significant differences between the subgroups regarding the other etiological factors for the development of heart failure. Analysis of the

Table 2: Baseline hematological and biochemical data with evaluated biomarkers of heart failure patients stratified to the category of the ejection fraction.

Parameter	HFrEF $n = 27$	HFmrEF $n = 23$	HFpEF $n = 25$	p^1
RBC (10^{12}/L)	4.76 ± 0.46	4.85 ± 0.59	4.70 ± 0.52	0.613[2]
WBC (10^9/L)	7.42 ± 1.82	8.14 ± 1.96	12.83 ± 27.39	0.445
Platelets (10^3/mm^3)	209.15 ± 57.85	257.83 ± 70.70	217.25 ± 76.84	0.137
Hemoglobin (g/L)	142.18 ± 14.36	141.96 ± 14.34	136.72 ± 11.44	0.272[2]
Hematocrit (%)	0.42 ± 0.04	0.42 ± 0.04	0.41 ± 0.03	0.418[2]
C-reactive protein (mg/L)	2.22 ± 5.81	0.52 ± 2.50	0.48 ± 2.40	0.259
ESR	17.13 ± 12.05	19.56 ± 12.86	18.56 ± 9.04	0.622
Creatinine (μmol/L)	109.73 ± 25.42	100.54 ± 20.26	99.78 ± 24.90	0.279
BUN (mmol/L)	8.69 ± 4.34[a,b]	6.61 ± 2.65	6.27 ± 1.65	0.031
Acidum uricum (mmol/L)	455.93 ± 129.26	395.26 ± 66.97	320.32 ± 91.23	<0.002
Glycemia (mmol/L)	6.37 ± 1.91	6.72 ± 2.01	6.59 ± 2.12	0.652
HbA1c (%)	7.70 ± 1.72	7.62 ± 2.62	9.24 ± 1.14	0.234
TC (mmol/L)	4.86 ± 1.14	4.92 ± 1.49	4.49 ± 1.45	0.321
Triglycerides (mmol/L)	1.61 ± 0.57	1.97 ± 0.79	1.46 ± 0.77	0.016
LDL (mmol/L)	3.05 ± 0.97	3.06 ± 1.32	2.86 ± 1.15	0.603
HDL (mmol/L)	1.09 ± 0.29	0.97 ± 0.13	1.02 ± 0.24	0.666
LDL/HDL	2.84 ± 0.81	3.10 ± 0.97	2.83 ± 0.99	0.533
TC/HDL	4.56 ± 0.93	5.04 ± 1.06	4.43 ± 1.22	0.147
TG/HDL	1.70 ± 0.87	2.02 ± 0.87	1.47 ± 0.85	0.050
eGFR (mL/min/1.73 m^2)	65.74 ± 22.84	76.09 ± 23.79	77.84 ± 22.54	0.148
Renalase (ng/mL)	147.33 ± 29.07[a,b]	118.58 ± 19.61	117.31 ± 32.83	0.007
BNP (pg/mL)	276.12 ± 200.73[a,b]	152.46 ± 27.53[c]	93.19 ± 19.31	<0.001

Continuous variables are expressed as mean ± standard deviation. [1]Kruskal-Wallis test, [2]ANOVA, [a]HFrEF vs. HFmrEF, [b]HFrEF vs. HFpEF, and [c]HFrEF vs. HFpEF. RBC: red blood cells; WBC: white blood cells; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; BUN: blood urea nitrogen; TC: total cholesterol; TG: triglycerides; LDL: low-density lipoprotein; LDL: low-density lipoprotein; HDL: high-density lipoprotein; eGFR: estimated glomerular filtration rate; BNP: brain natriuretic peptide.

clinical history showed that hyperlipidemia was a statistically significant parameter in all three subgroups ($p = 0.033$), being the most prevalent in HFpEF patients and least in HFrEF. However, family history of cardiovascular disease was similarly prevalent in HFrEF and HFpEF patients, compared to HFmrEF patients ($p = 0.033$). Regarding biochemical measurements, there were statistically significant differences in blood urea nitrogen (BUN) concentration ($p = 0.031$), acidum uricum ($p < 0.001$), and triglycerides ($p = 0.016$) between all three subgroups, as shown in Table 2. The evaluated biomarkers, renalase and BNP, were statistically different compared to the control group ($p < 0.001$) (data not shown in the tables). BNP concentration was significantly different in all three subgroups ($p < 0.001$), and renalase was statistically higher in HFrEF ($p = 0.007$) compared to the HFmrEF and HFpEF where its concentrations were similar, without statistical significance (Table 2).

Regarding echocardiographic parameters, HFmrEF had an intermediate profile in between HFrEF and HFpEF, as shown in Table 3. Statistical significance was obtained using the following parameters: left ventricle ejection fraction ($p < 0.001$), left ventricle mass ($p = 0.003$), LV mass index ($p = 0.005$), LV end-systolic volume ($p < 0.001$), LV end-diastolic volume ($p < 0.001$), left atrium ($p = 0.005$), mitral regurgitation ($p < 0.001$), tricuspid regurgitation ($p < 0.001$), TAPSE ($p < 0.001$), and right ventricle ($p = 0.003$). However, the E/A and systolic pressure of RV were different between the subgroups, although this was not statistically significant.

Renalase plasma concentration was demonstrated to be highly and positively associated with the left ventricle mass index in HFrEF ($p = 0.029$), as well as increased plasma concentration of BNP ($p = 0.006$). In the HFmrEF group of patients, the body mass index was positively associated with LVMI ($p = 0.05$), while in the patients with HFpEF, diabetes mellitus was demonstrated to have a positive association with LVMI ($p = 0.043$), as shown in Table 4 and Figure 1.

4. Discussion

The first finding of the study is that plasma renalase concentration was higher in HFrEF patients, while HFmrEF and HFpEF patients showed similar results, which are lower

Table 3: Echocardiographic parameters of heart failure patients stratified to the category of the ejection fraction.

Parameter	HFrEF $n = 27$	HFmrEF $n = 23$	HFpEF $n = 25$	p^1
LVEF (%)	26.30 ± 5.98[a,b]	41.43 ± 2.25[c]	53.84 ± 3.64	<0.001
LV mass (g)	288.00 ± 79.18[a]	230.70 ± 43.98[c]	229.00 ± 51.11	0.003
LV mass index (g/m^2)	147.52 ± 44.15[a,b]	117.87 ± 24.32	114.68 ± 27.55	0.005
LV end-systolic volume (mm)	48.28 ± 8.35[a,b]	36.78 ± 2.94	35.40 ± 3.08	<0.001
LV end-diastolic volume (mm)	63.70 ± 5.98[a,b]	55.65 ± 5.05	52.04 ± 4.57	<0.001
IV septum (mm)	10.87 ± 1.28	11.2 ± 1.27	11.59 ± 1.67	0.161
LV posterior wall (mm)	9.89 ± 1.24	9.93 ± 0.97	10.19 ± 1.14	0.539
Left atrium (mm)	47.02 ± 4.32[a,b]	44.26 ± 4.88	42.40 ± 5.90	0.001
Aortic root (mm)	34.54 ± 3.4	34.63 ± 4.1	34.51 ± 4.36	0.994
AR	0.41 ± 0.75	0.22 ± 0.52	0.23 ± 0.55	0.441
MR	1.93 ± 0.96[a,b]	1.48 ± 0.73[c]	0.91 ± 0.89	<0.001
TR	1.48 ± 0.70[b]	1.35 ± 0.65[c]	0.94 ± 0.68	0.006
TAPSE (mm)	18.39 ± 2.35[a,b]	21.83 ± 4.1[c]	24.23 ± 4.38	<0.001
Right ventricle (mm)	24.22 ± 2.74[1,2]	22.42 ± 2.59	22.32 ± 2.08	0.003
Systolic pressure of RV (mmHg)	36.96 ± 11.90	32.30 ± 8.92	31.56 ± 10.51	0.124
E/A	0.87 ± 0.27	0.83 ± 0.19	0.76 ± 0.14	0.442
LVMI > RF	22 (81.5)	13 (56.5)	14 (56.0)	0.089[2]

Continuous variables are expressed as mean ± standard deviation. [1]Kruskal-Wallis test, [2]chi-squared test, [a]HFrEF vs. HFmrEF, [b]HFrEF vs. HFpEF, and [c]HFmrEF vs. HFpEF. HFrEF: heart failure with reduced ejection fraction; HFmrEF: heart failure with mid-range ejection fraction; HFpEF: heart failure with preserved ejection fraction; LVEF: left ventricle ejection fraction; LV: left ventricle; IV: interventricular septum; AR: aortic regurgitation; MR: mitral regurgitation; TR: tricuspid regurgitation; TAPSE: tricuspid annular plane systolic excursion; RV: right ventricle; LVMI > RF: LVMI above reference value.

levels of renalase. However, we also documented that soluble renalase exerted a very strong positive association with the left ventricle mass index, making it a significant risk factor for left ventricle hypertrophy. To the best of our knowledge, this is the first study to assess the potential role of plasma renalase in chronic heart failure patients, in the form of heart failure subtype differentiation and as a possible biomarker for left ventricle hypertrophy. The concentration of BNP was significantly different in all three subgroups of heart failure patients and was also demonstrated to have a strong and positive association with LVMI in patients with reduced ejection fraction.

As previously reported, sympathetic overactivation is one of the main mechanisms involved in the pathogenesis of heart failure, with catecholamines exerting direct toxic effects to the cardiomyocytes, by stimulation of apoptosis and induction of ventricular arrhythmias [17]. As noted, extracellular renalase plays a very important role in maintaining plasma catecholamine concentration, by metabolizing it [9]; therefore, it may be the key protein in the regulation of the sympathetic tone. However, it was concluded that soluble renalase firstly circulates as an inactive, in the form of a proenzyme, waiting for an influx of catecholamines to be activated and then *de novo* synthetized and secreted [9]. The causal relation between heightened sympathetic tone and increased cardiovascular risk was documented in patients with end-stage renal disease, who lack renalase; therefore, it was proposed that the application of recombinant renalase may exert a cardioprotective role [8–13, 17], while in patients with advanced chronic kidney disease, soluble renalase was proven to be a predictor of all-cause mortality [11].

Considering heart failure itself, in experimentally induced heart failure in rats, the activity and renalase concentration in plasma were both elevated compared with control animals [17]. Our heart failure patients also had higher plasma renalase levels compared to healthy controls, while the highest concentration of renalase was in the group of heart failure patients with the ejection fraction below 40%. We postulated that renalase concentration rises as a compensatory effect of the kidneys, as heart failure progresses, and is probably parallel to the activity of the sympathetic nervous system. Similar observations were documented in previous research [17], where renalase concentration was the highest during the first week of the experiment, corresponding to the early phase of heart failure development, and afterwards, as cardiac function declined, renalase returned to its base level. The authors proposed that while renal perfusion was preserved, renalase concentration tended to be elevated and *vice versa*, so the cardiac decompensation phase would be presented with subbasal renalase levels [17]. This finding is in agreement with our research. We discovered that the cohort with the reduced ejection fraction had the highest renalase levels, probably because they were all clinically compensated with a preserved kidney function. Our model indicated that renalase could be used as a

Table 4: Association of evaluated parameters and LVMI in heart failure patients stratified to the category of the ejection fraction.

	Unstandardized coefficient		Standardized coefficient	
	B	Std. error	β	p
HFrEF				
Age	0.14	0.66	0.0	0.86
Gender	-1.21	14.07	-0.01	0.92
Renalase (ng/mL)	0.6	0.27	0.41	0.029
BNP (pg/mL)	0.11	0.04	0.52	0.006
Diabetes mellitus	-4.2	.5	-0.18	0.222
BMI (kg/m^2)	-1.6	1.	-0.1	0.20
Constant	94.28	6.47		0.011
Adjusted R^2		0.657		<0.001
HFmrEF				
Age	-0.67	0.65	-0.26	0.19
Gender	-11.8	14.8	-0.2	0.47
Renalase (ng/mL)	0.02	0.5	0.02	0.957
BNP (pg/mL)	0.25	0.26	0.28	0.42
Diabetes mellitus	0.6	2.84	0.05	0.828
BMI (kg/m^2)	-2.5	1.2	-0.45	0.05
Constant	204.18	59.82		0.004
Adjusted R^2		0.016		0.425
HFpEF				
Age	0.7	0.52	0.25	0.175
Gender	-11.	11.19	-0.18	0.24
Renalase (ng/mL)	0.18	0.15	0.21	0.28
BNP (pg/mL)	0.15	0.26	0.10	0.576
Diabetes mellitus	-5.24	2.40	-0.40	0.04
BMI (kg/m^2)	-2.44	1.7	-0.1	0.092
Constant	149.4	64.45		0.02
Adjusted R^2		0.66		0.02

LVMI: left ventricle mass index; HFrEF: heart failure with reduced ejection fraction; HFmrEF: heart failure with mid-range ejection fraction; HFpEF: heart failure with preserved ejection fraction; BNP: brain natriuretic peptide; BMI: body mass index.

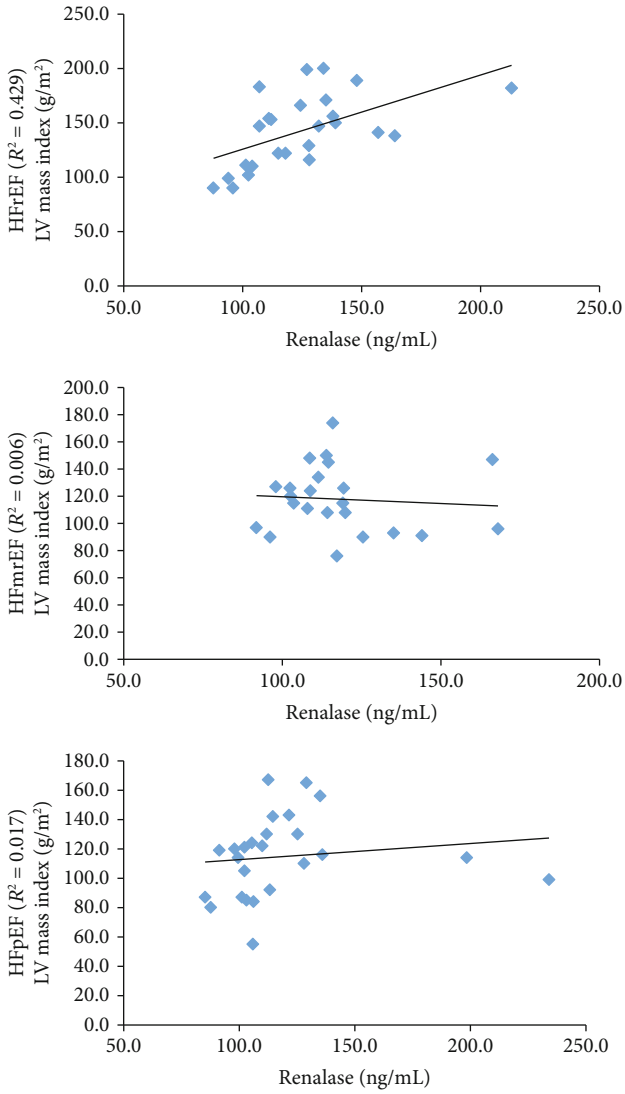

Figure 1: Association of renalase and left ventricle mass index stratified to the category of the ejection fraction. LV: left ventricle; HFrEF: heart failure with reduced ejection fraction; HFmrEF: heart failure with mid-range ejection fraction; HFpEF: heart failure with preserved ejection fraction.

differentiation biomarker in patients with ejection fraction below 40%. However, it could not be used, based on our findings, to differentiate heart failure patients with mid-range and preserved ejection fraction, since there was no statistical significance between plasma renalase concentrations in these two groups of patients. Impaired renal excretion also contributes to the rise of renalase concentration [18], but in our case, it is more likely that it reflects sympathetic activation [17], rather than the kidney dysfunction. However, further research is needed to decide the point at which renalase concentration starts to decline and the triggers of it.

Additional research has hypothesized that the increase in plasma renalase is due to its behaviour as a cytokine. However, some very important cytoprotective properties of renalase have been documented in the animal models of renal and cardiac injury [19]. In the experimental model of ischemic acute kidney injury, it was documented that after recombinant renalase was administered, the degree of kidney inflammation, apoptosis, and tubular necrosis was significantly reduced [20]. This protective effect of renalase against ischemia is probably provided by activation of renalase-dependent plasma membrane receptor, PMCA4b [21], with the final activation of mitogen-activated protein kinase (MAPK) and signal transducer and activator of transcription (STAT3) pathways [21]. Additionally, the same protective effect of renalase was observed in an animal model of ischemic myocardial damage [22]. The size and degree of an infarction area was attenuated after recombinant renalase was applicated. The administration of recombinant renalase was also demonstrated to prevent a significant fall in EF [22], after an ischemic injury. As reported,

renalase may represent a target gene of hypoxia-induced factor-1 alpha (HIF-1α) [23, 24], but its protective effects may also be expressed through attenuating inflammation and reduction of necrosis and apoptosis [25]. The same authors proposed that renalase might have an important role in local heart tissue and different roles in different organs and that it might serve as a new therapeutic target for ischemic damage [25]. Furthermore, renalase may be involved in the pathogenesis of cardiac hypertrophy, most probably in the context of its prevention. In the rats, who underwent subtotal nephrectomy (5/6), after administration of renalase, the left ventricle hypertrophy, as well as left ventricle papillary muscle dysfunction, was significantly reduced [26]. Similarly, the development of hypertension and remodeling of the heart were attenuated most probably by inhibiting the profibrotic gene expression and phosphorylation of ERK-1/2 [19, 21, 27]. Our results may be analyzed in the context of renalase concentration being positively associated with left ventricle hypertrophy. We demonstrated that soluble renalase presents a risk factor for an increased left ventricular mass index, regardless of the type of hypertrophy. In an experimental model, it was confirmed that cardiac and renal fibrosis, as well as cardiac remodeling, was attenuated by exogenous renalase, suggesting that renalase may be involved in the pathogenesis of fibrosis, thus remodeling organs in some manner [26, 27].

Subsequently, a strong association between functional polymorphism of the renalase gene (Glu37Asp) and cardiac hypertrophy, ventricular dysfunction, poor exercise capacity, and ischemia was demonstrated in patients with coronary disease [28], further confirming a link between renalase, cardiac hypertrophy, and different aspects of myocardial function. The study confirmed that Asp/Asp homozygosity at codon 37 was connected with and increased left ventricular mass index [28], which is similar to our results where plasma renalase was confirmed to be a risk factor for an increase of LVMI ($p = 0.029$, $\beta = 0.041$) in heart failure patients with reduced EF. Note that our cohort of HFrEF patients consisted mostly of patients with coronary artery disease (over 80%), with ischemia being the main underlying mechanism. Therefore, we may presume that the elevated plasma renalase may be associated with the risk of coronary artery disease. Similar research was conducted, but the investigators reported the opposite that the decrease in plasma renalase was a significant risk factor for cardiovascular disease [29], explaining that the absence of renalase promotes myocardial cell apoptosis, oxidative stress, and fibrosis. The most recent genetic testing on the GG genotype of rs2576178 polymorphism concluded that this particular genotype increases renalase levels and thereby contributes to increased risk of coronary artery disease [30]. Some investigators recently also confirmed the association of renalase gene polymorphism with cardiac hypertrophy in female patients with aortic stenosis [31], again suggesting a causal relationship between renalase and cardiac hypertrophy. The authors postulated the potential link knowing that in hypoxia, glycolysis is increased and that renalase is secreted in the manner of preserving primary metabolism [31].

All the evidence suggests an important role for renalase in the development of cardiovascular diseases. According to the proposed mechanism, an excess of renalase probably occurs as a result of an adrenergic overstimulation, aiming to protect the tissues, with renalase being upregulated following different kinds of stress conditions [25, 26]. However, some investigators proposed that even a 10-fold increase in plasma renalase may not be sufficient to alleviate catecholamine levels [32]. Whether this elevation is of clinical significance reflecting patients who are at a higher risk of complications is yet to be confirmed. In this paper, we did not discuss the connection between plasma levels of renalase and BNP, but our results indicate that plasma renalase was a noninferior marker in the stratification of heart failure patients compared to plasma BNP, when discussing the correlation with left ventricle mass index. Monitoring of patients with heart failure may be facilitated by synergistically using plasma BNP and renalase, together with clinical parameters, in terms of diagnosis and determination of prognosis. Multimarker approaches have shown relevant benefit over single biomarkers, but additional research is needed to conclude the best biomarker combination for HF management [33].

5. Conclusion

In summary, plasma renalase concentration was higher in patients with heart failure than in the control group and was highest in patients with a reduced ejection fraction, compared to HFmrEF and HFpEF, where renalase concentration showed similar result. These findings suggest that plasma renalase levels may be measured in order to differentiate patients with ejection fraction below 40% serving as a potent biomarker for identification of heart failure patients with reduced ejection fraction. We also demonstrated that high plasma renalase concentrations positively correlate with left ventricle hypertrophy in patients with reduced ejection fraction, being strongly associated with increased left ventricular mass index.

The exact pathophysiological link between renalase and heart failure and its potential use as a biomarker in identification or risk stratification in heart failure patients is yet to be established.

Data Availability

The data.xls used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors report no conflicts of interest.

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References

[1] E. D'Elia, M. Vaduganathan, M. Gori, A. Gavazzi, J. Butler, and M. Senni, "Role of biomarkers in cardiac structure phenotyping in heart failure with preserved ejection fraction: critical appraisal and practical use," *European Journal of Heart Failure*, vol. 17, no. 12, pp. 1231–1239, 2015.

[2] B. Ziaeian and G. C. Fonarow, "Epidemiology and aetiology of heart failure," *Nature Reviews Cardiology*, vol. 13, no. 6, pp. 368–378, 2016.

[3] P. Ponikowski, A. A. Voors, S. D. Anker et al., "2016 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure: the task force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC," *European Journal of Heart Failure*, vol. 18, no. 8, pp. 891–975, 2016.

[4] F. Triposkiadis, G. Karayannis, G. Giamouzis, J. Skoularigis, G. Louridas, and J. Butler, "The sympathetic nervous system in heart failure: physiology, pathophysiology, and clinical implications," *Journal of the American College of Cardiology*, vol. 54, no. 19, pp. 1747–1762, 2009.

[5] P. S. Chaggar, C. J. Malkin, S. M. Shaw, S. G. Williams, and K. S. Channer, "Neuroendocrine effects on the heart and targets for therapeutic manipulation in heart failure," *Cardiovascular Therapeutics*, vol. 27, no. 3, pp. 187–193, 2009.

[6] D. Chen, C. Assad-Kottner, C. Orrego, and G. Torre-Amione, "Cytokines and acute heart failure," *Critical Care Medicine*, vol. 36, pp. S9–16, 2008.

[7] G. V. Desir, "Regulation of blood pressure and cardiovascular function by renalase," *Kidney International*, vol. 76, no. 4, pp. 366–370, 2009.

[8] G. Desir, "Novel insights into the physiology of renalase and its role in hypertension and heart disease," *Pediatric Nephrology*, vol. 27, no. 5, pp. 719–725, 2012.

[9] G. Li, J. Xu, P. Wang et al., "Catecholamines regulate the activity, secretion, and synthesis of renalase," *Circulation*, vol. 117, no. 10, pp. 1277–1282, 2008.

[10] Y. B. Lv, Y. Wang, W. G. Ma et al., "Association of renalase SNPs rs2296545 and rs2576178 with the risk of hypertension: a meta-analysis," *PLoS One*, vol. 11, no. 7, article e0158880, 2016.

[11] S. H. Baek, R. H. Cha, S. W. Kang et al., "Circulating renalase predicts all-cause mortality and renal outcomes in patients with advanced chronic kidney disease," *Korean J Intern Med*, vol. 34, no. 4, pp. 858–866, 2017.

[12] X. Guo, L. Wang, H. Velazquez, R. Safirstein, and G. V. Desir, "Renalase: its role as a cytokine, and an update on its association with type 1 diabetes and ischemic stroke," *Current Opinion in Nephrology and Hypertension*, vol. 23, no. 5, pp. 513–518, 2014.

[13] L. Wang, H. Velazquez, G. Moeckel et al., "Renalase prevents AKI independent of amine oxidase activity," *American Society of Nephrology*, vol. 25, no. 6, pp. 1226–1235, 2014.

[14] R. M. Lang, L. P. Badano, V. Mor-Avi et al., "Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging," *European Heart Journal Cardiovascular Imaging*, vol. 16, no. 3, pp. 233–271, 2015.

[15] L. G. Rudski, W. W. Lai, J. Afilalo et al., "Guidelines for the echocardiographic assessment of the right heart in adults: a report from the American Society of Echocardiography. Endorsed by the European Association of Echocardiography, a registered branch of the European Society of Cardiology, and the Canadian Society of Echocardiography," *Journal of the American Society of Echocardiography*, vol. 23, no. 7, pp. 685–713, 2010.

[16] R Core Team, *A language and environment for statistical computing*, R Foundation for Statistical Computing, Vienna, Austria, 2014.

[17] R. Gu, W. Lu, J. Xie, J. Bai, and B. Xu, "Renalase deficiency in heart failure model of rats-a potential mechanism underlying circulating norepinephrine accumulation," *PLoS One*, vol. 6, no. 1, article e14633, 2011.

[18] D. Stojanovic, T. Cvetkovic, M. Stojanovic, V. Bojanic, N. Stefanovic, and I. Stojanovic, "The assessment of renalase: searching for the best predictor of early renal dysfunction by multivariate modeling in stable renal transplant recipients," *Annals of Transplantation*, vol. 20, pp. 186–192, 2015.

[19] Y. Wang, R. Safirstein, H. Velazquez et al., "Extracellular renalase protects cells and organs by outside-in signalling," *Journal of Cellular and Molecular Medicine*, vol. 21, no. 7, pp. 1260–1265, 2017.

[20] H. T. Lee, J. Y. Kim, M. Kim et al., "Renalase protects against ischemic AKI," *Journal of the American Society of Nephrology*, vol. 24, no. 3, pp. 445–455, 2013.

[21] L. Wang, H. Velazquez, J. Chang, R. Safirstein, and G. V. Desir, "Identification of a receptor for extracellular renalase," *PLoS One*, vol. 10, no. 4, article e0122932, 2015.

[22] Y. Wu, J. Xu, H. Velazquez et al., "Renalase deficiency aggravates ischemic myocardial damage," *Kidney International*, vol. 79, no. 8, pp. 853–860, 2011.

[23] M. Du, K. Huang, D. Huang et al., "Renalase is a novel target gene of hypoxia-inducible factor-1 in protection against cardiac ischaemia–reperfusion injury," *Cardiovascular Research*, vol. 105, no. 2, pp. 182–191, 2015.

[24] F. Wang, G. Zhang, T. Xing et al., "Renalase contributes to the renal protection of delayed ischaemic preconditioning via the regulation of hypoxia-inducible factor-1α," *Journal of Cellular and Molecular Medicine*, vol. 19, no. 6, pp. 1400–1409, 2015.

[25] X. Li, Z. Xie, M. Lin et al., "Renalase protects the cardiomyocytes of Sprague-Dawley rats against ischemia and reperfusion injury by reducing myocardial cell necrosis and apoptosis," *Kidney & Blood Pressure Research*, vol. 40, no. 3, pp. 215–222, 2015.

[26] A. Baraka and S. el Ghotny, "Cardioprotective effect of renalase in 5/6 nephrectomized rats," *Journal of Cardiovascular Pharmacology and Therapeutics*, vol. 17, no. 4, pp. 412–416, 2012.

[27] J. Yin, Z. Lu, F. Wang et al., "Renalase attenuates hypertension, renal injury and cardiac remodelling in rats with subtotal nephrectomy," *Journal of Cellular and Molecular Medicine*, vol. 20, no. 6, pp. 1106–1117, 2016.

[28] R. Farzaneh-Far, G. V. Desir, B. Na, N. B. Schiller, and M. A. Whooley, "A functional polymorphism in renalase (Glu37Asp) is associated with cardiac hypertrophy, dysfunction, and ischemia: data from the heart and soul study," *PLoS One*, vol. 5, no. 10, article e13496, 2010.

[29] B. He, J. Hao, W. Sheng et al., "Correlation between plasma renalase level and coronary artery disease," *Pakistan Journal of Medical Sciences*, vol. 30, no. 5, pp. 863–967, 2014.

[30] N. Hu, J. Wang, P. Hu, and Z. Li, "Investigation of renalase gene rs2576178 polymorphism in patients with coronary artery disease," *Bioscience Reports*, vol. 38, no. 5, article BSR20180839, 2018.

[31] E. Orlowska-Baranowska, L. Gadomska vel Betka, J. Gora et al., "Functional polymorphism of the renalase gene is associated with cardiac hypertrophy in female patients with aortic stenosis," *PLoS One*, vol. 12, no. 10, article e0186729, 2017.

[32] F. Boomsma and K. F. Tipton, "Renalase, a catecholamine-metabolising enzyme?," *Journal of Neural Transmission*, vol. 114, no. 6, pp. 775-776, 2007.

[33] C. Magnussen and S. Blankenberg, "Biomarkers for heart failure: small molecules with high clinical relevance," *Journal of Internal Medicine*, vol. 283, no. 6, pp. 530–543, 2018.

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Research Article

Association of Galectin-3 and Soluble ST2, and Their Changes, with Echocardiographic Parameters and Development of Heart Failure after ST-Segment Elevation Myocardial Infarction

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Purpose. To investigate the association of galectin-3 (Gal-3) and soluble ST2 (sST2) and their follow-up changes with the development of heart failure (HF) and echocardiographic parameters of HF (ejection fraction, atrial and ventricular size, left ventricular hypertrophy, e', and E/e') in patients with ST-segment elevation myocardial infarction (STEMI) treated with primary percutaneous coronary intervention (pPCI). *Methods*. A prospective, observational study, BIOSTRAT (Biomarkers for Risk Stratification After STEMI), enrolled 117 patients between October 2014 and April 2017. Gal-3 and sST2 serum collection and echocardiography were performed twice (during index hospitalization and on a control visit at one-year follow-up). The primary endpoint was HF onset at one-year follow-up. Secondary assessments included associations of biomarker concentration with echocardiographic indices of systolic and diastolic dysfunction at baseline and at one year. *Results*. Mean baseline concentrations of Gal-3 and sST2 (7.5 and 26.4 ng/mL, respectively) were significantly increased at one-year follow-up (8.5 ng/mL and $p < 0.001$ and 31.4 ng/mL and $p = 0.001$, respectively). Patients who reached the primary endpoint (50 patients (48%)) had significantly higher baseline concentrations of both biomarkers and a higher Gal-3 level at one year compared to patients who did not. Both Gal-3 and sST2 were predictors of the primary endpoint in univariate logistic regression analysis, but only Gal-3 remained significant in multivariate analysis. There was no clear association between both biomarkers and echocardiographic parameters. *Conclusions*. Baseline, but not one-year, changes of Gal-3 and sST2 concentrations may be useful for risk stratification after STEMI. However, only Gal-3 was the independent predictor of HF development at one-year observation. This trial is registered with NCT03735719.

1. Introduction

Acute myocardial infarction (AMI) initiates left ventricular remodeling (LVR) and may lead to the development of heart failure (HF) [1]. Accessible diagnostic tools commonly used in HF such as natriuretic peptides and New York Heart Association (NYHA) classification reflect already overt clinical HF [2, 3]. Troponin and creatine kinase reflect myocardial damage, but their usefulness in predicting long-term LVR is limited [3]. Recent guidelines on HF management stressed that HF onset may be delayed or prevented through certain interventions, such as pharmacotherapy, postinfarction rehabilitation, or modification of HF risk factors [3]. Therefore, it is important to identify potential markers, which would be more informative of HF preclinical stages to recognize patients with an increased risk of HF onset, and to start treatment in advance.

Numerous studies suggest that, in addition to natriuretic peptides, circulating galectin-3 (Gal-3) and soluble interleukin-1 receptor-like 1 (sST2) are independent markers of adverse outcomes in HF [4–10]. These two biomarkers have been already recommended for an additive risk stratification

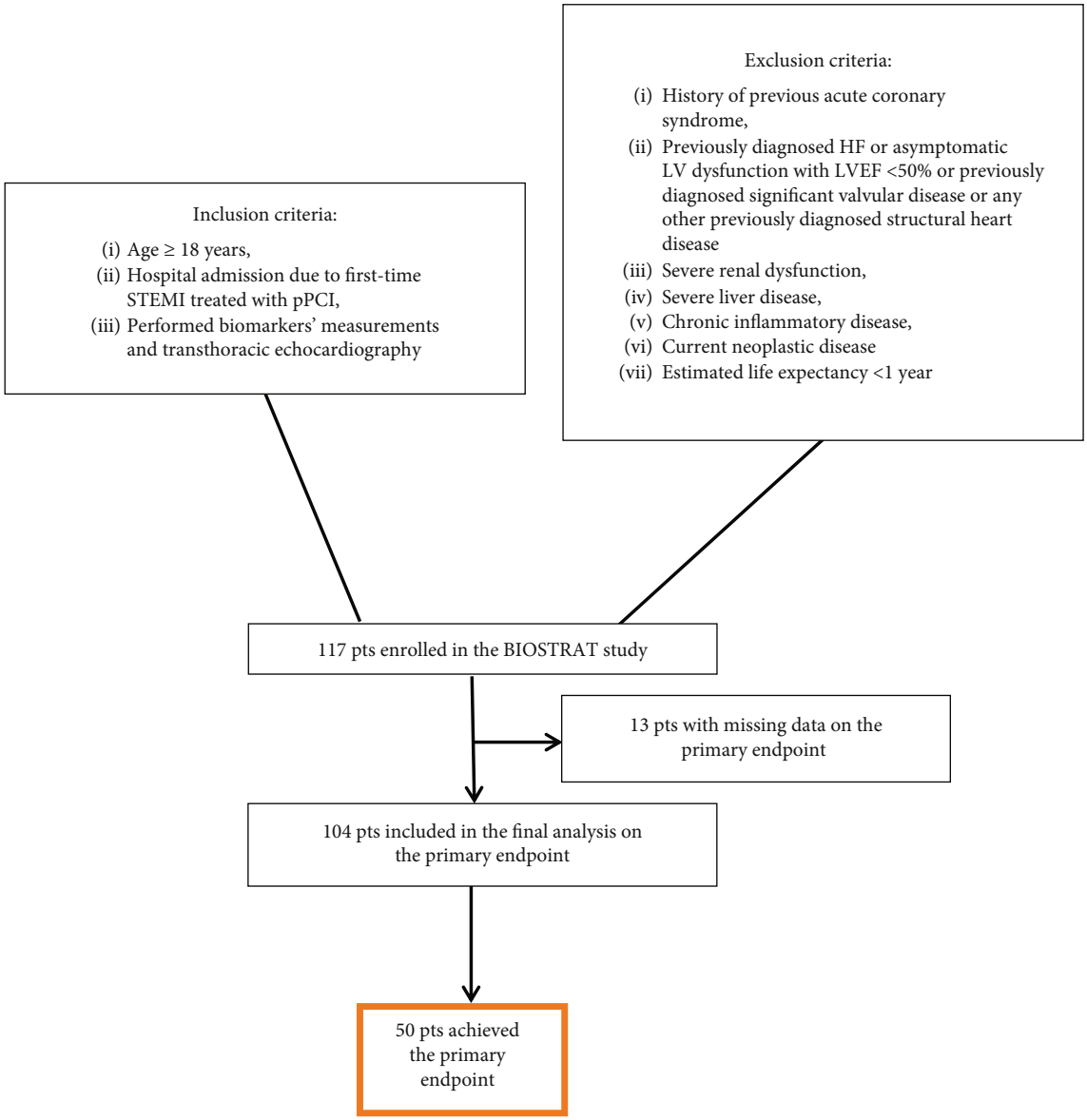

Figure 1: Flow chart of patient enrolment in the current analysis. HF: heart failure; pPCI: primary percutaneous coronary intervention; STEMI: ST-segment elevation myocardial infarction.

in the American guidelines for the management of HF [11]. These biomarkers represent pathophysiological pathways other than cardiac troponins or natriuretic peptides. Gal-3 participates in inflammation and profibrotic pathways, while sST2 is a biomarker of inflammation, cardiac mechanical strain, and tissue fibrosis, both of which may predict LVR [12, 13]. Both biomarkers are involved in many regulatory processes and might be useful in estimation of the risk of adverse cardiac remodeling and development of HF. Few small clinical studies have recently suggested a potential role of baseline Gal-3 and sST2 concentrations in predicting adverse outcomes after AMI [14–17], but there is insufficient data on the clinical usefulness of measurements of both biomarkers one year after AMI.

The aim of this study was to investigate the association of Gal-3 and sST2 concentrations and their changes at one-year follow-up with the development of clinically overt HF and echocardiographic indices of HF (left ventricular (LV) ejection fraction (LVEF), atrial and ventricular size, LV hypertrophy based on LV mass index (LVMI), diastolic tissue velocities at the mitral annulus (e'), and E/e' ratio) in patients after ST-segment elevation myocardial infarction (STEMI) treated with primary percutaneous coronary intervention (pPCI).

2. Methods

2.1. Study Design and Population. BIOSTRAT (Biomarkers for Risk Stratification After STEMI) was a prospective, observational, single-centre study, conducted between October 2014 and April 2017. The study included 117 consecutive patients admitted due to first-time STEMI treated by pPCI. STEMI was diagnosed based on standard algorithms [18]. Participants who agreed to sign informed consent were

included based on the following main criteria: (1) age ≥ 18 years and (2) hospital admission due to first-time STEMI treated with pPCI. The main exclusion criteria were (1) history of previous acute coronary syndrome, (2) previously diagnosed HF or asymptomatic left ventricular (LV) dysfunction with LVEF < 50% or previously diagnosed significant valvular disease or any other previously diagnosed structural heart disease, (3) severe renal dysfunction (plasma creatinine level > 220 mmol/L (≈2.5 mg/dL) and/or creatinine clearance < 30 mL/min), (4) severe liver disease, (5) chronic inflammatory disease, (6) current neoplastic disease, and (7) estimated life expectancy < 1 year. Figure 1 shows patient enrolment in the current study.

The study protocol was approved by the local ethics committee and registered at ClinicalTrials.gov (NCT03735719).

2.2. Data Collection and Gal-3 and sST2 Measurements. Data on baseline clinical characteristics, clinical examination, results of diagnostic tests performed during index hospitalization (ECG, echocardiography, coronary angiography, and biochemistry [including maximum concentrations of cardiac troponin I (cTnI), creatine kinase myocardial band (CK-MB), N-terminal pro-B-type natriuretic peptide (NT-proBNP), and high-sensitivity C-reactive protein (hs-CRP)]), and pharmacotherapy were collected.

Transthoracic echocardiography was performed within 48 hours of hospital admission in the Department's Echocardiography Laboratory using Philips EPIQ 7 Ultrasound Machines (Philips Medical Systems, Andover, Massachusetts, USA) by 3 certified echocardiographers (second degree accreditation in echocardiography of the Section of Echocardiography of the Polish Cardiac Society). LVEF was assessed by biplane Simpson's modified rule [19]. Other assessed parameters included atrial and ventricular size, LV posterior wall and septal thickness, LV hypertrophy based on LVMI: LVMI > 95 g/m^2 for female and LVMI > 115 g/m^2 for male, mitral inflow velocities, and early diastolic tissue velocities at the lateral and medial mitral annulus (E′ lat and E′ med). All measurements were performed according to the recommendations of the American Society of Echocardiography and the European Association of Cardiovascular Imaging [19, 20].

Blood samples for Gal-3 and sST2 were collected 72-96 hours after hospital admission. Separation of plasma was performed 1 hour after blood collection by centrifugation at 3500 rpm for 15 min at ambient temperature. Then, specimens were stored at −80°C until analyzed after trial completion. Serum concentrations of Gal-3 were assessed using a Human Galectin-3 Quantikine ELISA Kit® (BIOKOM, Janki, Poland) and of sST2 with a Presage ST2 Assay (Genloxa Sp. z o.o., Puck, Poland). Calibration and standardization of these assays were performed according to the manufacturers' protocols.

At the one-year follow-up, a control visit in our department was conducted. Clinical examination, echocardiography, and collection of blood samples for biomarkers' measurements were performed.

2.3. Study Endpoints. The primary endpoint of our study was new HF onset at one-year follow-up. New-onset HF was defined as LVEF below 40% or HF-related hospitalization or ambulatory diagnosis of HF.

Secondary assessments included associations of the baseline and follow-up biomarker concentrations with echocardiographic indices of systolic and diastolic dysfunction at baseline and at one year.

2.4. Statistical Analysis. On the basis of literature data [21] regarding Gal-3 concentration, the sample size of the study group was calculated. In the de Boer et al. study, the median concentration of Gal-3 in the entire study group ($n = 247$) was 13.4 (11.4-16.2 ng/mL; SD: 3.6). The study endpoint was LVEF < 50% after four months of observation since PCI performed in AMI. Patients who reached the study endpoint had higher median Gal-3 concentration 14.8 (12.5-18.2 ng/mL; SD: 4.2) comparing to those who had not (median 13.0 (11.2-15.4 ng/mL; SD: 3.1)). Therefore, theoretically (considering normal distribution and assuming 10% loss to follow-up) to reach statistical significance (with power of 80%), 114 patients should be included in the study.

Normally distributed continuous variables were presented as mean values and standard deviations, while ordinal variables and nonnormally distributed continuous variables, as median values and interquartile ranges (IQR). Categorical data were presented as numbers and percentages. Changes in concentrations of biomarkers were also calculated as the one-year level minus the baseline level and correlated with clinical variables. Significance of differences between groups was determined by Fisher's exact test for categorical variables and a Mann-Whitney U test for continuous and ordinal variables, respectively. The Wilcoxon signed-rank test was used to compare repeated measurement of biomarkers. The logistic regression model was performed to identify predictors of the primary endpoint. A p value of ≤0.05 was considered significant for all tests. In order to maintain an adequate events per predictor variable (EPV) value, we did not include all variables that were significant in the univariate analysis to the Cox proportional hazards regression model [22]. All tests were two-tailed. Pearson's and Spearman's correlation coefficients were used for parametric and nonparametric variables, respectively. Youden's J statistic was performed to determine the optimal biomarker's cut-off point for prediction of the primary endpoint. Receiver operating characteristic (ROC) curves were plotted for Gal-3 and sST2 in relation to the primary endpoint. Statistical analyses were performed using SPSS software, version 22 (IBM SPSS Statistics 22, New York, USA).

3. Results

3.1. Baseline Characteristics and One-Year Follow-Up. The final analysis included 104 patients who had completed the one-year follow-up. In all cases, the ischemia-related artery was revascularized using drug-eluting stents ($n = 109$) or balloon angioplasty alone ($n = 5$) during PCI. In most of the cases, the ischemia-related artery was the right coronary artery ($n = 49$) and the left anterior descending ($n = 47$), while less frequently was observed the stenotic circumflex artery ($n = 14$), diagonal artery ($n = 2$), obtuse marginal

Table 1: Baseline characteristics of patients with and without new HF onset at one-year follow-up.

Variable	$n = 104$		p value
	Patients without HF at 1 year ($n = 54$)	Patients with HF at 1 year ($n = 50$)	
Baseline characteristics			
Age (years)	58.0 (53.0-67.3)	64.0 (57.0-70.8)	**0.04**
Male gender	72.2%; 39/54	70.0%; 35/50	0.83
BMI (kg/m^2)	27.6 (24.0-29.7); $n = 51$	29.7 (25.2-33.2); $n = 42$	**0.04**
Moderate valve disease	1.9%; 1/54	8.0%; 4/50	0.19
Hypertension	59.3%; 32/54	64.0%; 32/50	0.69
Atrial fibrillation	0.0%; 0/54	10.0%; 5/50	**0.02**
Diabetes	13.0%; 7/54	30.0%; 15/50	0.053
Chronic kidney disease	14.8%; 8/54	22.0%; 11/50	0.45
COPD	5.6%; 3/54	6.0%; 3/50	1.00
Prior stroke or TIA	1.9%; 1/54	8.0%; 4/50	0.19
Peripheral artery disease	1.9%; 1/54	12.0%; 6/50	0.053
Current or former smoking	70.4%; 38/54	72.0%; 36/50	1.00
Clinical status at hospital admission			
Heart rate (b.p.m.)	75.0 (69.5-80.0)	80.0 (73.5-90.0)	**0.01**
SBP (mmHg)	130.0 (120.0-140.0)	130.0 (115.8-146.0)	0.49
DBP (mmHg)	70.0 (68.3-85.0)	80.0 (70.0-90.0)	0.16
Killip class	1 (1-1)	1 (1-2)	0.06
Intravenous diuretics during hospitalization	20.4%; 11/54	42.0%; 21/50	**0.02**
Extent of CAD, n (%)			
1-vessel	57.4%; 31/54	48.0%; 24/50	0.43
2-vessel	25.9%; 14/54	36.0%; 18/50	0.29
3-vessel	16.7%; 9/54	16.0%; 8/50	1.00
Laboratory findings at admission			
Hemoglobin (g/dL)	14.2 (13.5-15.1)	14.3 (13.6-15.7)	0.52
Serum creatinine (mg/dL)	0.96 (0.86-1.1)	0.9 (0.8-1.1)	0.69
eGFR (mL/min/1.73m^2)	89.2 (65.2-111.7)	89.1 (60.0-119.2)	0.95
Serum sodium (mmol/L)	140.0 (138.5-141.7)	139.6 (137.7-142.0)	0.64
Serum potassium (mmol/L)	3.9 (3.5-4.2)	4.0 (3.7-4.2)	0.31
Total cholesterol (mg/dL)	188.0 (161.5-230.5); $n = 52$	182.5 (148.8-211.5); $n = 48$	0.36
LDL (mg/dL)	120.0 (76.8-154.5); $n = 48$	108.0 (82.0-133.0); $n = 43$	0.50
HDL (mg/dL)	43.5 (36.0-51.8); $n = 52$	45.0 (32.0-55.0); $n = 47$	0.39
Triglycerides (mg/dL)	135.0 (94.0-170.0); $n = 51$	134.0 (86.0-200.0); $n = 47$	0.75
hs-CRP peak (mg/dL)	2.8 (1.1-7.1); $n = 51$	4.2 (2.1-10.1); $n = 48$	**0.03**
Troponin I peak (ng/L)	16.0 (1.6-65.1); $n = 51$	46.7 (9.4-111.5); $n = 49$	**0.03**
CK-MB peak (U/L)	45.2 (5.3-184.1); $n = 53$	91.9 (32.8-212.9)	0.07
NT-proBNP peak (pg/mL)	514.0 (192.0-1479.8); $n = 38$	1917.0 (850.5-4258.8); $n = 36$	**0.001**
Gal-3 (ng/mL)	6.9 (4.6-8.0)	7.8 (6.5-10.0)	**0.002**
sST2 (ng/mL)	23.4 (17.0-29.9)	25.7 (20.1-34.5)	**0.04**
Echocardiography			
LVEF (%)	51 (45-55)	43 (35-49)	**<0.001**
LVEDD (mm)	4.8 (4.5-5.1)	5.0 (4.6-5.3); $n = 49$	0.30
LVEDV (mL)	100.0 (80.0-121.0); $n = 33$	106.0 (72.0-131.0); $n = 39$	0.80
LVESV (mL)	47.0 (38.0-60.0); $n = 33$	61.0 (38.0-86.0); $n = 39$	0.17
LVH*	18.0%; 9/50	55.0%; 22/40	**<0.001**
LA dimension (mm)	3.8 (3.5-4.1)	3.9 (3.6-4.2); $n = 49$	0.15

Table 1: Continued.

Variable	$n = 104$ Patients without HF at 1 year ($n = 54$)	Patients with HF at 1 year ($n = 50$)	p value
E′ med (cm/s)	6.9 (6.0-7.8); $n = 33$	5.7 (4.2-7.7); $n = 33$	**0.03**
E/E′ med	11.0 (9.9-12.3); $n = 36$	12.4 (10.1-15.5); $n = 36$	0.06
E′ lat (cm/s)	8.9 (6.7-10.5); $n = 33$	6.2 (5.1-9.8); $n = 32$	**0.03**
E/E′ lat	8.4 (6.7-10.8); $n = 36$	10.0 (7.9-12.9); $n = 35$	0.08
TAPSE (mm)	21.0 (20.0-25.0); $n = 53$	22.0 (20.0-25.0)	0.85
Moderate mitral regurgitation	5.6%; 3/54	24.0%; 12/50	**0.01**
Clinical status and laboratory findings at discharge			
Heart rate (b.p.m.)	71.5 (63.0-80.0)	72.0 (67.5-83.0); $n = 49$	**0.002**
SBP (mmHg)	120.0 (110.0-131.0)	120.0 (110.0-136.0); $n = 49$	0.98
DBP (mmHg)	71.5 (63.0-80.00)	75.0 (65.5-80.00); $n = 49$	0.58
Hemoglobin (g/dL)	13.9 (12.8-14.6); $n = 49$	13.5 (12.3-14.7); $n = 45$	0.22
Serum creatinine (mg/dL)	1.0 (0.8-1.1); $n = 47$	0.9 (0.8-1.1); $n = 45$	0.78
eGFR (mL/min/1.73m^2)	91.4 (76.2-112.5); $n = 47$	86.1 (60.1-114.2); $n = 45$	0.30
Serum sodium (mmol/L)	141.8 (140.1-142.9); $n = 47$	140.7 (138.8-143.1); $n = 45$	0.21
Serum potassium (mmol/L)	4.3 (4.1-4.6); $n = 47$	4.5 (4.2-4.7); $n = 45$	0.21
*Pharmacotherapy at hospital discharge***			
ASA	100%; 54/54	100%; 49/49	1.00
Clopidogrel	94.4%; 51/54	83.7%; 41/49	0.11
Ticagrelor	5.6%; 3/54	16.3%; 8/49	0.11
Anticoagulant	3.7%; 2/54	14.3%; 7/49	0.08
Loop diuretic	5.6%; 3/54	46.9%; 23/49	**<0.001**
ACE-I	96.3%; 52/54	95.9%; 47/49	1.00
ARB	3.7%; 2/54	10.2%; 5/49	0.25
β-Blocker	90.7%; 49/54	95.9%; 47/49	0.44
Aldosterone antagonist	22.2%; 12/54	40.8%; 20/49	0.06
Ivabradine	0.0%; 0/54	4.1%; 2/49	0.22
Statin	98.1%; 53/54	95.9%; 47/49	0.60
Biomarkers at follow-up			
Gal-3 (ng/mL)	7.4 (5.3-9.5); $n = 46$	9.1 (7.4-11.2); $n = 43$	**0.01**
Increase in Gal-3 level from baseline	60.9%; 28/46	72.1%; 31/43	0.37
Change*** in Gal-3 (ng/mL)	0.5 (-0.7-2.0); $n = 46$	1.7 (-0.3-3.1); $n = 43$	0.17
sST2 (ng/mL)	28.5 (24.2-33.3); $n = 46$	33.1 (25.7-40.0); $n = 43$	0.11
Increase in sST2 level from baseline	63.0%; 29/46	67.4%; 29/43	0.82
Change*** in sST2 (ng/mL)	4.6 (-3.5-11.2); $n = 46$	5.2 (-4.2-13.7); $n = 43$	0.72

Bold values indicate p values < 0.05. *LVH was based on left ventricular mass index (LVMI): LVMI > 95 g/m^2 for female, LVMI > 115 g/m^2 for male. **In patients who survived to hospital discharge. ****Changes in biomarker concentrations were calculated as the one-year level minus the baseline level of each biomarker. ACE-I: angiotensin-converting-enzyme inhibitor; ARB: angiotensin II receptor blocker; ASA: acetylsalicylic acid; b.p.m.: beats per minute; BMI: body mass index; CK-MB: creatine kinase-muscle/brain; COPD: chronic obstructive pulmonary disease; DBP: diastolic blood pressure; E′ lat: lateral early diastolic mitral annular velocity; E/E′ lat: early mitral inflow divided by lateral early diastolic mitral annular velocity; E′ med: medial early diastolic mitral annular velocity; E/E′ med: early mitral inflow divided by medial early diastolic mitral annular velocity; eGFR: estimated glomerular filtration rate; Gal-3: galectin-3; HDL: high-density lipoprotein; HF: heart failure; hs-CRP: high-sensitivity C-reactive protein; LA: left atrium; LDL: low-density lipoprotein; LVEDD: left ventricular end-diastolic diameter; LVEDV: left ventricular end-diastolic volume; LVESV: left ventricular end-systolic volume; LVH: left ventricular hypertrophy; LVEF: left ventricular ejection fraction; n: number; NT-proBNP: N-terminal pro-B-type natriuretic peptide; SBP: systolic blood pressure; sST2: soluble interleukin-1 receptor-like 1; TAPSE: tricuspid annular plane systolic excursion; TIA: transient ischemic attack.

artery ($n = 2$), posterior descending artery ($n = 2$), and intermediate artery ($n = 1$). A total of 96 (92%) patients had echocardiography performed, and 89 (86%) patients had blood samples collected at the control visit at one year. Fifty out of 104 patients (48%) reached the primary endpoint. Table 1 presents clinical, biochemical, and echocardiographic characteristics of patients who reached and who did not reach the primary endpoint. During the follow-up, 4 patients

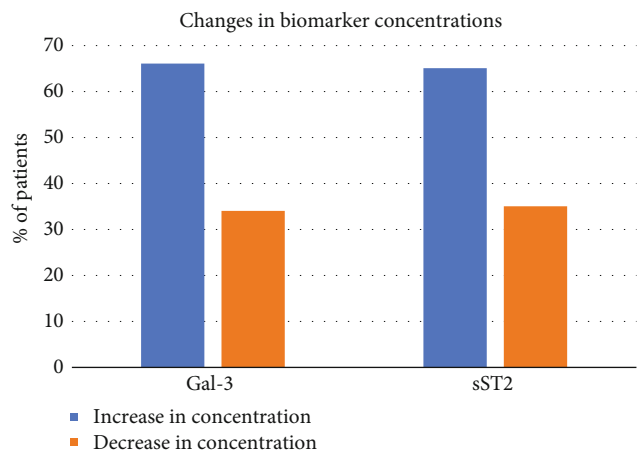

Figure 2: Changes* in biomarker concentrations. *Changes were calculated as the one-year level minus the baseline level of each biomarker.

died and 13 patients had HF-related hospitalizations (6 patients had hospitalization for HF worsening; 8 patients had cardioverter defibrillator implanted). Causes of death included HF in 2 patients, MI in 1 patient, and a noncardiovascular cause in 1 patient.

At baseline, the mean value of Gal-3 was 7.5 (2.0-19.3) ng/mL and sST2 was 26.4 (6.1-89.9) ng/mL, while at one year their concentrations significantly increased to 8.5 ng/mL (2.5-19.7, $p < 0.001$) and 31.4 ng/mL (13.0-57.2; $p = 0.001$), respectively. Gal-3 and sST2 levels increased over time in a majority of patients (66% for Gal-3 and 65% for sST2) (Figure 2). Compared to patients who did not reach the primary endpoint, patients who developed HF at one year had significantly higher baseline concentrations of both biomarkers and a higher Gal-3 level at one year. However, there were no significant differences in sST2 levels at one year or in changes of both biomarkers during follow-up between the two groups (Table 1).

Additionally, patients in the highest quartiles of Gal-3 and sST2 concentration at baseline were more likely to develop HF during follow-up than patients in lower quartiles. A similar association was observed for the highest quartile of Gal-3 measured at one year (Table 2).

In ROC analysis, the area under the curve (AUC) for Gal-3 and sST2 (for prediction of the primary endpoint) was 0.61 and 0.50. Gal-3 concentration of ≥8.74 ng/mL had a sensitivity of 38%, a specificity of 81%, a negative predictive value of 58%, and a positive predictive value of 65% for prediction of the primary endpoint at follow-up (Youden's index). sST2 concentration of ≥34.48 ng/mL had a sensitivity of 27%, a specificity of 83%, a negative predictive value of 55%, and a positive predictive value of 60% for prediction of the primary endpoint at follow-up (Youden's index).

Both Gal-3 and sST2 were predictors of the primary endpoint in univariate logistic regression analysis, but only Gal-3 remained significant in multivariate analysis (Table 3).

3.2. Association of Gal-3 and sST2 with Echocardiographic Parameters. We correlated Gal-3 and sST2 concentrations at baseline and after one year and their changes with echocardiographic parameters (Table S1). Correlation analysis revealed that higher baseline Gal-3 concentrations correlated inversely only with LV end-diastolic volume (LVEDV) at one year. There were no other significant correlations of baseline, follow-up, nor changes in Gal-3 concentration with echocardiographic parameters. Baseline sST2 values correlated positively with LV end-diastolic diameter (LVEDD), LV end-systolic volume (LVESV), and LV mass index (LVMI) and inversely with LVEF at one-year, but not with baseline echocardiographic parameters. Changes in sST2 concentration correlated positively only with LVEF at one year. There were no significant correlations of sST2 follow-up concentrations with echocardiographic parameters.

We also assessed echocardiographic parameters at follow-up by quartiles of baseline Gal-3 and sST2 concentrations, which is summarized in Table 2. Only participants with a higher sST2 level had lower LVEF at baseline and after one year, and patients with higher concentrations of both Gal-3 and sST2 at baseline were more likely to have LV hypertrophy initially and after one year. There was no clear association of rising quartiles with other echocardiographic parameters.

3.3. Correlation of Gal-3 and sST2 with Clinical Parameters. We performed a correlation analysis of Gal-3 and sST2 concentrations assessed at baseline and at one year and their changes with clinical parameters (Table S2). A significant positive correlation was found between Gal-3 and sST2 in regard to baseline, but not to follow-up, nor to changes in biomarker concentrations. Baseline Gal-3 and sST2, changes in sST2 concentration, and follow-up levels of Gal-3 correlated positively with NT-proBNP. Baseline and follow-up levels of Gal-3 and sST2 correlated negatively with the glomerular filtration rate. Baseline and follow-up levels of Gal-3 and sST2 positively correlated and changes in sST2 concentration negatively correlated with a longer stay in the intensive cardiac care unit. Baseline Gal-3 and follow-up levels of sST2 correlated positively with the Killip class, and baseline sST2 positively correlated with NYHA at one year. Only baseline and follow-up Gal-3 correlated positively with age. Only changes in sST2 concentration correlated negatively with cTnI.

3.4. Comparison of Patients with Preserved and Reduced Ejection Fraction at One Year. Patients with LVEF < 50% at one year (30% of patients) had higher baseline concentrations of NT-proBNP, cTnI, CK-MB, and hs-CRP. There were no significant differences between both LVEF groups in terms of baseline, follow-up, nor changes in Gal-3 and sST2 concentrations (Table 4).

4. Discussion

The main finding of our study is that baseline Gal-3 and sST2 presented potential clinical utility in predicting HF development at one year among patients admitted primarily due to STEMI treated with pPCI. In the total STEMI population, concentrations of both biomarkers increased in most of the

Table 2: Association of baseline and follow-up quartiles of Gal-3 (a) and sST2 (b) concentrations with the primary endpoint and echocardiographic parameters.

(a)

Variable	Baseline Gal-3 Quartiles 1	2	3	4	*p* value*	Follow-up Gal-3 Quartiles 1	2	3	4	*p* value*
Primary endpoint										
New-onset HF at follow-up	0.0%; 0/2	39.1%; 18/46	43.3%; 13/30	73.1%; 19/26	**0.01**	27.3%; 6/22	50.0%; 11/22	52.2%; 12/23	63.6%; 14/22	**0.02**
Baseline echocardiography										
LVEF (%)	44.5 (40.5-59.5); $n = 6$	48.0 (43.0-53.8); $n = 52$	50.0 (38.3-55.0); $n = 30$	45.0 (34.0-50.5); $n = 29$	0.09	49.0 (40.8-56.5); $n = 22$	49.0 (38.0-52.0); $n = 22$	48.0 (43.0-51.0); $n = 23$	45.0 (35.3-51.3); $n = 22$	0.26
Follow-up echocardiography										
LVEF (%)	45.0 (45.0-45.0); $n = 1$	56.0 (49.0-58.0); $n = 44$	56.0 (45.0-60.8); $n = 28$	53.0 (44.0-55.0); $n = 23$	0.25	56.0 (51.0-60.3); $n = 22$	54.0 (46.3-56.5); $n = 22$	55.0 (46.0-58.0); $n = 23$	55.0 (40.0-60.0); $n = 22$	0.58
LVEDV (mL)	142.0 (142.0-142.0); $n = 1$	108.5 (92.5-129.0); $n = 38$	105.0 (81.3-126.5); $n = 22$	84.0 (75.5-120.0); $n = 16$	0.33	115.5 (91.3-138.0); $n = 16$	114.0 (94.0-133.8); $n = 18$	101.0 (80.0-117.0); $n = 19$	87.0 (77.0-116.0); $n = 18$	0.22
LVESV (mL)	78.0 (78.0-78.0); $n = 1$	48.5 (40.0-62.0); $n = 38$	42.5 (31.5-61.5); $n = 22$	40.5 (33.0-66.5); $n = 16$	0.76	50.5 (35.5-73.5); $n = 16$	54.5 (38.0-77.0); $n = 18$	45.0 (30.0-62.0); $n = 19$	39.5 (32.8-63.0); $n = 18$	0.70
LVH*	0.0%; 0/1	35.0%; 14/40	44.0%; 11/25	64.7%; 11/17	**0.04**	28.6%; 6/21	47.4%; 9/19	47.4%; 9/19	47.1%; 8/17	0.23
E/E′ med	12.0 (12.0-12.0); $n = 1$	10.7 (8.6-12.6); $n = 42$	9.0 (8.0-11.9); $n = 27$	10.0 (8.2-15.3); $n = 22$	0.54	8.5 (10.0-11.9); $n = 20$	10.8 (9.2-13.4); $n = 22$	9.7 (8.3-12.7); $n = 23$	9.4 (7.9-12.6); $n = 22$	0.80
E/E′ lat	8.1 (8.1-8.1); $n = 1$	8.0 (7.0-10.3); $n = 42$	8.5 (6.2-11.4); $n = 27$	8.8 (6.7-10.9); $n = 22$	0.97	7.3 (6.9-9.6); $n = 20$	9.0 (7.0-12.0); $n = 22$	8.8 (6.5-11.5); $n = 23$	8.4 (6.7-10.4); $n = 22$	0.44

(b)

Variable	Baseline sST2 Quartiles 1	2	3	4	*p* value*	Follow-up sST2 Quartiles 1	2	3	4	*p* value*
Primary endpoint										
New-onset HF at follow-up	22.7%; 5/22	64.0%; 16/25	44.4%; 20/45	75.0%; 9/12	**0.004**	42.9%; 9/21	34.8%; 8/23	52.2%; 12/23	63.6%; 14/22	0.06
Baseline echocardiography										
LVEF (%)	50.0 (41.0-54.5); $n = 29$	47.0 (41.5-52.0); $n = 29$	48.5 (42.0-52.3); $n = 46$	44.0 (32.0-46.0); $n = 13$	**0.04**	49.0 (40.5-55.0); $n = 21$	48.0 (39.0-52.0); $n = 23$	48.0 (41.0-54.0); $n = 23$	46.5 (43.0-50.0); $n = 22$	0.75
Follow-up echocardiography										
LVEF (%)	56.0 (45.0-60.0); $n = 18$	56.0 (46.8-56.0); $n = 24$	55.0 (51.0-58.0); $n = 43$	48.0 (35.0-55.0); $n = 11$	**0.047**	56.0 (44.5-58.0); $n = 21$	55.0 (42.0-60.0); $n = 23$	55.0 (45.0-57.0); $n = 23$	56.0 (49.0-59.3); $n = 22$	0.80
LVEDV (mL)	98.0 (73.0-127.0); $n = 15$	95.0 (81.5-119.0); $n = 21$	111.5 (93.5-138.0); $n = 32$	117.0 (72.5-139.0); $n = 9$	0.58	110.0 (68.5-133.0); $n = 17$	109.5 (86.8-132.3); $n = 16$	101.0 (85.5-142.0); $n = 17$	101.0 (84.0-123.5); $n = 21$	0.82
LVESV (mL)	40.0 (28.0-60.0); $n = 15$	42.0 (34.5-53.5); $n = 21$	49.5 (42.0-65.0); $n = 32$	65.0 (33.0-89.0); $n = 9$	0.23	48.0 (28.0-71.5); $n = 17$	47.0 (35.8-75.8); $n = 16$	45.0 (32.5-82.5); $n = 17$	45.0 (32.5-62.0); $n = 21$	0.71
LVH**	14.3%; 2/14	55.0%; 11/20	43.6%; 17/39	60.0%; 6/10	**0.02**	35.3%; 6/17	42.1%; 8/19	47.6%; 10/21	42.1%; 8/19	0.45
E/E′ med	10.8 (7.8-12.6); $n = 18$	10.7 (9.0-15.0); $n = 23$	10.0 (8.2-12.1); $n = 41$	9.1 (8.0-14.1); $n = 10$	0.22	12.0 (9.7-13.5); $n = 20$	9.0 (7.8-10.7); $n = 22$	10.0 (8.0-11.7); $n = 23$	10.9 (8.4-15.3); $n = 22$	**0.03**
E/E′ lat	8.5 (6.6-8.5); $n = 18$	9.0 (7.2-11.6); $n = 23$	7.6 (6.3-10.0); $n = 41$	8.4 (6.7-13.5); $n = 10$	0.33	8.9 (7.0-10.5); $n = 20$	7.3 (6.1-10.1); $n = 22$	8.5 (7.0-10.0); $n = 23$	9.1 (6.8-12.8); $n = 22$	0.13

Bold values indicate p values < 0.05. *p values derived from statistical tests of equal means (or proportions) across quartiles using analysis of variance for continuous variables and the chi-square test for categorical characteristics. **LVH was based on left ventricular mass index (LVMI): LVMI > 95 g/m^2 for female, LVMI > 115 g/m^2 for male. E/E′ med: early mitral inflow divided by medial early diastolic mitral annular velocity; E/E′ lat: early mitral inflow divided by lateral early diastolic mitral annular velocity; Gal-3: galectin-3; HF: heart failure; LVEDV: left ventricular end-diastolic volume; LVESV: left ventricular end-systolic volume; LVH: left ventricular hypertrophy; LVEF: left ventricular ejection fraction; sST2: soluble interleukin-1 receptor-like 1.

Table 3: Predictors of the primary endpoint in univariate and multivariate analyses.

Variable	Univariate analysis	Multivariate analysis		
	p value	HR	95% CI	*p* value
Age (years)	**0.01**	1.11	1.03–1.19	**0.01**
Gal-3 (per 1 ng/mL)	**0.001**	1.61	1.07-2.42	**0.02**
sST2 (per 1 ng/mL)	**0.04**	1.01	0.94–1.08	**0.87**
Baseline LVEF (%)	**<0.001**	0.80	0.70–0.90	**<0.001**
NT-proBNP (per 100 ng/L)	**0.02**	0.99	0.95-1.01	0.33

Bold values indicate *p* values < 0.05. CI: confidence interval; Gal-3: galectin-3; HR: hazard ratio; LVEF: left ventricular ejection fraction; NT-proBNP: N-terminal pro-B-type natriuretic peptide; sST2: soluble interleukin-1 receptor-like 1.

patients and were significantly higher after one year compared to the baseline concentrations. Patients with new-onset HF had significantly higher baseline levels of Gal-3 and sST2 compared to patients without developed HF. Importantly, Gal-3, but not sST2, was a predictor of the primary endpoint in the multivariate analysis.

MI provokes the inflammatory response with migration of a multitude of cells into the myocardium which initiates structural and biochemical changes in the infarcted and noninfarcted areas leading to LVR. Pathological processes such as myocardial fibrosis, hypertrophy, and changes of ventricular size result in the impairment of LV diastolic and systolic function [1, 23]. Natriuretic peptides are considered as well-proven diagnostic and prognostic biomarkers in HF, but they are not always reliable. Concentrations of natriuretic peptides may be increased in various clinical situations and may also stay low until advanced stages of the disease [11, 24, 25]. LVR may continue for weeks or months; therefore, there is a need for biomarkers that can reflect myocardial fibrosis and early identify patients at risk of HF, which may have important implications for postdischarge follow-up. Considering the contribution of inflammation and fibrosis in the progression of HF, the clinical utility of Gal-3 and sST2 for prediction of new-onset HF is of interest.

Gal-3 is mainly located in the myocardial extracellular matrix and cardiac fibroblasts, whereupon myocardial stress induces cardiac remodeling. In experimental data, Sanchez-Mas et al. observed that Gal-3 increases in the myocardium after MI with the maximum concentration achieved in the infarcted area during the first week, with a gradual decrease in the following weeks [26]. Interestingly, Gal-3 also increased in noninfarcted areas, and it seems that the increase in concentrations of Gal-3 in the early phase after MI contributes to the activation of repair functions in the damaged zone in order to maintain the geometry and function of the heart [26]. However, in the long term, chronic activation leads to tissue fibrosis and accelerates adverse LVR [26].

In an experimental study, sST2 concentrations increased steadily after MI with a maximum expression at 12–18 h [13]. Both Gal-3 and sST2 provide robust prognostic information in patients with existing HF in predicting an increased risk of cardiovascular mortality and events [27–30].

A few previous studies have shown the clinical utility of a single measurement of Gal-3 and sST2 in the diagnosis of HF after acute coronary syndrome [14–17, 31–33]. In our study, patients who reached the primary endpoint had increased baseline concentrations of Gal-3 and sST2. However, in the multivariate logistic regression analysis, only Gal-3 persisted an independent predictor of the primary endpoint. Jenkins et al. concluded that high sST2 concentrations in patients after MI were also associated with an increased risk of HF development independently of other prognostic factors such as age, sex, comorbidities, Killip class, and troponin T in 5 years of observation, so the follow-up period was longer than in our study.

Moreover, previously, it was proven that serial measurements of Gal-3 and sST2 provide additional prognostic information. van der Velde et al. observed in large cohorts of patients with acute and chronic HF that repeated measurements of Gal-3 provide more accurate prognostic information when compared to a single measurement. Authors showed that >15% increase of Gal-3 concentrations between measurements was associated with a 50% higher risk for subsequent HF morbidity and mortality, independently of age, sex, diabetes mellitus, left ventricular ejection fraction, renal function, HF medication, and NT-proBNP ($p = 0.001$) [28]. In the TRIUMPH (Translational Initiative on Unique and novel strategies for Management of Patients with Heart failure) study, investigators showed that serial sST2 measurements were a strong predictor of all-cause mortality and HF rehospitalization in patients with acute HF, independently of NT-proBNP [29]. Moreover, based on the results of the PREVEND (Prevention of Renal and Vascular Endstage Disease) study, conducted among the general population, the researchers suggested that the presence of elevated concentration of Gal-3 may be a predictor of the development of HF. However, this relationship was observed only in patients with increased baseline cardiovascular risk [34]. In addition, serial biomarker measurements have been shown to provide more accurate prognostic information compared to a single Gal-3 measurement [34]. The same was observed by Ghorbani et al. that traditional cardiovascular risk factors (older age, hypertension, diabetes, and BMI) were associated with a rise in Gal-3 levels over time and the largest changes in Gal-3 were in regard to the development of chronic kidney disease, HF, and all-cause mortality [35].

We demonstrated that both biomarkers' concentrations increased during one-year observation in the total population after STEMI, regardless of the occurrence of HF. However, there was no significant difference in sST2 concentrations between the two cohorts after one year. In contrast, van der Velde et al. observed that in patients after AMI Gal-3 concentrations increased, while sST2 levels decreased. However, the authors observed patients for only the first 4 months, when it can be expected that changes in biomarkers' concentrations may be more pronounced. They also observed higher baseline concentrations of the biomarkers, but they recruited subjects regardless of the type

Table 4: Biomarker concentrations stratified to left ventricular ejection fraction after one year.

Variable	LVEF < 50% ($n = 29$)	LVEF ≥ 50% ($n = 67$)	p value
Baseline biomarkers			
Gal-3 (ng/mL)	7.1 (6.2-9.1); $n = 29$	7.1 (5.5-8.4); $n = 67$	0.34
sST2 (ng/mL)	24.5 (19.0-35.4); $n = 29$	24.0 (18.9-29.8); $n = 67$	0.37
hs-CRP peak (mg/dL)	6.3 (2.3-20.3); $n = 28$	2.7 (1.3-6.9); $n = 64$	**0.01**
Troponin I peak (ng/L)	69.7 (22.8-160.2); $n = 28$	14.4 (1.8-49.4); $n = 64$	**<0.001**
CK-MB peak (U/L)	135.0 (53.2-339.6); $n = 29$	46.5 (10.7-141.0); $n = 66$	**0.01**
NT-proBNP peak (pg/mL)	1921.5 (855.5-4262.0); $n = 22$	812.0 (287.5-1874.8); $n = 48$	**0.01**
Biomarkers at follow-up			
Gal-3 (ng/mL)	9.3 (6.8-10.7); $n = 26$	7.8 (6.1-10.0); $n = 63$	0.17
sST2 (ng/mL)	30.3 (24.9-34.9); $n = 26$	30.1 (24.4-36.7); $n = 63$	0.45
Changes of biomarker concentrations*			
Change in Gal-3 (ng/mL)	1.7 (-0.7-3.5); $n = 26$	0.6 (-0.6-2.8); $n = 63$	0.24
Change in sST2 (ng/mL)	3.9 (-5.5-11.8); $n = 26$	5.4 (-2.7-13.2); $n = 63$	0.73

Bold values indicate p values < 0.05. *Changes were calculated as the one-year level minus the baseline level of each biomarker. CK-MB: creatine kinase-muscle/brain; Gal-3: galectin-3; hs-CRP: high-sensitivity C-reactive protein; LVEF: left ventricular ejection fraction; NT-proBNP: N-terminal pro-B-type natriuretic peptide; sST2: soluble interleukin-1 receptor-like 1.

of acute coronary syndrome [21]. Accordingly, Sabatine et al. observed that baseline levels rather than subsequent values of sST2 appeared to be more predictive of cardiovascular death or HF. However, what one would expect is that subsequent values of NT-proBNP appeared to be more predictive than the initial value [36].

We observed that baseline but not follow-up sST2 concentrations significantly correlated with lower LVEF at follow-up. However, we also observed that the patients with increasing concentrations of sST2 had better LVEF than patients with decreasing sST2. This may reflect a greater mass of the vital myocardium and the reparative role of sST2 post-MI to prevent LV dilatation and preserve LVEF. A similar finding was observed by van der Velde in relation to Gal-3; however, in our study we did not observe a correlation between changes in Gal-3 concentration and LVEF.

The occurrence of HF with preserved ejection fraction (HFpEF) has risen significantly over the past decade and is characterized by the presence of diastolic dysfunction [3, 37–39]. Considering the contribution of Gal-3 and sST2 in inflammation and fibrosis and their significance in the progression of HF, we analyzed whether both biomarkers might be predictors of LVR and diastolic dysfunction. Our study showed an association between Gal-3 and sST2 and LV hypertrophy, but these biomarkers were not related to higher LV filling pressures (E/e' ratio) and diastolic tissue velocities at the mitral annulus (E').

4.1. Limitations. The study was mainly limited due to a relatively small number of patients, with approximately 10% lost to follow-up. In addition, some patients did not have a follow-up echocardiogram or did not have all parameters measured. Moreover, there are missing data on biomarker levels, including Gal-3 and sST2 at the control visit, and other biomarkers such as NT-proBNP from hospital stay.

5. Conclusions

These data highlight the potential role of Gal-3 and sST2 measurements after STEMI in the prediction of HF onset. However, baseline measurements of Gal-3 and sST2 showed greater clinical significance than values obtained after one year or changes in biomarker concentrations. In patients who achieved the primary endpoint, concentrations of both biomarkers were initially higher and Gal-3 was the predictor of the primary endpoint. In the entire study population, the biomarker levels were higher after one year. Both biomarkers were not associated with parameters of diastolic dysfunction; thus, it is unclear whether these biomarkers may be helpful in diagnosing and predicting HFpEF. There is a need for further studies to determine the predictive value and clinical utility of repeated measurements of sST2 and Gal-3 concentrations in patients after AMI.

Data Availability

The raw data of the study can be provided upon request with maintenance of confidentiality, privacy, and anonymity of the research participants.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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Supplementary Materials

Table S1: correlation of Gal-3 and sST2 concentrations at baseline and after one-year follow-up and changes of their concentrations with echocardiographic parameters. Table S2: correlation of Gal-3 and sST2 concentrations at baseline and after one-year follow-up and changes of their concentrations with clinical parameters. *(Supplementary Materials)*

References

[1] M. G. St John Sutton and N. Sharpe, "Left ventricular remodeling after myocardial infarction: pathophysiology and therapy," *Circulation*, vol. 101, no. 25, pp. 2981–2988, 2000.

[2] E. Roberts, A. J. Ludman, K. Dworzynski et al., "The diagnostic accuracy of the natriuretic peptides in heart failure: systematic review and diagnostic meta-analysis in the acute care setting," *BMJ*, vol. 350, article h910, 2015.

[3] P. Ponikowski, A. A. Voors, S. D. Anker et al., "2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC," *European Journal of Heart Failure*, vol. 18, no. 8, pp. 891–975, 2016.

[4] I. S. Anand, T. S. Rector, M. Kuskowski, A. Adourian, P. Muntendam, and J. N. Cohn, "Baseline and serial measurements of galectin-3 in patients with heart failure: relationship to prognosis and effect of treatment with valsartan in the Val-HeFT," *European Journal of Heart Failure*, vol. 15, no. 5, pp. 511–518, 2013.

[5] R. A. de Boer, D. J. Lok, T. Jaarsma et al., "Predictive value of plasma galectin-3 levels in heart failure with reduced and preserved ejection fraction," *Annals of Medicine*, vol. 43, no. 1, pp. 60–68, 2011.

[6] D. Gruson, T. Lepoutre, S. A. Ahn, and M. F. Rousseau, "Increased soluble ST2 is a stronger predictor of long-term cardiovascular death than natriuretic peptides in heart failure patients with reduced ejection fraction," *International Journal of Cardiology*, vol. 172, no. 1, pp. e250–e252, 2014.

[7] C. Llibre, E. Zamora, À. Caballero et al., "The real-life value of ST2 monitoring during heart failure decompensation: impact on long-term readmission and mortality," *Biomarkers*, vol. 21, no. 3, pp. 225–232, 2016.

[8] A. Bayes-Genis, M. de Antonio, J. Vila et al., "Head-to-head comparison of 2 myocardial fibrosis biomarkers for long-term heart failure risk stratification: ST2 versus galectin-3," *Journal of the American College of Cardiology*, vol. 63, no. 2, pp. 158–166, 2014.

[9] M. Tomaniak, G. Sygitowicz, K. J. Filipiak et al., "Dysregulations of miRNAs and galectin-3 may underlie left ventricular dilatation in patients with systolic heart failure," *Kardiologia Polska*, vol. 76, no. 6, pp. 1012–1014, 2018.

[10] G. Sygitowicz, M. Tomaniak, K. J. Filipiak, Ł. Kołtowski, and D. Sitkiewicz, "Galectin-3 in patients with acute heart failure: preliminary report on first Polish experience," *Advances in Clinical and Experimental Medicine*, vol. 25, no. 4, pp. 617–623, 2016.

[11] C. W. Yancy, M. Jessup, B. Bozkurt et al., "2017 ACC/AHA/HFSA focused update of the 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society of America," *Circulation*, vol. 136, no. 6, pp. e137–e161, 2017.

[12] R. Y. Yang, G. A. Rabinovich, and F. T. Liu, "Galectins: structure, function and therapeutic potential," *Expert Reviews in Molecular Medicine*, vol. 10, article e17, 2008.

[13] E. O. Weinberg, M. Shimpo, G. W. de Keulenaer et al., "Expression and regulation of ST2, an interleukin-1 receptor family member, in cardiomyocytes and myocardial infarction," *Circulation*, vol. 106, no. 23, pp. 2961–2966, 2002.

[14] E. W. Grandin, P. Jarolim, S. A. Murphy et al., "Galectin-3 and the development of heart failure after acute coronary syndrome: pilot experience from PROVE IT-TIMI 22," *Clinical Chemistry*, vol. 58, no. 1, pp. 267–273, 2012.

[15] G. Di Tano, G. Caretta, R. De Maria et al., "Galectin-3 predicts left ventricular remodelling after anterior-wall myocardial infarction treated by primary percutaneous coronary intervention," *Heart*, vol. 103, no. 1, pp. 71–77, 2017.

[16] G. Miñana, J. Núñez, A. Bayés-Genís et al., "ST2 and left ventricular remodeling after ST-segment elevation myocardial infarction: a cardiac magnetic resonance study," *International Journal of Cardiology*, vol. 270, pp. 336–342, 2018.

[17] W. S. Jenkins, V. L. Roger, A. S. Jaffe et al., "Prognostic value of soluble ST2 after myocardial infarction: a community perspective," *The American Journal of Medicine*, vol. 130, no. 9, pp. 1112.e9–1112.e15, 2017.

[18] P. G. Steg, S. K. James, D. Atar et al., "ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation," *European Heart Journal*, vol. 33, no. 20, pp. 2569–2619, 2012.

[19] R. M. Lang, L. P. Badano, V. Mor-Avi et al., "Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging," *European Heart Journal – Cardiovascular Imaging*, vol. 16, no. 3, pp. 233–271, 2015.

[20] S. F. Nagueh, O. A. Smiseth, C. P. Appleton et al., "Recommendations for the evaluation of left ventricular diastolic function by echocardiography: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging," *European Heart Journal – Cardiovascular Imaging*, vol. 17, no. 12, pp. 1321–1360, 2016.

[21] A. R. van der Velde, C. P. H. Lexis, W. C. Meijers et al., "Galectin-3 and sST2 in prediction of left ventricular ejection fraction after myocardial infarction," *Clinica Chimica Acta*, vol. 452, pp. 50–57, 2016.

[22] E. Vittinghoff and C. E. McCulloch, "Relaxing the rule of ten events per variable in logistic and Cox regression," *American Journal of Epidemiology*, vol. 165, no. 6, pp. 710–718, 2007.

[23] Y. Sun, "Myocardial repair/remodelling following infarction: roles of local factors," *Cardiovascular Research*, vol. 81, no. 3, pp. 482–490, 2009.

[24] A. Tymińska, A. Kapłon-Cieślicka, and K. J. Filipiak, "Heart failure — new biomarkers on the horizon?," *Choroby Serca i Naczyń*, vol. 15, no. 4, pp. 232–244, 2018.

[25] I. A. Sergeeva and V. M. Christoffels, "Regulation of expression of atrial and brain natriuretic peptide, biomarkers for heart development and disease," *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, vol. 1832, no. 12, pp. 2403–2413, 2013.

[26] J. Sanchez-Mas, A. Lax, M. C. Asensio-Lopez et al., "Galectin-3 expression in cardiac remodeling after myocardial infarction," *International Journal of Cardiology*, vol. 172, no. 1, pp. e98–e101, 2014.

[27] R. A. de Boer, A. A. Voors, P. Muntendam, W. H. van Gilst, and D. J. van Veldhuisen, "Galectin-3: a novel mediator of heart failure development and progression," *European Journal of Heart Failure*, vol. 11, no. 9, pp. 811–817, 2009.

[28] A. R. van der Velde, L. Gullestad, T. Ueland et al., "Prognostic value of changes in galectin-3 levels over time in patients with heart failure: data from CORONA and COACH," *Circulation: Heart Failure*, vol. 6, no. 2, pp. 219–226, 2013.

[29] L. C. van Vark, I. Lesman-Leegte, S. J. Baart et al., "Prognostic value of serial ST2 measurements in patients with acute heart failure," *Journal of the American College of Cardiology*, vol. 70, no. 19, pp. 2378–2388, 2017.

[30] J. L. Januzzi, A. Mebazaa, and S. Di Somma, "ST2 and prognosis in acutely decompensated heart failure: the International ST2 Consensus Panel," *The American Journal of Cardiology*, vol. 115, no. 7, pp. 26B–31B, 2015.

[31] R. A. P. Weir, C. J. Petrie, C. A. Murphy et al., "Galectin-3 and cardiac function in survivors of acute myocardial infarction," *Circulation: Heart Failure*, vol. 6, no. 3, pp. 492–498, 2013.

[32] T. H. Tsai, P. H. Sung, L. T. Chang et al., "Value and level of galectin-3 in acute myocardial infarction patients undergoing primary percutaneous coronary intervention," *Journal of Atherosclerosis and Thrombosis*, vol. 19, no. 12, pp. 1073–1082, 2012.

[33] I. Szadkowska, R. N. Wlazeł, M. Migała et al., "The association between galectin-3 and clinical parameters in patients with first acute myocardial infarction treated with primary percutaneous coronary angioplasty," *Cardiology Journal*, vol. 20, no. 6, pp. 577–582, 2013.

[34] A. R. van der Velde, W. C. Meijers, J. E. Ho et al., "Serial galectin-3 and future cardiovascular disease in the general population," *Heart*, vol. 102, no. 14, pp. 1134–1141, 2016.

[35] A. Ghorbani, V. Bhambhani, R. H. Christenson et al., "Longitudinal change in galectin-3 and incident cardiovascular outcomes," *Journal of the American College of Cardiology*, vol. 72, no. 25, pp. 3246–3254, 2018.

[36] M. S. Sabatine, D. A. Morrow, L. J. Higgins et al., "Complementary roles for biomarkers of biomechanical strain ST2 and N-terminal prohormone B-type natriuretic peptide in patients with ST-elevation myocardial infarction," *Circulation*, vol. 117, no. 15, pp. 1936–1944, 2008.

[37] A. Kapłon-Cieślicka, A. Tymińska, M. Peller et al., "Diagnosis, clinical course, and 1-year outcome in patients hospitalized for heart failure with preserved ejection fraction (from the Polish Cohort of the European Society of Cardiology Heart Failure Long-Term Registry)," *The American Journal of Cardiology*, vol. 118, no. 4, pp. 535–542, 2016.

[38] A. Tymińska, A. Kapłon-Cieślicka, K. Ozierański et al., "Anemia at hospital admission and its relation to outcomes in patients with heart failure (from the Polish Cohort of 2 European Society of Cardiology Heart Failure Registries)," *The American Journal of Cardiology*, vol. 119, no. 12, pp. 2021–2029, 2017.

[39] P. Balsam, K. Ozierański, A. Kapłon-Cieślicka et al., "Differences in clinical characteristics and 1-year outcomes of hospitalized patients with heart failure in ESC-HF Pilot and ESC-HF-LT registries," *Polish Archives of Internal Medicine*, vol. 129, no. 2, pp. 106–116, 2019.