Unlocking the Potential of microRNAs: Machine Learning Identifies Key Biomarkers for Myocardial Infarction Diagnosis

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**Abstract**

**Background:** MicroRNAs (miRNAs) play a crucial role in regulating adaptive and maladaptive responses in cardiovascular diseases, making them attractive targets for potential biomarkers. However, their potential as novel biomarkers for diagnosing cardiovascular diseases requires systematic evaluation.

**Methods:** In this study, we aimed to identify a key set of miRNA biomark- ers using integrated bioinformatics and machine learning analysis. We combined and analyzed three gene expression datasets from the Gene Expression Omnibus (GEO) database, which contains peripheral blood mononuclear cell (PBMC) sam- ples from individuals with myocardial infarction (MI), stable coronary artery disease (CAD), and healthy individuals. Additionally, we selected a set of miRNAs based on their area under the receiver operating characteristic curve (AUC-ROC) for separating the CAD and MI samples. We designed a two-layer

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architecture for sample classification, in which the first layer isolates healthy sam- ples from unhealthy samples, and the second layer classifies stable CAD and MI samples. We trained different machine learning models using both biomarker sets and evaluated their performance on a test set.

**Results:** We identified miR-21, miR-186, and miR-32 as the only miRNAs among the differentially expressed genes, and a set including miR-186, miR-21, miR-197, miR-29A, and miR-296 as the optimum set of miRNAs selected by their AUC- ROC. Both biomarker sets could distinguish healthy from not-healthy samples with complete accuracy. The best performance for the classification of CAD and MI was achieved with an SVM model trained using the biomarker set selected by AUC-ROC, with an AUC-ROC of 0.96 and an accuracy of 0.94 on the test data. **Conclusions:** Our study demonstrated that miRNA signatures derived from PBMCs could serve as valuable novel biomarkers for cardiovascular diseases.

**Keywords:** microRNA, Machine Learning, Myocardial Infarction, Bioinformatics, Biomarker

# 1 Introduction

Cardiovascular diseases (CVDs) are the leading cause of human mortality, accounting for 32% of all global deaths. It is estimated that approximately 85% of CVD mor- tality is due to myocardial infarction (MI) [[1](#_bookmark17)]. MI is an acute coronary syndrome characterized by sudden blockage and stenosis of the coronary artery and subsequent myocardial ischemia, leading to extensive cardiomyocyte damage and necrosis [[2](#_bookmark18)].

Over the last 50 years, numerous attempts have been made to use biomarkers to facilitate diagnosis, assess the risk, follow-up therapy, and determine therapeutic efficacy in CVD candidates. Based on released guidelines, cardiac troponins (cTns) are used as a highly sensitive and accurate approach for detecting MI. Despite these inherent advantages, the high sensitivity of cTn-based assays has also led to more false-positive results [[3](#_bookmark19)], necessitating the advent and development of new modalities with pathological value. To improve the diagnostic value of existing MI biomarkers, a combination of complementary biological markers, such as microRNAs (miRNAs) and other genetic factors, has been proposed. Previous research supports the notion that miRNAs exhibit great potential as alternative biomarkers for CVD detection and follow-up [[4](#_bookmark20)]. It has been suggested that miRNAs possess 18-22 nucleotides and play a crucial role in the regulation of gene expression. Evidence indicates that miRNAs are involved in the pathogenesis of cardiac tissue injury [[5](#_bookmark21)]. Several biological processes, such as angiogenesis, cardiomyocyte growth and contractility, lipid metabolism, plaque formation, and cardiac rhythm, are regulated by miRNAs [[6](#_bookmark22)]. These elements can easily circulate in biofluids and could be considered theranostic targets in terms of CVDs [[5](#_bookmark21)]. It has been postulated that the function and diagnostic properties of miRNAs are beyond the myocardium in patients with CVD. Specifically, the expression of miRNAs can vary in different biofluids and cell components such as serum and peripheral blood mononuclear cells (PBMCs) [[7](#_bookmark23)].

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PBMCs are a fraction of white blood cells, including monocytes, lymphocytes, macrophages, and other cells of the immune system [[8](#_bookmark24)]. Emerging data indicate that PBMCs can be used as a valid source of biomarkers for monitoring various patholog- ical conditions. Of note, the alteration of mRNAs and miRNAs under pathological conditions provides valuable information about different kinds of disorders. PBMCs can recapitulate the conditions of target tissues, thus providing a highly sensitive and specific source of biomarkers [[9](#_bookmark25)]. Combined with these conditions, these cells are repositories of dysregulated genes and miRNA expression profiles in CVDs [[8](#_bookmark24), [9](#_bookmark25)].

In recent years, the advent and application of machine learning (ML) has been an exciting prospect for advancing scientific research. Although the concept of ML and its initial algorithms were conceived many years ago, recent improvements in computing power and access to vast amounts of data have demonstrated that ML techniques outperform classical statistical methods in various fields. Furthermore, the progress made in omics technologies has enabled the analysis of massive and intricate biological datasets, consisting of hundreds to thousands of samples, which makes it possible for ML to extract valuable biological insights and information from such data [[10](#_bookmark26)]. Consequently, ML provides innovative methods for merging and interpreting diverse types of omics data, leading to the identification of new biomarkers. These biomarkers can aid in precise disease prediction, patient stratification, and the development of novel therapeutic approaches [[11](#_bookmark27)].

In this study, we aimed to identify potential miRNA biomarkers in patients with MI by combining and analyzing three different microarray datasets from PBMCs. The integration of omics data with bioinformatics and ML techniques could be a promis- ing tool in the discovery of new and more accurate biomarkers for monitoring MI. Additionally, this approach can deepen our understanding of the underlying mecha- nisms of MI and aid in the development of valid diagnostic biomarkers and patient stratification.

# Methods

## Microarray data collection

Microarray datasets were obtained from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>). To obtain sufficient classification power between MI, healthy, and CAD samples, a relatively large sample size is needed Therefore, GSE59867 for the MI and CAD samples and GSE56609 and GSE54475 for the healthy samples were selected. All samples were produced using Affymetrix Human Gene 1.0 ST Array platform (GPL6244). Only healthy, CAD, and early-stage MI sam- ples were selected from these datasets for further analysis. The basic information for the three datasets evaluated in this study is provided in Table [1](#_bookmark0). Bioinformatics anal- yses were conducted using R, ver. 4.2.0 [[12](#_bookmark28)], RStudio [[13](#_bookmark29)]. All plots and graphics of these sections were created using the ggplot2 R package [[14](#_bookmark30)].

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**Table 1** Sample information on the GEO microarray dataset.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Dataset | Platform | Healthy | CAD | MI | Reference |
| GSE59867 | GPL6244 | - | 46 | 111 | [[15](#_bookmark31)] |
| GSE56609 | GPL6244 | 46 | - | - | [[16](#_bookmark32)] |
| GSE54475 | GPL6244 | 5 | - | - | [[17](#_bookmark33)] |

## Preprocessing

The raw data in the form of CEL files from all datasets were obtained from GEO. To prepare the data for analysis, we utilized the fRMA package [[18](#_bookmark34)] to facilitate preprocessing of individual microarray samples and their consistent combination. For each dataset, background correction was applied using the RMA algorithm, fol- lowed by quantile normalization based on the reference distribution. To account for probe-specific effects, batch effects were eliminated during summarization and gene expression variances were estimated accordingly. In cases where multiple probe sets matched the same gene, the mean log-fold change was retained. Consequently, fRMA can serve as a technique to remove batch effects across diverse datasets generated by identical microarray platforms [[19](#_bookmark35)]. To ensure the effectiveness of the batch effect removal, we employed principal component analysis (PCA) and relative log expression (RLE) plots to visualize the data before and after applying fRMA.

## Differential expression analysis

The barcode algorithm was introduced by McCall et al. [[20](#_bookmark36)], aimed to convert actual expression values into binary barcode values. Extensive sample collections were gathered and normalization was performed using fRMA across multiple platforms, including the Affymetrix Human Gene 1.0 ST Array (GPL6244) platform. By utiliz- ing these normalized datasets, the distribution of the observed intensities for both the expressed and unexpressed genes was estimated. The determination of whether a gene was expressed or not was based on the following equation, where a value of 1 indicates expression and a value of 0 indicates non-expression:

(1 if *xij ≥ µne* + *C × σne*

0 otherwise

*x*ˆ*ij* =

(1)

In the barcode algorithm, the normalized intensity of gene *i* in sample *j* is denoted as *xij*. A user-defined parameter, *C*, was introduced along with the standard deviation (*σne*) and mean (*µne*) of the non-expressed distribution. Based on these values, the barcode representation of a sample was generated as a vector consisting of ones and zeros, representing the estimated expression (ones) and non-expression (zeros) of each gene. The barcode function within the R fRMA package was employed to implement the barcode algorithm, utilizing the default value of *C*.

To assess the differences in expressed ratios between the MI and healthy control groups, Fisher’s exact test was performed on the barcode values of individual genes. Genes that exhibited a false discovery rate (FDR) below 0*.*05, calculated using the Benjamini-Hochberg procedure to account for multiple testing issues were identified

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as differentially expressed genes (DEGs). The same procedures were applied to the CAD versus healthy control comparison, as well as to the MI versus CAD group, to identify DEGs specific to each comparison.

## Functional and pathway enrichment analyses

The R clusterProfiler package [[21](#_bookmark37)] was utilized to perform Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis and Gene Ontology (GO) functional annotation on the set of DEGs. GO analysis encompassed three categories: biological process (BP), cellular component (CC), and molecular function (MF). For statistical significance, an adjusted p-value threshold of less than 0.05 was employed. Enrichment analyses were conducted separately for DEGs specific to the MI-healthy and CAD-healthy comparisons. All the default parameters provided by the package were used in the analyses.

## ML procedure

ML analysis was performed using Python software, ver. 3.9, Numpy [[22](#_bookmark38)], Pandas [[23](#_bookmark39)], and Scikit-Learn packages [[24](#_bookmark40)]. Whenever hypertuning was needed, the Scikit- opt package [[25](#_bookmark41)] was used. In all ML analyses, the datasets were divided into training and test sets at a 0.7:0.3 ratio, and all reported results are the average of 10-fold cross-validation.

Two different approaches were used to select miRNAs for model training. The first approach was to use differentially expressed miRNAs. In the second approach, miRNAs with individual area under the receiver operating characteristic curves (AUC- ROCs) over 0.8 for separating MI from CAD were selected. The results of these two approaches can provide an informative comparison between the predictive capabilities of sets of miRNAs selected with different logics.

### miRNAs in DEGs

In this approach, a two-layer architecture is deployed to the data to maximize the prediction values. The first layer predicted whether a sample was healthy or not, and the second layer separated MI from CAD in the samples that were predicted as not healthy in the first layer. To this end, a distinct ML model was trained for each layer. Because, there were a limited number of miRNAs in the DEGs, both layers were trained with all of them. For further comparison with the models’ performance, the ROC curve of each miRNA for classifying healthy and not-healthy, as well as CAD and MI, was generated using a logistic regression model.

#### First layer for the isolation of healthy and not-healthy samples:

A support vector machine (SVM) model using RBF kernels was trained and hyper- tuned using all miRNAs in the DEGs. To handle the severe imbalance in the number of samples (51 for the healthy group and 157 for the not-healthy group), the sample weights for the healthy and the not-healthy samples were set to 1 and 0.5, respectively. The ROC curve and confusion matrix for the model are reported.

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#### Second layer for separating the MI and CAD samples:

Different models were investigated to achieve the highest classification performance. To do so, SVM (with linear, polynomial, and RBF kernels), logistic regression (LR), random forests (RF), k-nearest neighbor (kNN), gradient boosting (GB), XGBoost (XGB) and decision tree (DT) models were trained. All models were trained with their preset parameters using 10-fold cross-validation. The criteria for selecting the best model were the highest accuracy and AUC-ROC for the test set. The best model was hypertuned using the scikit-opt package [[25](#_bookmark41)] for the best classification performance. The ROC curve and confusion matrix for the best model are reported.

### miRNAs with the highest AUC-ROC

As in the previous approach, a two-layer strategy was employed. The first layer classi- fied samples into healthy and not-healthy, and the second layer separated the MI and CAD samples. However, to keep the number of miRNAs as low as possible, miRNAs were selected from the second layer and their performance was evaluated in the first layer. The AUC-ROC of all miRNAs for classifying MI and CAD samples was calcu- lated, and miRNAs with AUC-ROC *>* 0*.*8 were selected. ROC curves for each selected miRNA for separating healthy samples from not-healthy samples and MI from CAD samples were also plotted for further comparison.

#### First layer for the isolation of healthy and not-healthy samples:

An SVM model with an RBF kernel is trained using the selected set of miRNAs. Additionally, the model was hypertuned to find the hyperparameters for the highest AUC-ROC and accuracy. The same sample weights as in the previous approach (1 for healthy and 0.5 for not-healthy samples) were used. The ROC curve and confusion matrix for the model were reported.

#### Second layer for separating the MI and CAD samples:

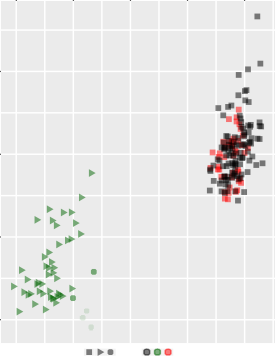
The selected miRNA set was used to train different algorithms to determine the best model. Similar to the previous approach, the SVM (with linear, polynomial, and RBF kernels), LR, RF, kNN, GB, XGB, and DT models were trained. All models were trained with their preset parameters using 10-fold cross-validation. The models with the highest AUC-ROC and accuracy on the test set were selected and hypertuned using the scikit-opt package [[25](#_bookmark41)]. The ROC curve and confusion matrix for the best model were reported.

# Results

## Preprocessing

The PCA plots of the samples are shown in Figures [1](#_bookmark1)A and B. Healthy samples were separated from the CAD or MI samples in the primary data and after conducting fRMA. In the RLE plot, there was a distinct difference between the dataset means for all samples before fRMA was performed (Figure [1](#_bookmark1)C). All datasets were rearranged to approximately 0 in the RLE plot after fRMA was conducted (Figure [1](#_bookmark1)D). Moreover,

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PC2

**A**

0.05

0.00

−0.05

−0.10

−0.15

0.064

0.068

PC1

**C**

1

0

−1

**D**

1

0

−1

**Fig. 1**

the relative log expression plots for (C) primary data and (D) the data after fRMA.

there was an apparent change in the interquantile distances, but the values were still

greater than 0.1.

**3.2 Differential expression analysis**

According to the cutoff criterion of *FDR <* 0*.*05, there were 860 DEGs between MI and healthy samples. Among them, 323 were up-regulated, and 537 were down- regulated in the MI group compared to the healthy group. In the CAD and healthy group comparison, we found 670 DEGs, of which 262 and 408 DEGs were up- and down-regulated, respectively, in CAD samples. In the MI and CAD groups, the number of DEGs was 260, and the numbers of up- and down-regulated genes in MI samples

**B**

CAD

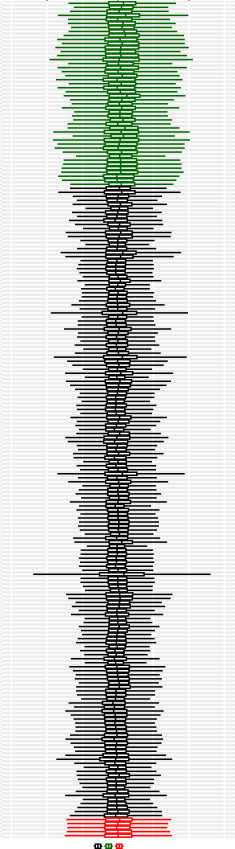
Healthy MI

**Dataset**

GSE54475 GSE56609 GSE59867

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Principal component analysis plots for (A) primary data and (B) the data after fRMA, and

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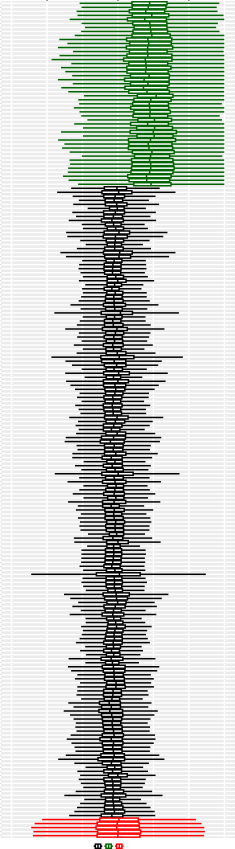
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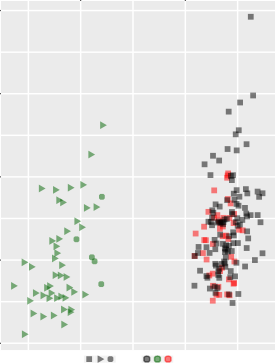
**75.GSM1316373.H**

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**Dataset**

GSE54475 GSE56609 GSE59867

PC2

0.0

CAD

Healthy MI

**Dataset**

GSE54475 GSE56609 GSE59867

0.072

0.076

0.0625

0.0650

0.0675

PC1

0.0700

**Table 2** Total, up-, and down-regulated DEGs and differentially expressed miRNAs.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Total DEGs | Up-regulated DEGs | Down-regulated DEGs | miRNAs |
| MI vs. Healthy | 860 | 323 | 537 | miR-186, miR-21, miR-32 |
| CAD vs. Healthy | 670 | 262 | 408 | miR-186, miR-21, miR-32 |
| MI vs. CAD | 260 | 144 | 116 | miR-186 |

CAD/Healthy MI/Healthy

59

489

238

42

80

91

47

MI/CAD

**Fig. 2** Venn diagram for DEGs in CAD/Healthy, MI/Healthy, and MI/CAD.

were 144 and 116, respectively, compared to CAD samples. The data are summarized in Table [2](#_bookmark2).

The Venn diagram in Figure [2](#_bookmark3) shows that the CAD and MI samples shared most of their DEGs. From 860 DEGs of MI/healthy and 670 DEGs of CAD/healthy, 531 genes were common, which is 62% of MI/healthy DEGs and 79% of CAD/healthy DEGs.

## GO and KEGG enrichment analyses of the DEGs

To explore the biological classification of the DEGs, we performed GO and KEGG pathway enrichment analyses on the MI/healthy and CAD/healthy DEGs. For MI/healthy, GO enrichment analysis in the BP category suggested that the DEGs were enriched in “immune response-regulating signaling pathway,” “lymphocyte differ- entiation,” “immune response-regulating cell surface receptor signaling pathway,” and “leukocyte activation involved in immune response” (Figure [3](#_bookmark4)A). In the CC category, DEGs were enriched in “secretory granule membrane,” “azurophil granule,” “ficolin-1- rich granule,” “tertiary granule,” and “ficolin-1-rich granule membrane” (Figure [3](#_bookmark4)B). In the MF category, DEGs were involved in “cadherin binding” and “MHC class I protein binding” (Figure [3](#_bookmark4)C). KEGG pathway analysis indicated that the DEGs were related to the following pathways: “Chemokine signaling pathway,” “Lipid and atherosclerosis,” and “Hematopoietic cell lineage” (Figure [3](#_bookmark4)D).

8

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immune response−regulating

signaling pathway

regulation of cell−cell

adhesion

positive regulation of cytokine production

negative regulation of immune system process



**Count**

10

15

20

**B**

cell−substrate junction

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focal adhesion membrane raft membrane microdomain



**Count**

10

15

20

lymphocyte differentiation

activation of immune response

cell activation involved in immune response

positive regulation of binding

platelet activation response to antibiotic

**p.adjust**

1e−04



8e−05

6e−05

4e−05

secretory granule membrane ficolin−1−rich granu primary lysosome azurophil granule endopeptidase complex proteasome complex

**p.adjust**

0.0125



0.0100

0.0075

0.0050

0.0025

**C**

transcription coregulator

activity GTPase regulator activity

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0.02 0.03 0.04 0.05 0.06

**GeneRatio**

**D**

Huntington disease Parkinson disease

0.02 0.03 0.04 0.05

**GeneRatio**

nucleoside−triphosphatase

regulator activity cadherin binding

disordered domain specific

binding

core promoter sequence−specific DNA binding

protein folding chaperone transcription corepressor

binding

purinergic nucleotide receptor activity

nucleotide receptor activity

0.01

0.02

0.03



0.04

0.05

**Count**

10

15

20

**p.adjust**



0.024

0.022

0.020

0.018

Chemokine signaling pathway

Transcriptional misregulation in cancer

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Lipid and atherosclerosis

Protein processing in endoplasmic reticulum

Thyroid hormone signaling

pathway Chronic myeloid leukemia

Primary immunodeficiency Endocrine and other

factor−regulated calcium

reabsorption

0.03 0.04 0.05 0.06 0.07

**Count**

10

15

20

**p.adjust**

0.015



0.010

0.005

**GeneRatio GeneRatio**

**Fig. 3** Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enriched with the MI and healthy DEGs. (A) Biological process terms. (B) Cellular component terms.

(C) Molecular function terms. (D) KEGG analysis.

The enrichment results for the CAD/healthy DEGs were as follows. In the BP cat- egory, GO enrichment suggested that the DEGs were enriched in “positive regulation of defense response,” “positive regulation of innate immune response,” “mononuclear cell differentiation,” and “positive regulation of response to external stimulus” (Figure [4](#_bookmark5)A). In the CC category, DEGs were enriched in “azurophil granule,” “ficolin-1-rich granule,” and “ficolin-1-rich granule membrane” (Figure [4](#_bookmark5)B). In the MF category, DEGs were involved in “lipoprotein particle receptor binding” and “NF-*κ*B binding” (Figure [4](#_bookmark5)C). KEGG pathway analysis showed that the DEGs were related to the fol- lowing pathways: “Chemokine signaling pathway,” “Lipid and atherosclerosis,” and “Hematopoietic cell lineage” (Figure [4](#_bookmark5)D).

9

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positive regulation of

**B**

 cell−substrate junction



response to external stimulus

negative regulation of immune system process

regulation of cell−cell

adhesion

mononuclear cell differentiation

regulation of apoptotic signaling pathway

cellular response to chemical stress

positive regulation of defense response

myeloid leukocyte activation negative regulation of

cell−cell adhesion

positive regulation of innate immune response

0.03

0.04

0.05

0.06

**Count**

10

15

20

**p.adjust**

0.005



0.004

0.003

0.002

focal adhesion vacuolar membrane

ficolin−1−rich granule primary lysosome azurophil granule peptidase complex

endopeptidase complex

proteasome complex ficolin−1−rich granule

membrane

0.02

0.03

0.04

0.05

**Count**

10

15

20

**p.adjust**

0.0125



0.0100

0.0075

0.0050

0.0025

**C**

transcription coregulator

activity

**GeneRatio**



**D**

Huntington disease Lipid and atherosclerosis



**GeneRatio**

transcription corepressor

|  |  |  |  |  |  |  |  |  |  |
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activity

protein folding chaperone

lipoprotein particle receptor binding

NF−kappaB binding

0.01 0.02 0.03 0.04 0.05 0.06

**GeneRatio**

**Count**

15



18

21

24

**p.adjust**



0.040

0.038

0.036

Parkinson disease

Chemokine signaling pathway Human cytomegalovirus

infection Yersinia infection

Hematopoietic cell lineage Endocrine and other

factor−regulated calcium

reabsorption Proteasome

Legionellosis

0.03 0.04 0.05 0.06 0.07 0.08

**GeneRatio**

**Count**

10

15

20

**p.adjust**

0.025



0.020

0.015

0.010

**Fig. 4** Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enriched with the CAD and healthy DEGs. (A) Biological process terms. (B) Cellular component terms. (C) Molecular function terms. (D) KEGG analysis.

## Machine Learning

### miRNAs in DEGs

Among the DEGs, miR-186, miR-32, and miR-21 were identified as differentially expressed miRNAs. The expression profiles of the three miRNAs are shown in Figure [5](#_bookmark7). The ROC curves of each miRNA in each layer are presented in Figure [6](#_bookmark8). Using the logistic regression model, the AUC-ROC values of miR-21, miR-32, and miR-186 for separating healthy and not-healthy samples were 0.98, 0.99, and 0.90, respectively (Figure [6](#_bookmark8)A). The accuracy of each miRNA for classifying the samples into healthy and not-healthy groups on the test set for miR-21, miR-32, and miR-186 was 0.92, 0.98, and 0.89, respectively. The ROC curve of each miRNA for classifying MI and CAD samples is presented in Figure [6](#_bookmark8)B. The AUC-ROC and accuracy for miR-21, miR- 32, and miR-186 in the test set were 0.85; 0.70; and 0.86, and 0.78; 0.67; and 0.74, respectively.

10

**Table 3** Investigated miRNAs log fold-change and adjusted p-values for CAD samples relative to healthy, MI samples relative to healthy, and MI samples relative to CAD.

CAD/Healthy MI/Healthy MI/CAD

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | logFC | adj. p-value |  | logFC | adj. p-value |  | logFC | adj. p-value |  |
| miR-186 | 1.4 | 3.60e-25 |  | 0.9 | 6.76e-20 |  | -0.5 | 1.05e-09 |  |
| miR-21 | 1.4 | 1.31e-17 |  | 2.3 | 2.07e-47 |  | 0.8 | 2.96e-11 |  |
| miR-32 | 2.5 | 8.39e-43 |  | 2.2 | 3.10e-59 |  | -0.3 | 7.60e-04 |  |
| miR-197 | 0.5 | 2.95e-20 |  | 0.7 | 1.59e-47 |  | 0.2 | 8.58e-09 |  |
| miR-29A | 0.7 | 7.76e-29 |  | 0.1 | 1.70e-01 |  | -0.5 | 2.14e-10 |  |
| miR-296 | -0.1 | 5.00e-02 |  | 0.1 | 2.00e-02 |  | 0.2 | 6.15e-06 |  |

12

Healthy

CAD MI

10



Expression Value (log2)

8

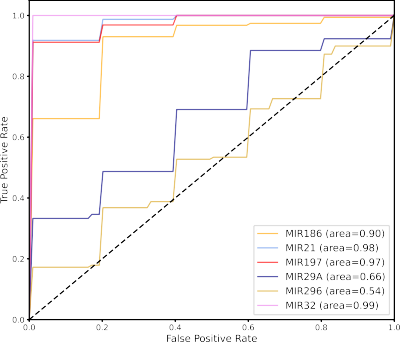
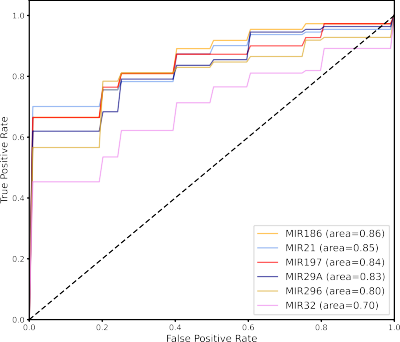


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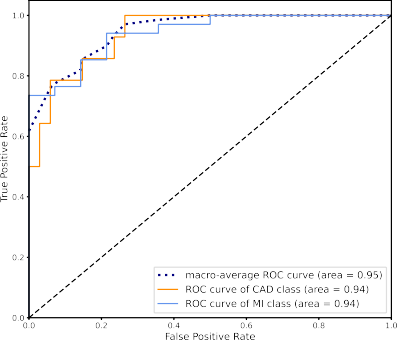
MIR186 MIR21 MIR197 MIR29A MIR296 MIR32

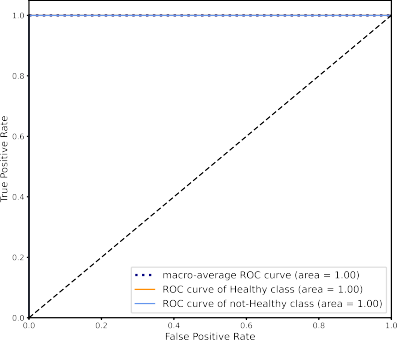
**Fig. 5** Expression profile of all miRNAs in two approaches in different sample classes.

A B

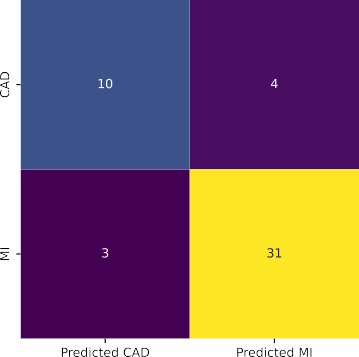
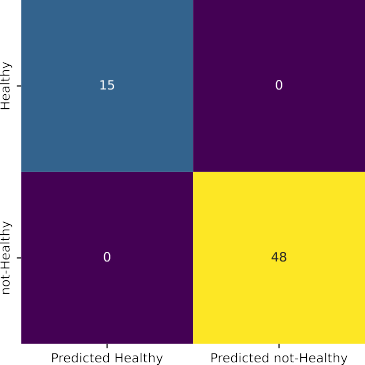
**Fig. 6** ROC curve for single miRNAs on test set classification for (A) healthy and not-healthy samples and (B) CAD and MI samples.

11

A B



**Fig. 7** ROC curve for the model trained with miRNAs in DEGs on test set classification; (A) An SVM model with RBF kernel for healthy and not-healthy and (B) An SVM model with linear kernel for CAD and MI sample classification.

A B

**Fig. 8** Confusion matrix for the model trained with miRNAs in DEGs on test set classification; (A) An SVM model with RBF kernel for healthy and not-healthy and (B) An SVM model with linear kernel for CAD and MI sample classification.

#### First layer for the isolation of healthy and not-healthy samples:

Although single miRNAs had an acceptable performance for this layer, their predictive value could be further improved by using them as a set. The ROC curve for the SVM model with an RBF kernel trained with all three miRNAs is presented in Figure [7](#_bookmark9)A. The model had a better performance in classification than single miRNAs.The AUC- ROC for the model was 1, and its accuracy on the test set was also 1. In Figure [8](#_bookmark10)A, the confusion matrix for the model is presented.

#### Second layer for separating the MI and CAD samples:

Different models were trained using the expression values of three differentially expressed miRNAs. The models’ AUC-ROC and the accuracy of the test set are shown in Figure [9](#_bookmark11). The best model from both the AUC-ROC and accuracy points of view

12

0.95

Test Set Accuracy

Test Set AUC

0.90

0.85

0.80

Accuracy/AUC

0.75

0.70

0.65

0.60

**Fig. 9** Area under the receiver operating characteristic curve and accuracy of different models trained with three miRNAs in DEGs.

**Table 4** AUC-ROC and accuracy for SVM with a linear kernel as the best model trained with differentially expressed miRNAs on the training and test sets before and after hypertuning

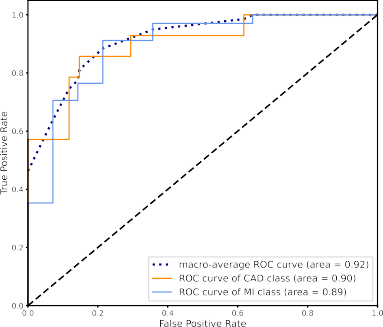
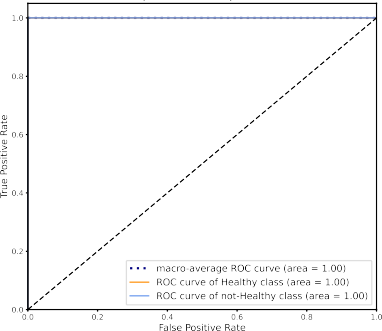
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| --- | --- | --- | --- | --- | --- | --- |
|  | | Preset | parameters |  | Hypertuned |  |
| Model | Metrics | train | test |  | train test |
| SVM-linear | AUC-ROC | 0.91 | 0.93 |  | 0.92 0.95 |  |
|  | Accuracy | 0.83 | 0.82 |  | 0.84 0.85 |  |

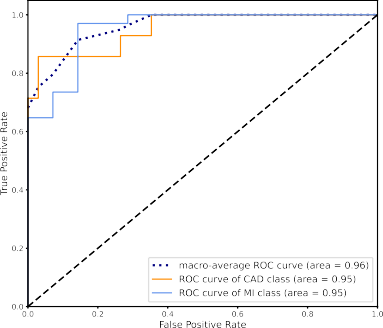
was the SVM model with a linear kernel. The AUC-ROC and accuracy for this model with its preset values were 0.93 and 0.82, respectively. The model was hypertuned for C and gamma hyperparameters, and therefore the model showed better perfor- mance. The ROC curve of the hypertuned model is presented in Figure [7](#_bookmark9)B. For this model, the AUC-ROC reached 0.95, and the accuracy was improved to 0.85 (Table [4](#_bookmark12)). Moreover, the sensitivity and specificity for the model on the test set were 0.91 and 0.71, respectively. The confusion matrix for the hypertuned model is illustrated in Figure [8](#_bookmark10)B.

### AUC-ROC approach

After calculating the AUC-ROC for each miRNA to classify of MI and CAD samples, the miRNAs with AUC-ROC *>* 0*.*8 were selected. The miRNAs selected were miR- 29a, miR-197, miR-186, miR-21, and miR-296. The expression levels of these miRNAs in healthy, CAD, and MI samples are presented in Figure [5](#_bookmark7). The ROC curves of the selected miRNAs in both layers are shown in Figure [6](#_bookmark8).

13

A B

C

**Fig. 10** ROC curve for models trained with the set of miRNAs selected by AUC-ROC on test set classification; (A) SVM with RBF kernel for healthy and not-healthy samples classification. (B) Logistic regression model for CAD and MI sample classification. (C) SVM with polynomial kernel for CAD and MI sample classification.

#### First layer for the isolation of healthy and not-healthy samples:

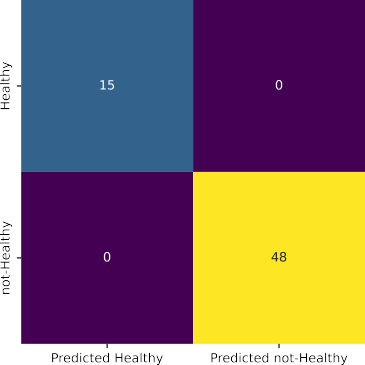
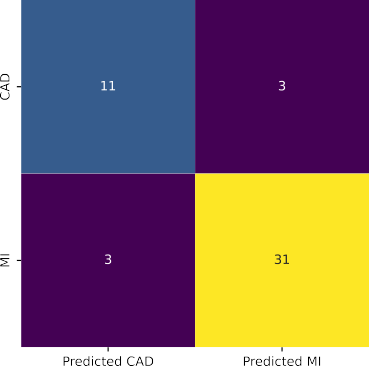
Using the selected set, an SVM model with an RBF kernel was trained to sepa- rate healthy and not-healthy samples. The ROC curve for the model is presented in Figure [10](#_bookmark13)A, and the confusion matrix is illustrated in Figure [11](#_bookmark14)A. Both the AUC-ROC and accuracy of the model on the test set were 1.

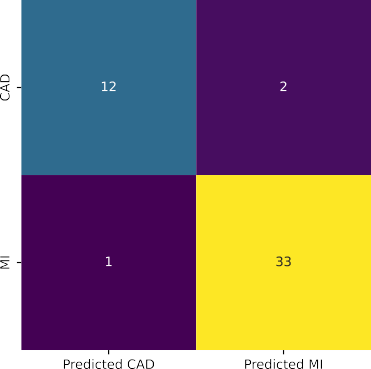
#### Second layer for separating the MI and CAD samples:

To find the best model for this set of miRNAs, different models were trained using their preset values. The AUC-ROC and accuracy results for the test set are presented in Figure [12](#_bookmark15). The best model from the AUC-ROC point of view was the SVM with a linear kernel, and from the accuracy point of view, it was the SVM model with an RBF kernel. For the SVM-linear model, the AUC-ROC and accuracy were 0.93 and 0.82, respectively; and for the SVM-RBF, the values were 0.92 and 0.84, respectively. Both models were hyper-tuned, and the ROC curve for their best performance is presented in Figure [10](#_bookmark13)B and C. The AUC-ROC and accuracy for the SVM-linear model were

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A B

C

**Fig. 11** Confusion matrix for models trained with the set of miRNAs selected by AUC-ROC on test set classification; (A) SVM with RBF kernel for healthy and not-healthy samples classification. (B) Logistic regression model for CAD and MI sample classification. (C) SVM with polynomial kernel for CAD and MI sample classification.

modified to 0.92 and 0.88, respectively. For the SVM-RBF, these values increased to

0.96 and 0.94, respectively (Table [5](#_bookmark16)). The sensitivities for the SVM-linear and SVM- RBF models were 0.91 and 0.97, respectively; and their specificities were 0.79 and 0.86, respectively. The confusion matrix for both models is illustrated in Figure [11](#_bookmark14)B and C.

# Discussion

The prevalence of MI can lead to high mortality rates in the clinical setting. How- ever, early diagnosis and the application of suitable treatment protocols can reduce mortality and improve MI prognosis ([[1](#_bookmark17), [3](#_bookmark19), [26](#_bookmark42)]). Studies have suggested that changes in miRNA expression may play a significant role in the progression of MI and the subsequent remodeling [[27](#_bookmark43)]. It is believed that miRNA expression is altered during the various biological processes correlated with MI within the myocardium or other related tissues [[28](#_bookmark44)]. Although several studies have focused on examining free circu- lating miRNAs in serum samples for the detection of cardiac tissue injuries [[29](#_bookmark45)],

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0.95

Test Set Accuracy

Test Set AUC

0.90

0.85

0.80

Accuracy/AUC

0.75

0.70

0.65

0.60

**Fig. 12** Area under the receiver operating characteristic curve and accuracy of different models trained with AUC-selected miRNAs.

**Table 5** AUC-ROC and accuracy for SVM with the linear and RBF kernels as the best models trained with miRNAs selected based on their AUC-ROC on the train and test sets before and after hypertuning

Preset parameters Hypertuned

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Model | Metrics | Train | Test |  | Train | Test |  |
| SVM-linear | AUC-ROC | 0.91 | 0.93 |  | 0.93 | 0.92 |  |
|  | Accuracy | 0.85 | 0.82 |  | 0.90 | 0.88 |  |
| SVM-RBF | AUC-ROC | 0.90 | 0.92 |  | 0.96 | 0.96 |  |
|  | Accuracy | 0.86 | 0.84 |  | 0.96 | 0.94 |  |

more information is needed to fully comprehend the miRNAs found in different blood subcomponents, such as plasma, platelets, and PBMCs. Based on previous findings, PBMCs play a crucial role in the destabilization and rupture of plaques as well as in the initial inflammatory reactions in individuals experiencing myocardial infarction (MI) [[9](#_bookmark25), [30](#_bookmark46)]. Moreover, PBMCs have specific miRNA profiles that are altered under certain pathological conditions, making them great candidates as disease biomarkers [[9](#_bookmark25)].

PBMCs can respond to several insulting conditions, such as MI, in the shortest possible time with notable changes in their miRNA profile [[9](#_bookmark25)]. Considering their reg- ulatory roles, subtle changes in the transcription of miRNAs can be monitored even before alterations in mRNA and protein levels [[4](#_bookmark20)]. These features make miRNAs a valid early-stage diagnostic tool for the detection of minor and major cell injuries. To date, few studies have compared the miRNA profiles in PBMCs from patients with

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MI and other CADs and healthy samples to find a robust set of identical miRNAs to differentiate these pathological conditions.

In this study, we combined three GEO datasets for healthy, CAD, and MI samples. Having these sample sets alongside bioinformatics analysis and ML methods enabled us to identify potential biomarker sets and effective therapeutic targets. The results of the DEG analysis (Table [2](#_bookmark2) and Figure [2](#_bookmark3)) prove the close relationship between the MI and CAD samples. Interestingly, functional enrichment analysis demonstrated that DEGs in both CAD/healthy and MI/healthy were strongly correlated with the immune cell response, which is a major part of PBMCs. Two sets of miRNAs were selected as biomarker sets for sample classification. miR-21; miR-32; and miR-186 were selected as differentially expressed miRNAs, and miR-186; miR-21; miR-29a; miR-197; and miR-296 were selected based on their AUC-ROC values. As shown in Figure [6](#_bookmark8), all miRNAs selected with both approaches had AUC-ROCs *>* 0*.*9 for isolating healthy and not-healthy samples except for miR-296 and miR-29a. The data confirmed that the real challenge was to classify CAD and MI samples because of the close overlap. Of the six miRNAs under investigation in both approaches, except for miR-32, all miRNAs had an AUC-ROC *>* 0*.*8 for the discrimination of CAD and MI samples. As expected, the high AUC-ROC values of the miRNAs confirmed their high potential as biomarkers.

ML models trained with miRNA sets selected by both DEG and AUC-ROC approaches, showed better classification performance than each miRNA. To avoid unwanted complexity and poor predictive values, a two-layer architecture was designed. The first layer was used to discriminate between healthy and not-healthy samples, and the second layer was was used to separate CAD from MI candidates. As expected, in both approaches, a hypertuned SVM model could flawlessly sepa- rate healthy and not-healthy samples using distinct miRNA sets. ML models are also capable of effectively separating CAD from MI patients. Although both miRNA sets had nearly the same AUC-ROC using the best model, their accuracy, sensitivity, and specificity were different. The model trained with AUC-selected miRNAs showed bet- ter performance in all predictive values, which is logical because of the higher number of miRNAs in the set.

Numerous studies have reported that different biological processes can affect the miRNA expression in PBMCs. However, the exact role of miRNAs in the function of immune cells and the correlation between specific pathological conditions and miRNA profiles remain controversial. Several studies have proven the activation of particular miRNA types in PBMCs under cardiovascular events [[31](#_bookmark47)]. For instance, there is evi- dence that elevation of miR-186 suppresses the expression of cystathionine-*γ*-lyase, leading to the subsequent secretion of pro-inflammatory cytokines and cellular lipid accumulation. In addition, macrophage-derived miR-186 may promote atherosclerotic plaque formation [[32](#_bookmark48)]. In line with this claim, we found that miR-186 was up-regulated in both CAD and MI candidates compared to their control counterparts. Surprisingly, the obtained data indicated that the expression of miR-186 was higher in patients with CAD than in patients with MI (Figure [5](#_bookmark7)). Specifically, miR-186 was the only differentially expressed miRNA between CAD and MI, with a clear up-regulation in CAD, indicating its main role in the promotion of atherosclerosis.

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As mentioned before, miR-21 was also up-regulated in both MI and CAD patients compared to healthy controls. Moreover, the expression value of miR-21 was signifi- cantly higher in the MI group than in the CAD group (Table [3](#_bookmark6)). It is thought that the up-regulation of miR-21 in PBMCs is a compensatory reaction to reduce the T*reg* lymphocyte number in response to the reduction in TGF*β*1 secretion into the plasma through a TGF*β*1/smad-independent pathway. In line with the previous and present data, miR-21 can modulate the activity of PBMCs following the occurrence of cardiovascular diseases [[33](#_bookmark49)].

Recent data have supported the elevation of miR-32 levels in CAD samples with calcification of the coronary artery. Notably, miR-32 promotes vascular smooth muscle calcification in mice by controlling the activity of several proteins, including bone morphogenetic protein-1, runt-related transcription factor-2 (RUNX2), osteopontin, and bone-specific phosphoprotein matrix GLA protein [[34](#_bookmark50)]. Likewise, some reports are associated with the activity of miR-32 in PBMCs in several pathologies [[35](#_bookmark51), [36](#_bookmark52)]. The exact role of miR-32 in PBMCs after cardiovascular events remains unclear.

Molecular analyses have indicated the regulatory role of miRNAs selected using the AUC-ROC approach in PBMCs after a cardiovascular event. The biological impor- tance of two common miRNAs in the DEGs and AUC-ROC approaches, miR-21 and miR-186, have already been discussed. Based on numerous reports, miR-29a can be activated in different diseases [[37](#_bookmark53)]. Data analysis indicated that miR-29a was signifi- cantly up-regulated in CAD patients compared to the healthy and MI groups (Table [3](#_bookmark6)). Increased miR-29a is associated with the progression of atherosclerosis, and the com- bination of miR-29a and ox-LDL has been suggested as a valid biomarker set for paraclinical classification [[38](#_bookmark54)]. However, the role of miR-29a in the function of PBMCs from patients with CAD has not been thoroughly examined.

The data indicated that miR-197 was significantly up-regulated in both the CAD/healthy and MI/healthy groups. Previous studies have demonstrated that miR- 197 may play a crucial role in controlling the anti-inflammatory response of IL-35 by influencing the secretion of cytokines that can either promote or suppress inflamma- tion, the ratio of M1/M2 macrophages, and the proliferation of T*reg* lymphocytes, which are responsible for suppressing immune responses [[39](#_bookmark55)]. Alongside our findings, it can be concluded that miR-197 could be a useful diagnostic tool for predicting adverse cardiovascular events.

The findings of this study demonstrate the potential of miR-296 as a biomarker with high discriminatory power to distinguish between samples from individuals with MI and CAD. MiR-296 has been identified as a key regulator in the development and advancement of atherosclerosis by controlling the expression of target genes associ- ated with various biological processes, including angiogenesis, cholesterol metabolism, inflammation, cellular proliferation, hypertension, and apoptosis [[31](#_bookmark47)]. In a previous study, miR-296 expression levels were found to be significantly increased in the PBMCs of CAD patients compared to healthy controls, suggesting its involvement in regulat- ing proinflammatory cytokines such as IL-6 and TNF-*α* [[40](#_bookmark56)]. These findings suggested that miR-296 may have a significant impact on the pathogenesis of atherosclerosis and could potentially serve as a diagnostic biomarker for CAD or MI.

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# Conclusion

In summary, we derived a set of miRNA biomarkers by comparing MI samples with both healthy and CAD samples. We found that the SVM model performed best in both the first layer, which separated healthy and unhealthy samples, and the second layer, which classified the MI/CAD samples. The set of miRNAs selected based on their AUC-ROC values performed better in the second layer. Overall, our two-layer structure achieved an accuracy of 0.96. This demonstrates the potential of combining bioinformatics and machine learning techniques to identify novel biomarkers and gain a deeper understanding of myocardial infarction.

# Abbreviations

**CVD**: Cardiovascular disease **MI**: Myocardial infarction **cTn**: Cardiac troponin **miRNA**: microRNA

**PBMC**: Peripheral blood mononuclear cell

**ML**: Machine learning

**GEO**: Gene Expression Omnibus **PCA**: Principal component analysis **RLE**: Relative log expression

**FDR**: False discovery rate

**DEG**: Differentially expressed gene

**KEGG**: Kyoto Encyclopedia of Genes and Genomes

**GO**: Gene Ontology **BP**: Biological process **CC**: Cellular component **MF**: Molecular function

**SVM**: Support vector machine

**LR**: Logistic regression **RF**: Random forests **kNN**: K-nearest neighbor **GB**: Gradient boosting **XGB**: XGBoost

**DT**: Decision tree

**AUC-ROC**: Area under the receiver operating characteristic curve

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# Declarations

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## Competing interests

The authors declare that they have no competing interests.

## Ethics approval

The study was approved by the research ethics committee of Tabriz University of Medical Sciences (approval ID: IR.TBZMED.VCR.REC.1399.388, date of approval: 2021/1/11).

## Consent for publication

All authors gave consent for the publication of the article.

## Availability of data and materials

The datasets generated and/or analysed during the current study are available in the Gene Expression Omnibus (GEO, <https://www.ncbi.nlm.nih.gov/geo/>), reference

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## Authors’ contributions

F.B. and K.K. conceived the idea and coordinated the project. M.S. researched, collected the data, performed the analyzes, assembled the results, and drafted the manuscript. Z.P. and M.E.contributed to data analyze. C¸ .B.A. was foreign supervisor and collaborator. F.B., R.R. and K.K. edited and revised the manuscript. F.B. and

K.K. are author responsible for contact and ensures communication.

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