Research Article

Novel Biomarker MicroRNAs for Subtyping of Acute Coronary Syndrome: A Bioinformatics Approach

Yujie Zhu,1,2,3 Yuxin Lin,1 Wenyong Yan,1 Zhandong Sun,1 Zhi Jiang,4 Bairong Shen,1 Xiaoqian Jiang,2 and Jingjing Shi5

1Center for Systems Biology, Soochow University, Suzhou 215006, China
2Biomedical Informatics Division, UC San Diego, La Jolla, CA, USA
3Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, Jiangsu 210008, China
4School of Medicine, Soochow University, Suzhou 215123, China
5Department of Cardiovascular Internal Medicine, Wuxi Third People’s Hospital, Wuxi 214041, China

Correspondence should be addressed to Jingjing Shi; jjshi.wuxi3yuan@163.com

Received 20 October 2016; Accepted 27 October 2016

Acute coronary syndrome (ACS) is a life-threatening disease that affects more than half a million people in the United States. We currently lack molecular biomarkers to distinguish the unstable angina (UA) and acute myocardial infarction (AMI), which are the two subtypes of ACS. MicroRNAs play significant roles in biological processes and serve as good candidates for biomarkers. In this work, we collected microRNA datasets from the Gene Expression Omnibus database and identified specific microRNAs in different subtypes and universal microRNAs in all subtypes based on our novel network-based bioinformatics approach. These microRNAs were studied for ACS association by pathway enrichment analysis of their target genes. AMI and UA were associated with 27 and 26 microRNAs, respectively, nine of them were detected for both AMI and UA, and five from each subtype had been reported previously. The remaining 22 and 21 microRNAs are novel microRNA biomarkers for AMI and UA, respectively. The findings are then supported by pathway enrichment analysis of the targets of these microRNAs. These novel microRNAs deserve further validation and will be helpful for personalized ACS diagnosis.

1. Introduction

Acute coronary syndrome (ACS) is a life-threatening disease that affects more than half a million people in the United States. We currently lack molecular biomarkers to distinguish the unstable angina (UA) and acute myocardial infarction (AMI), which are the two subtypes of ACS. MicroRNAs play significant roles in biological processes and serve as good candidates for biomarkers. In this work, we collected microRNA datasets from the Gene Expression Omnibus database and identified specific microRNAs in different subtypes and universal microRNAs in all subtypes based on our novel network-based bioinformatics approach. These microRNAs were studied for ACS association by pathway enrichment analysis of their target genes. AMI and UA were associated with 27 and 26 microRNAs, respectively, nine of them were detected for both AMI and UA, and five from each subtype had been reported previously. The remaining 22 and 21 microRNAs are novel microRNA biomarkers for AMI and UA, respectively. The findings are then supported by pathway enrichment analysis of the targets of these microRNAs. These novel microRNAs deserve further validation and will be helpful for personalized ACS diagnosis.
is therefore urgent to discover more effective biomarkers to precisely diagnose the subtypes of ACS.

MicroRNAs (miRNAs) are a class of small noncoding RNAs with the posttranscriptional role of regulating about 60% of human protein-coding genes [15]. Currently there are more than 2500 mature human miRNAs listed in miRBase (release 21) [16]. They play functions in a wide variety of biological processes such as cell proliferation [17, 18], development [19], and apoptosis [20], which contribute to various physiological and pathological conditions, including cardiovascular diseases such as the acute coronary syndrome [21, 22].

Until now, very few studies have looked at the two ACS subtypes, AMI and UA, in terms of similarities and differences, and in particular miRNA expression levels have not been well studied. To better understand the disease pathogenesis of these two subtypes, we applied an in-house regulatory model termed improved Pipeline of Outlier MicroRNA Analysis (POMA) [23, 24] to identify miRNAs specific to each subtype or shared by both subtypes (see Figure 1). The model focused on miRNAs’ independent regulatory power and the biological functions of their targets. Two measures, novel out degree (NOD) and transcription factor percentage (TFP) of genes, were defined, where NOD was equivalent to the number of genes that were uniquely targeted by a single miRNA and TFP represented the percentage of all transcription factor (TF) genes that were targeted. According to the statistical evidences described in our previous work, miRNAs with larger NOD and TFP values were more likely to be candidate biomarkers and represented biomarker miRNAs that had strong abilities to regulate genes independently and, meanwhile, regulate more TF genes. The application of biomarker discovery for prostate cancer [23, 25], sepsis [26], clear cell renal cell carcinoma [27], and pediatric acute myeloid leukemia [24] demonstrated its great predictive power.

2. Materials and Methods

2.1. Dataset Collection. The miRNA expression datasets (GSE31568 and GSE49823) were downloaded from Gene Expression Omnibus (GEO) [28]. GSE31568 contained 454 samples and we extracted 70 controls and 21 AMI samples [29], and GSE49823 contained 13 controls and 13 UA samples. The details of the two datasets are listed in Table 1. We then

![Figure 1: Schematic diagram for the identification of candidate miRNA biomarkers in acute myocardial infarction (AMI) and unstable angina (UA). Here, “DE” is the abbreviation of “differentially expressed.”](image-url)
identified differentially expressed (DE) miRNAs based on linear models in Limma R package [30, 31]; the empirical Bayes (eBayes) method was performed to calculate the p value and other parameters. The Benjamini-Hochberg method was applied to adjust and correct p values. The adjusted p value <0.05 was chosen as the cut-off criteria.

We also collected the reported miRNAs for AMI and UA from PubMed by the search criteria “(Acute Myocardial Infarction OR AMI) AND (miRNA OR microRNA) AND (biomarker OR marker)” and “(Unstable Angina OR UA) AND (miRNA OR microRNA) AND (biomarker OR marker)”. We only took published reports from the past five years and all of the samples were extracted with human data in consideration. The information of biomarkers including miRNA ID, biomarker type, expression pattern, study design, publication date, and PMID are summarized in Tables S1 and S2, in Supplementary Material available online at http://dx.doi.org/10.1155/2016/4618323.

2.2. Prediction of Putative miRNA Biomarkers for AMI and UA. Based on two significantly DE miRNA sets, we employed improved POMA to predict miRNA biomarkers for AMI and UA [24]. In the pipeline, two important measures NOD and TFP were defined. NOD is the number of genes uniquely targeted by a certain miRNA and TFP is the percentage of TF genes of all targets of the miRNA. The main idea of the improved POMA model is that miRNAs with larger NOD values and targeting more TF genes are more likely to be biomarkers. The POMA and improved POMA methodologies were elaborated in our previous studies [23, 24].

Using this pipeline, the AMI- and UA-specific miRNA-mRNA networks were constructed by mapping relevant DE miRNAs onto human miRNA-mRNA network (reference network). Then, NOD and TFP were measured for each miRNA in the condition-specific network of AMI and UA, respectively. Finally, miRNAs with significantly large NOD and TFP values (Wilcoxon signed-rank test, p value <0.05) were selected as candidate biomarkers.

We calculated the percentage of reported AMI/UA biomarker miRNAs in the whole predicted set and defined it as the prediction precision for evaluating the accuracy of our model.

2.3. Functional Enrichment Analysis of the Target Genes of Candidate miRNA Biomarkers. We performed functional enrichment analysis of the genes uniquely regulated by candidate biomarker miRNAs from the two condition-specific miRNA-mRNA networks by MetaCore™ software. The significantly enriched pathways and diseases ontologies were ranked by p value (<0.05), which was calculated by hypergeometric test. FDR adjustment was used for multiple test correction.

3. Results

3.1. Identification of Candidate miRNA Biomarkers for AMI and UA. Based on AMI and UA miRNA expression datasets, we identified 292 and 182 deregulated miRNAs in AMI and UA, respectively. Employing our in-house model improved POMA [24], and a total of 27 miRNAs for AMI and 26 miRNAs for UA were screened (see Figure 2(a), Wilcoxon signed-rank test, p value < 0.05). These miRNAs were predicted to be candidate biomarkers for the two subtypes of ACS by our model. The substructural characteristics of these biomarker miRNAs in the miRNA regulatory network, including the number of whole targets (termed N), NOD, and TFP values, are listed in Table 2.

As listed in Table 2, nine miRNA biomarkers were shared by both AMI and UA subtypes, indicating that these miRNAs (miR-126, miR-142-3p, miR-145, miR-240*, miR-346, miR-34a, miR-93, and let-7g) could be universal biomarkers for both AMI and UA. The remaining 18 and 17 miRNAs could be putative biomarkers specific for AMI and UA, respectively.

3.2. Literature-Based Validation of Identified miRNA Biomarkers. We collected AMI- and UA-specific miRNA biomarkers by analysis of citations in PubMed, as shown in Figure 2(b). Altogether, 30 miRNAs have been reported to be biomarkers for AMI and 25 of them are diagnostic. Two miRNAs (miR-155 and miR-300*) [32] and a cluster of miR-16-27a/101/150 [33] were reported to be prognostic indicators. Two miRNAs (miR-208b and miR-133a) were reported to be valuable for both diagnosis and prognosis in AMI (see Table S1).

For UA, 15 miRNAs have been reported to be biomarkers, 13 of them were diagnostic, including a cluster of three miRNAs (miR-132/150/186) [34], and two were reported to be effective for both diagnosis and prognosis (miR-133a and miR-208b) [35] (see Table S2). We then compared literature reported miRNAs with ones we identified and found five that were the same in the AMI set (prediction precision: 18.5%): miR-155, miR-34a, miR-27a, miR-101, and miR-126 (see Figure 2(c)). Among them, miR-155 expression was increased approximately 4-fold in patients with a high-risk of cardiac death after discharge and could be a biomarker for cardiac death in post-AMI patients [32]; miR-34a was investigated for its role as a p53 responsive miRNA and confirmed as predicator for the risk of heart failure after AMI [36]. Elevated miR-27a expression was included in the panel of prognostic miRNAs for outcome after AMI; downregulation of miR-101 was also included in this panel. However, miR-101 was also reported to be upregulated in another study [33].

There were also five biomarker miRNAs (miR-106b, miR-25, miR-590-5p, miR-132, and miR-126) for UA found from our analysis and the reported list (see Figure 2(d), prediction precision: 19.2%). Among them, miR-106b, miR-25, and miR-590-5p were upregulated when compared with the control group [37]. The significantly elevated expression levels of the miR-106b/25 cluster and miR-21/590-5p family could be used as an indicator of coronary artery disease. A panel that consisted of miR-132, miR-150, and miR-186 showed the highest discriminatory power (AUC = 0.91) [34]. miR-126 was a unique biomarker that was found both in our analysis and in previous studies for both AMI and UA. However,
miR-126 was upregulated in the AMI dataset while it was reported to be downregulated in the literature [22]. In UA, the regulation pattern of the overlapping miRNAs was found to be consistent between our study and the previous reported work [38].

3.3. Functional Enrichment Analysis of Target Genes of Candidate miRNA Biomarkers. We further explored the roles of uniquely regulated genes of the identified miRNAs in AMI and UA by functional enrichment analysis using the MetaCore software [39–44]. In pathway analysis, we found 35 significantly enriched pathways in AMI and 18 in UA ($p$ value < 0.05 and FDR < 0.05; see Figures 3(a) and 3(b)). There were nine pathways significantly enriched by the targets of candidate miRNA biomarkers for both AMI and UA (see Tables S3 and S4).

In general, the significantly enriched pathways were grouped into immune response, development, cell adhesion, signal transduction, apoptosis, and survival, and others as shown in Figures 3(c) and 3(d). In AMI, pathways in immune response (34%) and development (26%) account for 60% of the pathways. In UA, immune response and developmental pathways also play a role, with 11% and 17% of the miRNA-regulated pathways belonging to these categories, respectively. Besides these two, apoptosis and survival pathways accounted for a combined 22% of all miRNA targets.

We then evaluated the relevance of these pathways in AMI and UA by searching PubMed for published papers describing the role of constituent network objects of pathways in AMI and UA. As shown in Table S3, 28 of the 35 AMI pathways were reported to be involved with AMI and 10 of them are in the group of immune responses, such as CD40 signaling [45, 46]. Many interleukin (IL) factors were also reported in immune response pathways related to AMI and UA [47, 48, 49]. IL-12/27a/101/150 (b) miRNA biomarkers collected from published literature. IDs in bold mean they were prognostic, those in italic meant they were functional for both diagnosis and prognosis, and the remaining ones were reported to be significantly enriched pathways in AMI and UA ($p$ value < 0.05 and FDR < 0.05; see Figures 3(a) and 3(b)). There were nine pathways significantly enriched by the targets of candidate miRNA biomarkers for both AMI and UA (see Tables S3 and S4).

In general, the significantly enriched pathways were grouped into immune response, development, cell adhesion, signal transduction, apoptosis, and survival, and others as shown in Figures 3(c) and 3(d). In AMI, pathways in immune response (34%) and development (26%) account for 60% of the pathways. In UA, immune response and developmental pathways also play a role, with 11% and 17% of the miRNA-regulated pathways belonging to these categories, respectively. Besides these two, apoptosis and survival pathways accounted for a combined 22% of all miRNA targets.

We then evaluated the relevance of these pathways in AMI and UA by searching PubMed for published papers describing the role of constituent network objects of pathways in AMI and UA. As shown in Table S3, 28 of the 35 AMI pathways were reported to be involved with AMI and 10 of them are in the group of immune responses, such as CD40 signaling [45, 46]. Many interleukin (IL) factors were also reported in immune response pathways related to AMI such as IL-9 [47], IL-10 [48], IL-17 [49], IL-18 [50], and IL-33 [51]. In the development group, there were five pathways related to AMI, including WNT [52], G-CSF [53], SDF-1 [54], NF-kB [55], PEDF [56], and VEGF [57].

In the 18 pathways found in UA, 12 had been reported previously to relate to UA. The most important pathways were...
### Table 2: The identified miRNA biomarker candidates for acute myocardial infarction (AMI) and unstable angina (UA).

| miRNA ID | AMI | UA |
|----------|-----|-----|
| miR-126 | N   | N   | NOD | NOD | TF (TFP) | TF (TFP) | Pathways (percentage) | Pathways (percentage) |
| miR-155 | 185 | 64  | 39  | 64  | 0.21     | 0.46     | 16 (0.46)             | 16 (0.46)             |
| miR-30e | 109 | 15  | 32  | 15  | 0.46     | 0.21     | 5 (0.14)              | 5 (0.14)              |
| miR-98  | 255 | 18  | 0   | 18  | 0.06     | 0.14     | 2 (0.06)              | 2 (0.06)              |
| miR-23b | 185 | 15  | 32  | 15  | 0.14     | 0.46     | 5 (0.14)              | 5 (0.14)              |
| miR-204 | 198 | 15  | 32  | 15  | 0.29     | 0.14     | 10 (0.29)             | 10 (0.29)             |
| miR-34a | 151 | 32  | 24  | 24  | 0.16     | 0.46     | 39 (0.21)             | 39 (0.21)             |
| miR-34a | 151 | 32  | 24  | 24  | 0.16     | 0.46     | 39 (0.21)             | 39 (0.21)             |
| let-7g  | 199 | 13  | 34  | 34  | 0.17     | 0.69     | 24 (0.69)             | 24 (0.69)             |
| miR-576-3p | 133 | 13  | 23  | 23  | 0.17     | 0.69     | 12 (0.17)             | 12 (0.17)             |
| miR-346 | 31  | 13  | 5   | 5   | 0.14     | 0.21     | 2 (0.14)              | 2 (0.14)              |
| miR-454 | 298 | 13  | 43  | 43  | 0.14     | 0.21     | 2 (0.14)              | 2 (0.14)              |
| miR-532-3p | 112 | 12  | 18  | 18  | 0.14     | 0.36     | 2 (0.14)              | 2 (0.14)              |
| miR-145 | 55  | 11  | 11  | 11  | 0.30     | 0.21     | 8 (0.30)              | 8 (0.30)              |
| miR-340-3p | 256 | 11  | 37  | 37  | 0.21     | 0.69     | 12 (0.21)             | 12 (0.21)             |
| miR-126 | 87  | 8   | 18  | 18  | 0.21     | 0.69     | 2 (0.21)              | 2 (0.21)              |
| miR-31  | 34  | 7   | 8   | 8   | 0.21     | 0.30     | 6 (0.21)              | 6 (0.21)              |
| miR-600 | 127 | 7   | 23  | 23  | 0.30     | 0.69     | 1 (0.30)              | 1 (0.30)              |
| miR-491-3p | 119 | 6   | 21  | 21  | 0.69     | 0.30     | 0 (0.69)              | 0 (0.69)              |
| miR-603 | 149 | 6   | 32  | 32  | 0.69     | 0.30     | 2 (0.69)              | 2 (0.69)              |
| miR-93  | 394 | 6   | 68  | 68  | 0.69     | 0.30     | 0 (0.69)              | 0 (0.69)              |
| miR-340-5p | 72  | 5   | 12  | 12  | 0.69     | 0.30     | 0 (0.69)              | 0 (0.69)              |
| miR-934-3p | 103 | 5   | 18  | 18  | 0.69     | 0.30     | 0 (0.69)              | 0 (0.69)              |
| miR-101 | 69  | 4   | 18  | 18  | 0.69     | 0.30     | 0 (0.69)              | 0 (0.69)              |
| miR-128 | 22  | 4   | 4   | 4   | 0.69     | 0.30     | 0 (0.69)              | 0 (0.69)              |

**Notes.** The miRNAs were ranked based on their NOD values. miRNA IDs in bold have been reported in published studies and those with underlines were shared by both AMI and UA.

In AMI, miR-126 (83%), let-7g (69%), and miR-155 (46%) were the top three miRNAs that regulated more than 30% of the significantly enriched pathways (as listed in Table 2). miR-126 (72%), let-7g (72%), and miR-34a (33%) were the top three miRNAs involved in UA. Both in AMI and in UA, let-7g and miR-126 regulated more than half of the pathways, which indicated that they were functionally important to both of the subtypes. This observation is helpful for understanding the molecular mechanisms of ACS common or specific to the AMI and UA subtypes.

**3.4. The Percentage of Pathways Potentially Regulated by Each Biomarker miRNA.** Analyzing the biological processes for each subtype revealed mechanistic relationships. Some of the pathways may result in atherosclerosis progression and atherosclerotic lesion rupture. However, some may contribute to the development of coronary collateral vessels, and some may even have their roles in inhibiting the formation of the thrombus. In order to explore the role of miRNAs in the pathways, we calculated the percentage of pathways that were regulated by miRNA in all significantly enriched pathways (see Table 2).

In AMI, miR-126 (83%), let-7g (69%), and miR-155 (46%) were the top three miRNAs that regulated more than 30% of the significantly enriched pathways (as listed in Table 2). miR-126 (72%), let-7g (72%), and miR-34a (33%) were the top three miRNAs involved in UA. Both in AMI and in UA, let-7g and miR-126 regulated more than half of the pathways, which indicated that they were functionally important to both of the subtypes. This observation is helpful for understanding the molecular mechanisms of ACS common or specific to the AMI and UA subtypes.

**4. Discussion**

Understanding the mechanism and identifying the biomarkers specific to AMI and UA are important for ACS diagnosis and treatments. In this study, miRNA biomarkers are identified for AMI and UA using our improved POMA model. The model enriches fragile sites in the miRNA-mRNA network, focusing on miRNAs that regulate important genes involved with apoptosis and survival (FAS signaling cascades [58], TNFRI signaling pathway [59], and NGF activation of NF-kB [60]). The other correlated pathways that had been previously reported were the PPAR pathway [61] and TCR and CD28 costimulation in activation of NF-kB [62] (see Table S4).
Figure 3: Significantly enriched pathways by targets of microRNA biomarkers for acute myocardial infarction (AMI) and unstable angina (UA). (a) Top 10 enriched pathways in AMI. (b) Top 10 enriched pathways in UA. (c) Pie plot of category enriched pathways in AMI. (d) Pie plot of category enriched pathways in UA.
for these disease subtypes. We defined measures NOD and TFP to quantify whether a miRNA could be a candidate biomarker. The former reflected the power of a miRNA to regulate genes independently whereas the latter indicated the potential of regulating TF genes. TF genes are chosen because TFs are important regulators and are kernels of many crucial biological processes. According to our previous studies [23, 24], biomarker miRNAs tended to have large NOD and TFP values. Based on the evidence, we identified 27 and 26 miRNAs as candidate biomarkers for AMI and UA, respectively, and nine of them were shared by the two ACS subtypes. Five AMI and five UA candidates had been previously reported as miRNA biomarkers.

In order to explore the roles of miRNAs in ACS, we performed a functional enrichment analysis for the targets of candidate miRNA biomarkers. In AMI, 35 pathways were significantly enriched and 28 (80%) have been reported to be related to AMI. Many of the pathways enriched in AMI were correlated with the immune response, and let-7g, miR-155, miR-101, miR-126, and miR-145 were closely relevant (see Table S3). A deregulated immune system is considered not only a trigger but also a factor amplifying an uncontrolled immune response in AMI [45]. CD40 signaling was reported to be upregulated in the pathogenesis of AMI patients [46]. The levels of IL-18 were upregulated in patients with AMI and the inhibition of its activity promoted cardiac function and reduced scar formation and infarct size [50, 63]. The predictive values of IL-6 and IL-10 were also shown to be elevated after AMI, which suggested a role for the formation of coronary collateral vessels, providing adequate blood supply and preventing death of cardiomyocytes [78]. Many studies have found that serum VEGF concentrations were elevated in ACS, which can be a surrogate marker of myocardial infarction [79, 80]. Serum VEGF-A was shown to be elevated after AMI, which suggested a role for the development of coronary collateral vessels [57, 81]. VEGF-A is also a target gene of miR-126, which mapped in the pathway. The other common developmental pathway in AMI and UA was PEDF signaling. PEDF, a 50-kDa glycoprotein, has anti-inflammatory, antioxidant, antiangiogenic, antiatherosclerotic, anti-tumorigenic, and neuroprotective properties [82] and is widely expressed throughout the human body. In ACS patients, plasma PEDF concentrations were significantly lower than the control group and associated with adverse cardiac outcomes after ACS [83]. PEDF can block platelet activation and aggregation [84] through its anti-inflammatory and antioxidative properties, leading to the inhibition of the vascular inflammation and the formation of a thrombus [85].

We also calculated the percentage of pathways that were potentially regulated by the miRNAs in all significantly enriched pathways (see Tables 2, S3, and S4). A novel miRNA (let-7g), which had never been reported as an important factor in ACS before, deserves further investigation, as it participated in regulating 69% (24/35) of the pathways in AMI and 72% (13/18) in UA. About half of these enriched pathways were closely associated with the immune response, especially in AMI (see Tables S3 and S4). Notably, it was previously reported that a miRNA together with its targets was differentially regulated in E2F1-deficient mice, and the E2F1 transcription factor played important roles in the immune response to systemic Escherichia coli lipopolysaccharide (LPS) [86]. The conclusions demonstrated the significance of let-7g in the immune response, which may represent a latent therapeutic target for the treatment of immunological diseases as well as ACS. More clinical validations of this hypothesis will be needed in the future.
We noticed that the miRNA datasets selected for our comparison in this study were inconsistent. The AMI dataset was obtained on whole blood (GSE31568) whereas the UA was on plasma (GSE49823). As we know, the concentration of miRNAs in whole blood is higher than that in plasma; thus the prediction based on the two data sources has some limitations. It would be better if we could obtain AMI and UA samples chosen from the same source type (both were from plasma or whole blood) and compare with the same control group. Unfortunately, the miRNA expression data that could be used for analyses were quite limited. On the other hand, we considered that plasma is an important component of whole blood, where miRNAs could present in a remarkably stable form [87]. Hence further expression data analyses and clinical validations need to be done when more and better datasets are available for in-depth studies.

5. Conclusions

In this study, we applied our improved POMA model to identify miRNA biomarkers for subtyping ACS, finding 18 and 17 miRNAs to be specific biomarkers for AMI and UA, respectively. Nine miRNAs were found in both subtypes, which implied that they could be universal molecular markers for ACS. These findings were further verified by enrichment analysis and compared with previous publications. For future translational application, further experimental and clinical verifications are necessary.

Abbreviations

DE: Differentially expressed
TF: Transcription factor
POMA: Pipeline of Outlier MicroRNA Analysis
NOD: Novel out degree
TFP: Transcription factor gene percentage.

Competing Interests

The authors declare that there is no conflict of interests.

Authors’ Contributions

Yuje Zhu and Yuxin Lin contributed equally to this work.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant nos. 31470821, 31400688, 81471488, and 81271378) and the Natural Science Foundation of Jiangsu Province, China (Grant no. BK20130290).

References

[1] R. A. Nishimura, C. M. Otto, R. O. Bonow et al., “2014 AHA/ACC guideline for the management of patients with valvular heart disease: executive summary: a report of the American college of cardiology/American heart association task force on practice guidelines,” Journal of the American College of Cardiology, vol. 63, no. 22, pp. 2438–2488, 2014.
[2] D. Mozaffarian, E. J. Benjamin, A. S. Go et al., “Heart disease and stroke statistics—2015 update: a report from the American Heart Association,” Circulation, vol. 131, no. 4, pp. e29–e322, 2015.
[3] D. C. Angus, W. T. Linde-Zwirble, J. Lidicker, G. Clermont, J. C. Carcillo, and M. R. Pinsky, “Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care,” Critical Care Medicine, vol. 29, no. 7, pp. 1303–1310, 2001.
[4] D. M. Kolansky, “Acute coronary syndromes: morbidity, mortality, and pharmacoeconomic burden,” American Journal of Managed Care, vol. 15, no. 2, pp. S36–S41, 2009.
[5] M. Torres and S. Moayedi, “Evaluation of the acutely dyspneic elderly patient,” Clinics in Geriatric Medicine, vol. 23, no. 2, pp. 307–325, 2007.
[6] V. Čulić, D. Eterović, D. Mireć, and N. Silić, “Symptom presentation of acute myocardial infarction: influence of sex, age, and risk factors,” American Heart Journal, vol. 144, no. 6, pp. 1012–1017, 2002.
[7] E. M. Antman, M. Cohen, P. J. L. M. Bernink et al., “The TIMI risk score for unstable angina/non-ST elevation MI: a method for prognostication and therapeutic decision making,” Journal of the American Medical Association, vol. 284, no. 7, pp. 835–842, 2000.
[8] B. Lee, A. M. Chang, A. C. Matsuura, S. Maroon, and J. E. Hollander, “Comparison of cardiac risk scores in ED patients with potential acute coronary syndrome,” Critical Pathways in Cardiology, vol. 10, no. 2, pp. 64–68, 2011.
[9] J. Sanchis, V. Bodí, J. Núñez et al., “New risk score for patients with acute chest pain, non-ST-segment deviation, and normal troponin concentrations: a comparison with the TIMI risk score,” Journal of the American College of Cardiology, vol. 46, no. 3, pp. 443–449, 2005.
[10] D. K. Slater, M. A. Hlatky, D. B. Mark, F. E. Harrell Jr., D. B. Pryor, and R. M. Califf, “Outcome in suspected acute myocardial infarction with normal or minimally abnormal admission electrocardiographic findings,” The American Journal of Cardiology, vol. 60, no. 10, pp. 766–770, 1987.
[11] J. A. Goldstein, K. M. Chinnaiyan, A. Abidov et al., “The CT-STAT (coronary computed tomographic angiography for systematic triage of acute chest pain patients to treatment) trial,” Journal of the American College of Cardiology, vol. 58, no. 14, pp. 1414–1422, 2011.
[12] W. B. Gibler, L. M. Lewis, R. E. Erb et al., “Early detection of acute myocardial infarction in patients presenting with chest pain and nondiagnostic ECGs: serial CK-MB sampling in the emergency department,” Annals of Emergency Medicine, vol. 19, no. 12, pp. 1359–1366, 1990.
[13] K. Thygesen, J. S. Alpert, and H. D. White, “Universal definition of myocardial infarction,” Journal of the American College of Cardiology, vol. 50, no. 22, pp. 2173–2195, 2007.
[14] P. Valensi, L. Lorgis, and Y. Cottin, “Prevalence, incidence, predictive factors and prognosis of silent myocardial infarction: a review of the literature,” Archives of Cardiovascular Diseases, vol. 104, no. 3, pp. 178–188, 2011.
[15] M. Esteller, “Non-coding RNAs in human disease,” Nature Reviews Genetics, vol. 12, no. 12, pp. 861–874, 2011.
[16] A. Kozomara and S. Griffiths-Jones, “mirBase: annotating high confidence microRNAs using deep sequencing data,” Nucleic Acids Research, vol. 42, no. 1, pp. D68–D73, 2014.
[50] D. Kawasaki, T. Tsujino, S. Morimoto et al., “Plasma interleukin-18 concentration: a novel marker of myocardial ischemia rather than necrosis in humans,” Coronary Artery Disease, vol. 16, no. 7, pp. 437–441, 2005.

[51] A. M. Miller, "Role of IL-33 in inflammation and disease," Journal of Inflammation, vol. 8, article 22, 2011.

[52] B. Assmus, M. Iwasaki, V. Schächinger et al., “Acute myocardial infarction activates progenitor cells and increases Wnt signalling in the bone marrow,” European Heart Journal, vol. 33, no. 15, pp. 1911–1919, 2012.

[53] S. Vandervelde, M. J. A. van Luyk, R. A. Tio, and M. C. Harmens, "Signaling factors in stem cell-mediated repair of infarcted myocardium," Journal of Molecular and Cellular Cardiology, vol. 39, no. 2, pp. 363–376, 2005.

[54] K. Stellos, B. Bigalke, H. Langer et al., "Expression of stromal-cell-derived factor-1 on circulating platelets is increased in patients with acute coronary syndrome and correlates with the number of CD34+ progenitor cells," European Heart Journal, vol. 30, no. 5, pp. 584–593, 2009.

[55] X. X. Liao, X. Li, Z. F. Ma et al., "Role of nuclear factor-XB in endothelial injury in acute myocardial infarction," Zhongguo Wei Zhong Bing Ji Ji You Xue, vol. 20, no. 7, pp. 413–415, 2008.

[56] K. Distelmaier, C. Adlbrecht, J. Jakowitsch et al., "Proteomic profiling of acute coronary thrombosis reveals a local decrease in pigment epithelium-derived factor in acute myocardial infarction," Clinical Science, vol. 123, no. 2, pp. 111–119, 2012.

[57] A. Kranz, C. Rau, M. Kochs, and J. Waltenberger, "Elevation acute myocardial infarction," European Journal of Cell Biology, vol. 32, no. 1, pp. 65–72, 2000.

[58] A. Bossovaska, A. Boskowski, and B. Galar, "Analysis of apoptotic markers Fas/Fasl (CD95/CD95L) expression on the lymphocytes in patients with acute coronary syndrome," Kardiologia Polska, vol. 65, no. 8, pp. 883–889, 2007.

[59] P. Aukrust, W. J. Sandberg, K. Otterdal et al., "Tumor necrosis factor superfamily molecules in acute coronary syndromes," Annals of Medicine, vol. 43, no. 2, pp. 90–103, 2011.

[60] M. E. Ritchie, "Nuclear factor-XB is selectively and markedly activated in humans with unstable angina pectoris," Circulation, vol. 98, no. 17, pp. 1707–1713, 1998.

[61] J. Yang, C. Liu, L. Zhang et al., "Intensive atorvastatin therapy attenuates the inflammatory responses in monocytes of patients with unstable angina undergoing percutaneous coronary intervention via peroxisome proliferator-activated receptor γ activation," Inflammation, vol. 38, no. 4, pp. 1415–1423, 2015.

[62] L. Cominacini, M. Anselmi, U. Garbin et al., "Enhanced plasma levels of oxidized low-density lipoprotein increase circulating nuclear factor-kappa B activation in patients with unstable angina," Journal of the American College of Cardiology, vol. 46, no. 5, pp. 799–806, 2005.

[63] Z. Mallat, C. Heymes, A. Corbaz et al., "Evidence for altered interleukin 1α (IL-1α) pathway in human heart failure," The FASEB Journal, vol. 18, no. 14, pp. 1752–1754, 2004.

[64] E. Ammirati, C. V. Cannistraci, N. A. Cristell et al., "Identification and predictive value of interleukin-6+ interleukin-10 and interleukin-6 interleukin-10 cytokine patterns in ST-elevation acute myocardial infarction," Circulation Research, vol. 110, no. 10, pp. 1336–1348, 2012.

[65] Y.-Z. Lin, B.-W. Wu, Z.-D. Lu et al., "Circulating Th22 and Th9 levels in patients with acute coronary syndrome," Mediators of Inflammation, vol. 2013, Article ID 635672, 2013.

[66] S. Sanada, D. Hakuno, L. J. Higgins, E. R. Schreiter, A. N. J. McKenzie, and R. T. Lee, "IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system," Journal of Clinical Investigation, vol. 117, no. 6, pp. 1538–1549, 2007.

[67] K. Zhang, X.-C. Zhang, Y.-H. Mi, and J. Liu, "Predicting value of serum soluble ST2 and interleukin-33 for risk stratification and prognosis in patients with acute myocardial infarction," Chinese Medical Journal, vol. 126, no. 19, pp. 3628–3631, 2013.

[68] Y. Maekawa, T. Anzai, T. Yoshikawa et al., "Prognostic significance of peripheral monocytosis after reperfused acute myocardial infarction: a possible role for left ventricular remodeling," Journal of the American College of Cardiology, vol. 39, no. 2, pp. 241–246, 2002.

[69] T. Takahashi, Y. Hiasa, Y. Ohara et al., "Relationship of adhesion neutrophil count to microvascular injury, left ventricular dilatation, and long-term outcome in patients treated with primary angioplasty for acute myocardial infarction," Circulation Journal, vol. 72, no. 6, pp. 867–872, 2008.

[70] X. Cheng, Y.-H. Liao, H. Ge et al., "TH1/TH2 functional imbalance after acute myocardial infarction: coronary arterial inflammation or myocardial inflammation," Journal of Clinical Immunology, vol. 25, no. 3, pp. 246–253, 2005.

[71] V. E. A. Stoneman and M. R. Bennett, "Role of apoptosis in atherosclerosis and its therapeutic implications," Clinical Science, vol. 107, no. 4, pp. 343–354, 2004.

[72] M. M. Kockx and A. G. Herman, "Apoptosis in atherogenesis: implications for plaque destabilization," European Heart Journal, vol. 19, pp. G23–G28, 1998.

[73] Y. Li, G. Takemura, K.-I. Kosai et al., "Critical roles for the Fas/Fas ligand system in postinfarction ventricular remodeling and heart failure," Circulation Research, vol. 95, no. 6, pp. 627–636, 2004.

[74] M. Shimizu, K. Fukuo, S. Nagata et al., "Increased plasma levels of the soluble form of Fas ligand in patients with acute myocardial infarction and unstable angina pectoris," Journal of the American College of Cardiology, vol. 39, no. 4, pp. 585–590, 2002.

[75] N. Ferrara, "Vascular endothelial growth factor," European Journal of Cancer Part A, vol. 32, no. 14, pp. 2413–2422, 1996.

[76] K. Harada, M. Friedman, J. J. Lopez et al., "Vascular endothelial growth factor administration in chronic myocardial ischemia," American Journal of Physiology—Heart and Circulatory Physiology, vol. 270, no. 5, pp. H1791–H1802, 1996.

[77] L. F. Brown, K.-T. Yeo, B. Berse et al., "Expression of vascular permeability factor (vascular endothelial growth factor) by epidermal keratinocytes during wound healing," The American Journal of Pathology, vol. 134, no. 4, pp. 1415–1423, 1992.

[78] T. Sugimoto, K. Inui, and Y. Shimazaki, "Gene therapy for myocardial angiogenesis: with direct intramuscular gene transfer of naked deoxyribonucleic acid encoding vascular endothelial growth factor and cell transplantation of vascular endothelial growth factor transfecting H9c2 myoblast," Japanese Journal of Thoracic and Cardiovascular Surgery, vol. 51, no. 5, pp. 192–197, 2003.

[79] A. Konopka, J. Janas, W. Piotrowski, and J. Stepinska, "Concentration of vascular endothelial growth factor in patients with acute coronary syndrome," Cytokine, vol. 61, no. 2, pp. 664–669, 2013.

[80] C. Gui, S.-K. Li, Q.-L. Nong, F. Du, L.-G. Zhu, and Z.-Y. Zeng, "Changes of serum angiogenic factors concentrations in
patients with diabetes and unstable angina pectoris," *Cardiovascular Diabetology*, vol. 12, no. 1, article 34, 2013.

[81] Y. Wang, H. E. Johnsen, S. Mortensen et al., "Changes in circulating mesenchymal stem cells, stem cell homing factor, and vascular growth factors in patients with acute ST elevation myocardial infarction treated with primary percutaneous coronary intervention," *Heart*, vol. 92, no. 6, pp. 768–774, 2006.

[82] D. Orlic, J. Kajstura, S. Chimenti et al., "Bone marrow cells regenerate infarcted myocardium," *Nature*, vol. 410, no. 6829, pp. 701–705, 2001.

[83] J. Liu, S. Wang, J. Shi et al., "The association study of plasma levels of pigment epithelium-derived factor with acute coronary syndrome in the Chinese Han population," *Cardiology*, vol. 127, no. 1, pp. 31–37, 2014.

[84] K. Takenaka, S.-I. Yamagishi, T. Matsui et al., "Pigment epithelium-derived factor (PEDF) administration inhibits occlusive thrombus formation in rats: a possible participation of reduced intraplatelet PEDF in thrombosis of acute coronary syndromes," *Atherosclerosis*, vol. 197, no. 1, pp. 25–33, 2008.

[85] S.-I. Ueda, S.-I. Yamagishi, T. Matsui, Y. Jinnouchi, and T. Imaizumi, "Administration of pigment epithelium-derived factor inhibits left ventricular remodeling and improves cardiac function in rats with acute myocardial infarction," *American Journal of Pathology*, vol. 178, no. 2, pp. 591–598, 2011.

[86] L. A. Warg, J. L. Oakes, R. Burton et al., "The role of the E2F1 transcription factor in the innate immune response to systemic LPS," *American Journal of Physiology—Lung Cellular and Molecular Physiology*, vol. 303, no. 5, pp. L391–L400, 2012.

[87] P. S. Mitchell, R. K. Parkin, E. M. Kroh et al., "Circulating microRNAs as stable blood-based markers for cancer detection," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 30, pp. 10513–10518, 2008.