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Machine Learning-Based Prediction of the Genetic Background of Colorectal Liver Metastasis Using Mirna Expression Data

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Abstract

Aim: Understanding the underlying mechanisms in colorectal cancer, especially in the case of liver metastasis, investigating sensitive and specific molecules associated with metastasis is very important to improve patient clinical outcomes. This study aims to classify open-access microRNA data of patients with colorectal cancer liver metastasis and without liver metastases using the XGBoost method, one of the machine learning methods, and reveal important genes that may cause liver metastases.

Methods: This retrospective study considered the open-access microRNA expression data of patients with colorectal cancer liver metastasis and without liver metastases. The dataset consists of miRNA data from 10 CRC tissues from patients with liver metastases and 10 CRC tissues from patients without liver metastases. XGBoost was constructed for the classification *via* ten-fold cross-validation. Accuracy, balanced accuracy, sensitivity, specificity, positive predictive value, and negative predictive value performance metrics were evaluated for model performance.

Results: With respect to the feature selection method, 23 microRNA were selected, and modeling was performed with these input variables. Accuracy, balanced accuracy, sensitivity, specificity, positive predictive value, negative predictive value, and F1 score obtained from the XGBoost model were 95%, 95%, 90%, 100%, 100%, 90.9%, and 94.7%, respectively. Based on the variable importance findings acquired from the XGBoost, microRNA 4306, microRNA 2115, and microRNA 4708 can be employed as potential biomarkers for liver metastases.

Conclusion: As a result of the study, genes that could be biomarkers for colorectal cancer liver metastases were identified using a machine learning-based prediction approach. Following clinical validation of the obtained microRNAs in subsequent research, therapeutic procedures based on this microRNA scan be established, and their usefulness in clinical practice can be documented.

Keywords: Colorectal cancer; Liver metastases; Machine learning; Biomarker

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Introduction

The fourth most common cause of cancer-related deaths worldwide is Colorectal Cancer (CRC), one of the three most prevalent cancers [1]. 1.9 million new cases of CRC were diagnosed in 2020, while 935,000 CRC patients died [2]. The worldwide incidence of CRC has been rising at a 3.2% yearly rate, starting with 783,000 cases in 1999 and rising to 1.8 million by 2020. This trend is expected to persist [2-6]. The improvement of diagnostic procedures and treatment modalities, such as surgery, adjuvant chemotherapies, and palliative medicines, has resulted in a decline in CRC mortality rates in recent decades. After curative treatment, the metastasis of colorectal cancer is still a big problem, and it is the leading cause of mortality due to CRC. Tumor metastasis remains the most significant barrier to CRC treatment and prognosis. The most prevalent site of distant spread is liver metastasis, and around 15% to 25% of CRC patients will have distant metastases at the time of original diagnosis [7,8]. Liver metastasis of CRC may be related to factors such as the presence of the portal vein system, which directly connects the colorectal and liver and is associated with abundant blood supply, the location of the primary tumor and its histological type [9,10].

Curative resection and chemotherapy are standard treatment modalities in patients with Colorectal Cancer Liver Metastasis (CCLM) [11]. However, due to factors such as the location and

size of the tumor, the presence of extrahepatic disease, unresectable disease, or comorbidities of the patients, the surgical treatment option can be applied only in 10% to 20% of the cases, and the 5-year survival rate is very low [12,13]. Those who are unsuitable for surgery also have a worse prognosis. It is, therefore, necessary to find more effective targeted therapies for CCLM. Therefore, understanding the mechanisms underlying CRC and investigating the highly sensitive and specific molecules associated with metastasis, particularly during colorectal liver metastasis, is crucial to improve patient's clinical outcomes and overall survival [14]. In this process, it is very important to understand the molecular mechanisms and to carry out purposeful studies. Although many previous studies have identified various molecules involved in the initiation and development of colorectal liver metastases, the mechanisms still remain unclear [15].

There is still no holistic understanding of the process of carcinogenesis and metastasis in CRC, and studies for the causes of metastasis have focused on limited molecular markers. Next-generation sequencing, a high-throughput technology, is widely used to detect complex genetic changes to gain a more comprehensive understanding of the molecular pathophysiology of diseases and to identify various biomarkers to determine prognosis and treatment efficacy. Thanks to these genomic technologies used, molecular mechanisms related to diseases can be illuminated, and genes, RNAs, etc., that may be disease-related biomarkers can be detected [16].

Aiming to develop computer algorithms that can be developed with experience, machine learning aims to provide descriptive information to researchers in the analysis of large, complex datasets of computers. When exposed to new data, machine learning learns based on previous data and makes predictions about new data. In the past ten years, thanks to the availability of big datasets and increased processing power, machine learning approaches have achieved outstanding performance in a number of research. Therefore, machine learning is perhaps most useful for analyzing and interpreting large genomic datasets and may be used to describe a wide variety of genomic sequence elements [17,18].

In this study, in order to identify microRNAs that may be biomarkers for CCLM; by making bioinformatic analyzes from an open-access microRNA dataset belonging to CCLM is aimed to model the data set formed by overexpressed RNAs with XGBoost, which is a machine learning model, and to determine the possible biomarker RNAs with the variable importance values obtained.

Material and Methods

Data set and variables

This current research was conducted using data from a case-control study published by Qiaoming Zhi et al. [14]. The dataset consists of miRNA data from 10 CRC tissues from patients with liver metastases and 10 CRC tissues from patients without liver metastases. In the current study, this dataset was used to identify miRNAs differentially expressed between the two groups by bioinformatic analysis and to identify miRNAs that could be biomarkers using the machine learning method.

Bioinformatics analysis phase

For CCLM, which was examined for miRNA expression profiles, differential expression analyses were performed using the limma package available in the R programming language [19]. Differential expression analysis is known as the statistical analysis of normalized read count data to find the quantitative differences present in expression activities in different group situations. As a result of the analysis, a gene table in order of importance and a volcano plot to visualize differentially expressed genes are obtained. Log2FC>1 was used to identify upregulated genes, and log2FC<-1 to identify downregulated genes in the analyzes performed [20]. Up-regulated miRNAs are shown in red, down-regulated miRNAs are shown in blue in the volcano plot used to visualize genes with differential regulation, and miRNAs that do not show any change in expression between the 2 groups are shown in black.

Modelling phase

The modeling phase starts with variable selection. In the bioinformatics analysis, LASSO variable selection method was used in the variable selection stage made from differently expressed miRNAs. The variable selection phase is one of the most important steps in any predictive modeling project. Basically, variable selection is the process of deciding which data/variables to include in the study while developing a statistical model. Variable selection is a feature identification process on a data set that has an effect on the dependent variable. The high dimensionality of the explanatory variables that have an effect on the dependent variable can lead to both long computation times and over-learning of the data. For this reason, before performing statistical modeling, the variables with the most important features, that is, the variables that explain the model the most, should be selected [21]. The datasets obtained by gene expression also have very large volumes. Therefore, variable selection methods are applied before modeling with these datasets. The LASSO variable selection method used in this study imposes a constraint on the sum of the absolute values of the model parameters, and this sum must be less than a fixed value (upper limit). To achieve this, the method uses a throttling (narrowing-regulating) operation that penalizes the coefficients of the regression variables and causes some of them to drop to zero [22]. Modeling was done with the data set obtained by the variable selection. In the modeling phase, XGBoost, which is a tree-based method from machine learning methods, was used. XGBoost is a machine learning method based on gradient boosting and decision tree methods and has found application in many areas. This method, which has a very high predictive power compared to other algorithms, is 10 times faster than other methods and includes a series of regularizations that can improve the overall performance of the model and reduce overfitting and overlearning [23].

In order to ensure model validity, the n-fold cross-validation method, one of the resampling methods, was used in this study. In this method;

- First, the dataset is divided into n pieces, and the model is applied to n pieces.
- In the second step, one of the n parts is used for the testing process, while the other n-1 parts are used in the training process.
- In the last stage, the average of the values obtained from the models is evaluated for the cross-validation method.

Accuracy, balanced accuracy, sensitivity, specificity, positive predictive value, negative predictive value, and F1-score metrics were used to evaluate the performance obtained as a result of the modeling. Finally, in order to identify miRNAs that could be biomarkers, variable importance values were calculated, giving information about how much the input variables explain the output variable.

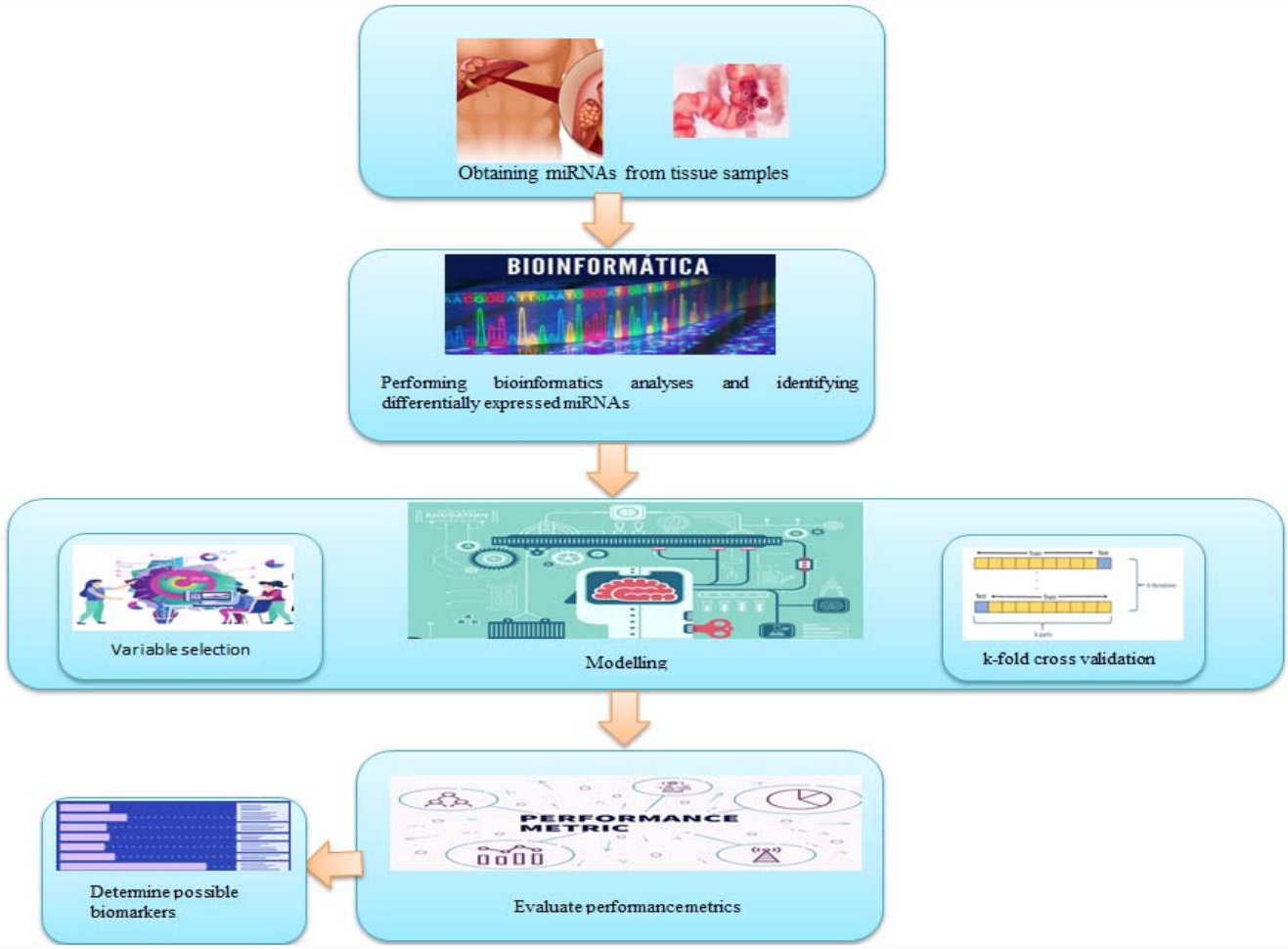

Figure 1: The process flow diagram of the used models.

Biostatistical analysis phase

Shapiro Wilk test of normality was employed to determine whether the variables had a normal distribution. Data were summarized as mean ± standard deviation. Independent samples t-test was employed to compare data. p-value <0.05 was considered statistically significant. IBM SPSS Statistics 25.0 program was used in the analysis.

The process flow diagram

The process flow diagram of the used models is also shown in Figure 1 for a better understanding of the system.

Results

There are 9 female and 11 male patients in the current study. The mean age of the patients was 63.4 ± 11.51. While the mean age of patients without liver metastasis is 64.9 ± 5.85, the mean age of patients with metastasis is 61.9 ± 15.50 years.

When the data obtained as a result of the bioinformatics analysis were examined, 72 microRNAs with down- and 24 up-regulation were found. According to the table in which the microRNAs with the smallest adjacent P. value are listed, 4 of the first 10 microRNAs were downregulated, 5 of them were upregulated, while 1 of them did not. The results obtained according to the bioinformatics analysis are tabulated in Table 1.

The volcano plot, where we can see all differentially expressed genes, is as follows (Figure 2).

According to the results of the biostatistical analysis performed with 23 microRNAs selected by the variable selection method, statistically significant differences were obtained between the groups with and without liver metastasis with CRC. Mentioned 23 microRNAs show differences for 2 groups. Biostatistical analysis results are given in Table 2.

The values of the performance metrics obtained as a result of modeling with the XGBoost method are as follows (Table 3).

The graphical presentation of the metrics is as follows (Figure 3).

According to the variable significance values obtained as a result of the modeling, the 3 microRNAs were the most differentiated microRNAs among the 2 groups. The graphic showing of these microRNAs according to the obtained variable importance values is given in Figure 4.

Discussion

CRC is still frequent cancer. Most cancers, including CRC, may have a single clonal origin in the early stages of the disease. Still, a malignant tumor contains multiple populations of cells with distinct characteristics such as growth rate, karyotype, immunogenicity, drug susceptibility, and ability to expand. And develop metastases.

Table 1: The results of the bioinformatics analysis.

ID	adj. P. Val	P. Value	t	B	Lo2FC	miRNA_ID	Official Full Name	Diff expressed
169028	0.0472	7.38E-05	48,14,448	1.627	13,54,761	hsa-miR-4708-3p	microRNA 4708	up
147722	0.0472	0.000147	45,37,742	1.022	0,854155	hsa-miR-4306	microRNA 4306	no
145798	0.0472	0.000156	-4,51,324	0.969	-1,47,049	hsa-miR-142-5p	microRNA 142	down
168802	0.0472	0.000168	44,85,198	0.907	14,91,169	hsa-miR-4516	microRNA 4516	up
42934	0.0472	0.000178	-4,46,127	0.855	-1,28,299	hsa-miR-345-5p	microRNA 345	down
42614	0.057	0.000258	-4,31,308	0.529	-13,399			down
169010	0.1276	0.000738	-3,89,021	-0.397	-13,389	hsa-miR-2681-3p	microRNA 2681	down
147834	0.1276	0.000846	-3,83,515	-0.517	-1,19,189			down
148143	0.1276	0.000866	-3,82,554	-0.538	-1,10,441			down
145950	0.1589	0.001209	-3,69,041	-0.831	-2,35,191	hsa-miR-33b-5p	microRNA 33b	down

Table 2: The results of the biostatistical analysis.

ID	Group		
	With liver metastasis	without liver metastasis	
	Mean ± Sd	Mean ± Sd	p*
11053	1.511 ± 1.065	0.564 ± 0.481	0.024
13132	1.134 ± 0.496	1.794 ± 0.578	0.013
145798	10.71 ± 5.798	3.69 ± 1.644	0.004
146089	0.089 ± 0.037	0.039 ± 0.023	0.002
146161	1.221 ± 0.356	0.701 ± 0.126	0.001
147722	1.202 ± 0.185	2.184 ± 0.416	<0.001
147793	0.239 ± 0.15	0.08 ± 0.054	0.009
147834	0.325 ± 0.129	0.15 ± 0.088	0.002
147862	0.084 ± 0.031	0.025 ± 0.016	<0.001
148143	0.208 ± 0.078	0.1 ± 0.051	0.002
148241	0.196 ± 0.06	0.096 ± 0.042	<0.001
148570	0.075 ± 0.062	0.22 ± 0.133	0.006
148690	0.617 ± 0.188	1.116 ± 0.522	0.011
169028	1.977 ± 0.536	5.445 ± 2.912	0.004
169274	0.558 ± 0.144	0.404 ± 0.122	0.019
169312	0.771 ± 0.134	0.486 ± 0.17	0.001
17503	0.311 ± 0.188	0.126 ± 0.106	0.017
17537	0.61 ± 0.161	0.918 ± 0.266	0.006
17626	0.173 ± 0.097	0.064 ± 0.032	0.006
27558	0.254 ± 0.137	0.124 ± 0.122	0.038
32608	0.321 ± 0.153	0.144 ± 0.058	0.003
42614	0.386 ± 0.162	0.156 ± 0.07	0.001
46514	5.095 ± 1.422	9.977 ± 4.508	0.008

Sd: Standard deviation; *: Independent samples t test

Table 3: Metrics obtained as a result of the XGBoost model.

Metrics	Value (%) (95% CI)
Accuracy	95 (85.1-100)
Balanced Accuracy	95 (85.1-100)
Sensitivity	90 (55.5-99.1)
Specificity	100 (69.2-100)
Positive predictive value	100 (66.4-100)
Negative predictive value	90.9 (58.7-99.8)
F1 score	94.7 (85-100)

Liver metastasis is the leading cause of mortality in CRC patients. The treatment of advanced CCLM remains a significant challenge [15,24]. Recent advancements in diagnosis and therapy have allowed clinicians to save many patients' lives in the early stages of the disease, but the prognosis for patients with advanced disease or systemic metastases remains very dismal [16]. The major obstacle to the effective treatment of CRC is metastasis, with liver metastasis being the most common cause of death. Therefore, many researchers have tried to understand the genetic characteristics of metastatic clones, since clones with metastatic potential are genetically different from non-metastatic clones. And it aimed to create treatments in this direction. In many studies, KRAS2, p53, p21/WAF/CEP1, CD44, COX-2, cytokeratin-19, proliferating cell nuclear antigen, MMP-9, cyclin D1, VEGF-C, and E-cadherin have been identified as biomarkers for CRC [25-27]. Although these biomarkers for CRC have been identified, there is still no fully established understanding of the metastasis process. Because studies have focused on limited molecular markers [16].

Although these biomarkers for CRC have been identified, there is still no fully established understanding of the metastasis process. Because studies have focused on limited molecular markers. Therefore, more comprehensive studies on molecular pathophysiology are needed to gain a more comprehensive understanding of the metastasis status and to determine the prognosis and treatment efficacy [16]. Detecting complex genetic changes will be useful for identifying various biomarkers. With these identified markers, it will be possible to search for highly sensitive and specific molecules associated with metastasis, especially during colorectal liver metastasis. In fact, these studies are crucial for improving patients' clinical outcomes and even survival rates.

The current study aimed to find biomarkers based on microRNAs by using machine learning techniques for CCLM and aimed to support researchers in developing treatment by revealing the genetic mechanism.

According to the bioinformatic analyzes, it was determined that 72 microRNAs showed different regulation in the CCLM state compared to the CCR. Of these, microRNA 4708, microRNA 4306, microRNA 142, microRNA 4516, microRNA 345, id=42614, microRNA 2681, id=147834, id=148143, microRNA 33b are the microRNAs with the smallest adj p-value values and the most differentiated. When the log2FC values obtained were examined, microRNA 4708 and microRNA 4516 in CCLM had 2.54- and 2.80-fold higher gene

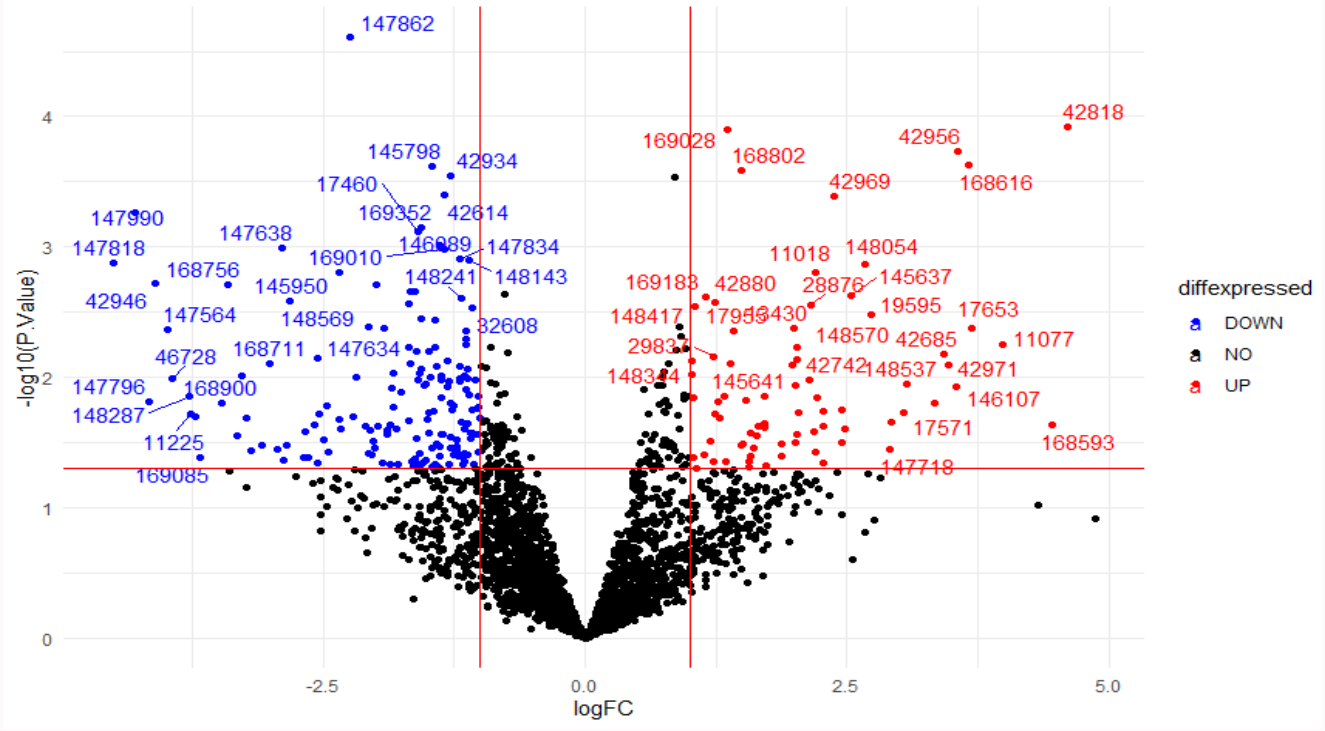

Figure 2: The volcano plot.

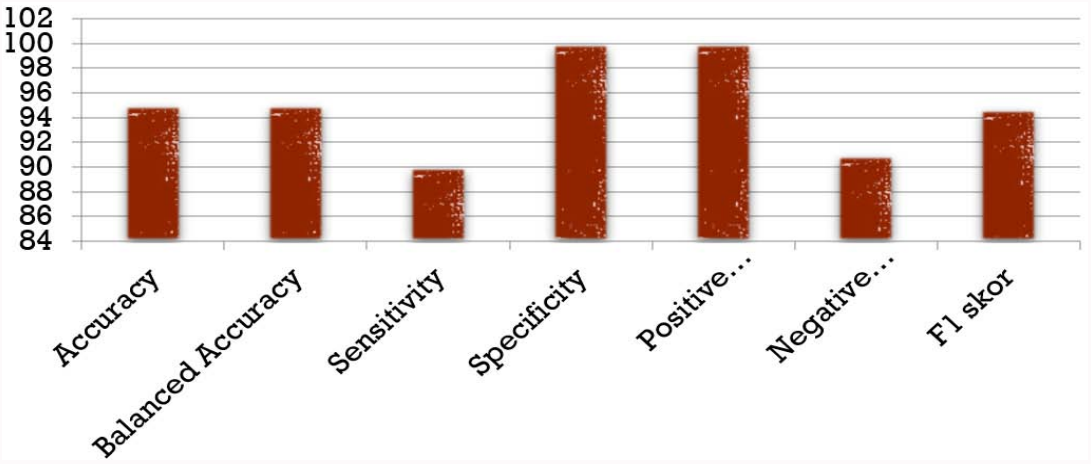

Figure 3: Presentation of the metrics.

expression than those with CRC, respectively. When the log2FC values obtained were examined, microRNA 4306, microRNA 345, id=42614, microRNA 2681, id=147834, id=148143, microRNA 33b microRNAs in CCLM had 2.77, 2.42, 2.51, 2.51, 2.28, 2.14 and 5.09 fold lower gene expression than those with CRC, respectively. microRNA 4306 had the same expression between the two groups.

Statistically significant differences were obtained between the groups with and without CCLM according to the biostatistical analyzes performed with the remaining 23 microRNAs with the choice of the variable. These selected microRNAs are microRNAs that differentiate between the 2 groups and have a distinctive feature between the 2 groups. The performance criteria values obtained by modeling 23 microRNAs with the machine learning method XGBoost were obtained as accuracy 95%, balanced accuracy 95%, Sensitivity 90%, Specificity 100%, Positive predictive value 100%, Negative predictive value 90.9%, F1 score 94.7%, respectively. Based on the performance criteria, the suggested XGBoost could correctly categorize two groups of patients using an artificial intelligence technique. When the variable significance values obtained as a result of the modeling are examined, it can be said that 3 microRNAs are the determining microRNAs between the 2 groups. These microRNAs are microRNAs called microRNA 4306, microRNA 2115, and microRNA 4708 and will be associated with the state of metastasis.

One study mentioned that MicroRNA (miR)-4306 is associated with tumor growth and cancer, and its relationship with CRC was investigated. According to the results obtained, it was determined that miR-4306 expression was decreased in tissues with CRC [28]. microRNA 4708 has been investigated for many disease states such as

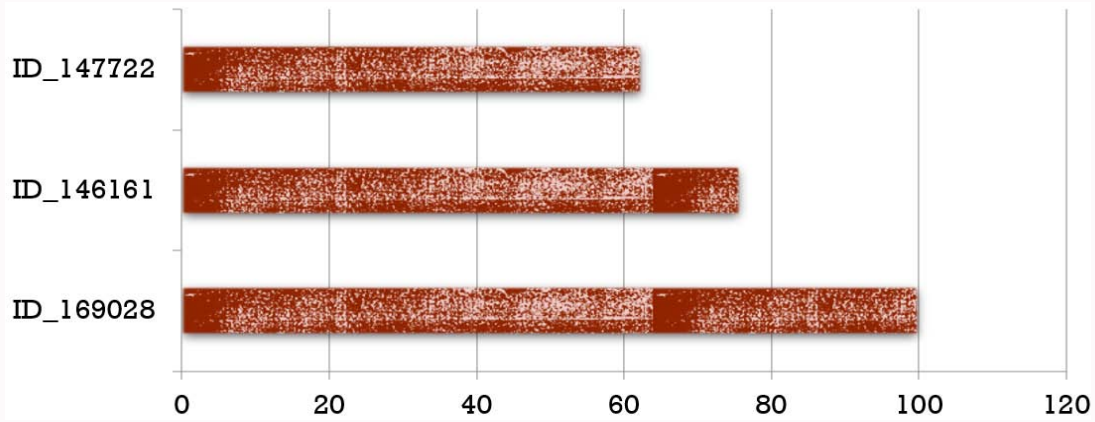

Figure 4: Graphical representation of microRNAs that differed most between the two groups.

breast cancer, pancreatic cancer and has been found to be associated with cancer [29,30].

In this study, the classification of patients with and without CCLM was provided using the machine learning model. According to the results obtained from the model, microRNAs that may be possible biomarkers for CCLM have been determined. With comprehensive analyses and studies to be conducted, these microRNAs can be associated with metastasis, so patients' care procedures can be regulated. It can be determined whether metastasis will develop or not.

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