Original Research

ONCOLOGY

Investigation of selected MiRNA as potential biomarker in chronic myeloid leukemia patients

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Abstract

Background and aim. Chronic myeloid leukemia (CML) is a type of blood cancer caused by a genetic abnormality. Meanwhile, circulating microRNAs (miRNAs) are emerging as potential noninvasive cancer biomarkers.
This research aims to establish whether miR-192-5p and miR-766-5p provide an accurate assessment of CML diagnosis, and whether these miRNAs have predictive ability on an individual (i.e., independent) and combined basis.

Methods. One hundred (n=100) study participants (i.e., 50 patients with diagnosed CML and 50 healthy controls) were recruited and consented to study participation. Plasma samples were obtained from all subjects, and miRNA levels were measured using qRT-PCR and analyzed. Group differences were examined, and receiver operating characteristic (ROC) curves and multivariate logistic regression were used for statistical analysis.

Results. The plasma of CML participants showed downregulation ($p < 0.0001$) of both miR-192-5p and miR-766-5p in comparison to control participants. miR-192-5p had good diagnostic performance (i.e., AUC = 0.7958, sensitivity 74%, specificity 72%). However, miR-766-5p had moderate diagnostic performance (i.e., AUC = 0.7164, sensitivity 74%, specificity 64%). In the multivariate logistic regression analysis, miR-192-5p remained a statistically significant, independent CML predictor (OR = 0.48, 95% CI: 0.31-0.69, $p < 0.001$); whereas, miR-766-5p did not. Smoking status and age (OR = 0.23, $p < 0.01$) were associated with CML. The combined multivariable model (i.e., plasma miRNA and demographic/clinical variables) yielded enhanced diagnostic performance (AUC = 0.864; sensitivity 86%, specificity 76%, accuracy 81%); thus, the combined model had better discriminative ability than either miRNA alone.

Conclusion. Downregulation appeared clearly in both miR-192-5p and miR-766-5p among CML patients. Still, after adjusting for multiple variables, only miR-192-5p kept its predictive strength on its own. Nevertheless, when used together, accuracy increased - shown by an AUC reaching 0.864 - which suggests that combining markers may work better than using one alone.

Keywords: CML, miR-192-5p, miR-766-5p, qRT-PCR, ROC analysis, multivariate logistic regression

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Introduction

Cancer remains one of the top causes of death worldwide, with a recent sharp increase in new cases. [1]. Tumors may appear as solid masses - such as carcinoma, sarcoma, melanoma, or lymphoma - or take the form of leukemia, which does not produce a defined tumor [2]. Unchecked growth of cells defines these diseases, along with failure to undergo programmed cell death, spread to distant sites, and the development of blood vessels that feed malignant tissue [3,4].

Leukemia is a white blood cell malignancy that arises when abnormal clones multiply in the bone marrow, disrupting normal activity [5]. This condition is classified into four types based on the progression rate and the specific stem cells involved: acute lymphoblastic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia [6].

One adult out of every hundred thousand develops CML each year, making it a rare form of blood cancer that represents about 15 percent of new leukemia diagnoses in adults [7]. Though uncommon, this condition stems from a genetic shift within one versatile blood-forming stem cell, triggering unchecked growth of early-stage white blood cells - especially those meant to become neutrophils [8-11].

MicroRNA (miRNA), meanwhile, gain a role in cancer detection and patient outcomes, because levels shift with tumor types, severity, and survival paths [12]. Since researchers spotted free-floating miRNAs in blood back in 2008, over 79 types have turned up in serum or plasma, tied to neoplasms in the stomach, lung, breast, throat, bowel, skin, ovary, and prostate [13]. Some small regulators, such as miR-766 or miR-192, attract notice thanks to their wide influence across malignant diseases [14,15]. miR-766 flips behavior, driving tumor growth or halting growth based on tissue context. When cervical squamous cells turn malignant, this RNA rises, driving the disease forward through the silencing of HOXA9. Moreover, in some cancers, such as colorectal, liver, kidney, and bone tumors, it slows down tumor development and spread by controlling specific genes: HNF4G, Wnt3a/PRC1, SF2, and BCL9L [13,14,16]. Notably, miRNA-192, a stable molecule abundant in liver tissue, appears across multiple cancer types and liver conditions, ranging from viral infections to metabolic damage and malignancies. Its presence links closely to disorders affecting the liver, yet also reaches into broader disease patterns [15,17-20].

Given the urgent need for dependable non-invasive biomarkers to enable early detection and monitor disease progression in CML patients, the present study aimed to examine the expression levels of miR-192-5p and miR-766-5p in patients with CML and to assess their diagnostic potential using ROC curve analysis and multivariate logistic regression analysis.

Methods

Participants

The present study included 100 participants, 50 patients with CML, who were recruited from Al-Basheer Hospital (Amman, Jordan) and 50 healthy controls which were matched for age and sex. Exclusion criteria were applied to participants with other hematological diseases or receiving non-standard treatments.

Ethics statement

This study received approval from the Scientific Research Committee of the Faculty of Applied Medical Sciences at Al-Ahliyya Amman University and was conducted following the guidelines of the Declaration of Helsinki (DOH). All ethical standards for medical research on human subjects were strictly observed, and informed consent was obtained from all participants.

Blood sample collection

Venous blood, 2-3 mL, was drawn into EDTA tubes. A portion of the sample was reserved for the complete blood count test. The remaining blood was centrifuged at 15,000 rpm for 10 minutes, and then the plasma was stored at -80°C.

miRNA extraction

Total miRNA was extracted and concentrated from each sample with the miRNeasy Serum/Plasma Advanced Kit (Qiagen, Germany) following the manufacturer's instructions. The kit uses a silica membrane to capture all miRNA during the process. RNA quantity of eluted samples was measured using a NanoDrop spectrophotometer. (Nabi, Korea) at 260 and 280 nm. The samples were stored at ultra-low temperatures following the quantification.

Complementary deoxyribonucleic acid (cDNA) synthesis

The miRCURY® LNA® RT Kit from Qiagen (Germany) prepared a 10 µL reverse transcription mix with miRNA and water, as per instructions. cDNA synthesis used a standard PCR thermal cycler from Applied Biosystems (Singapore).

Relative quantification of plasma miRNA levels

Serum microRNA concentrations were quantified using a qRT-PCR assay. The reactions were carried out in 10 µL volumes with the miRCURY LNA SYBR Green PCR Kit (Qiagen, Germany) on an Applied Biosystems Thermal Cycler. Data analysis followed the Pfaffl method [21].

Statistical analysis

Data analysis was carried out using GraphPad Prism and SPSS version 22. Results are shown as mean ± standard deviation. To compare the two groups, either an independent samples t-test or a Mann-Whitney U test was employed, based on the data distribution. ROC curve analysis evaluated the diagnostic accuracy of the findings, calculating sensitivity, specificity, and the area under the curve. A P value of less than 0.05 was deemed statistically significant. Multivariate logistic regression identified independent predictors of CML, including miR-192-5p, miR-766-5p, smoking status, and age. Findings are presented as odds ratios with 95% confidence intervals.

Results

Study sample characteristics

As shown in Figure 1, various demographic and hematological parameters were compared between CML patients and healthy controls. It was observed that a statistically significant age difference was present, with CML patients older on average than healthy controls ($p < 0.01$). Regarding red blood cell parameters, CML patients showed a highly significant reduction in RBC and Hb values compared with healthy controls ($p < 0.0001$). In addition, CML patients demonstrated a highly significant reduction in PCV values, indicating anemia in CML patients, ($p < 0.0001$).

However, CML patients and healthy controls showed no differences in MCV and MCH values, nevertheless, CML patients showed highly significant increases in RDW values, indicating anisocytosis in CML group ($p < 0.001$).

Regarding platelet parameters, CML patients showed no differences in MPV, plateletcrit, or platelet distribution width compared with healthy controls. In addition, CML patients showed no differences in mixed-cell population values, indicating platelet stability.

In conclusion, CML patients showed highly significant alterations in erythropoiesis, characterized by reduced RBC, Hb, and PCV, and increased RDW parameters, whereas platelet parameters were relatively unchanged.

Smoking status distribution

A significant difference in the distribution of smoking status between patients with CML and healthy controls was noted. For instance, a greater percentage of healthy controls were smokers compared to patients with CML. The percentage of healthy controls who were smokers was 62%, whereas for patients with CML, it was 26%. The results were found to be significant ($\chi^2 = 13.15$, $p = 0.0003$) (Table I). The results of the odds ratio test showed that smoking status is not positively associated with CML (OR = 0.215, 95% CI: 0.096–0.521) (Table I).

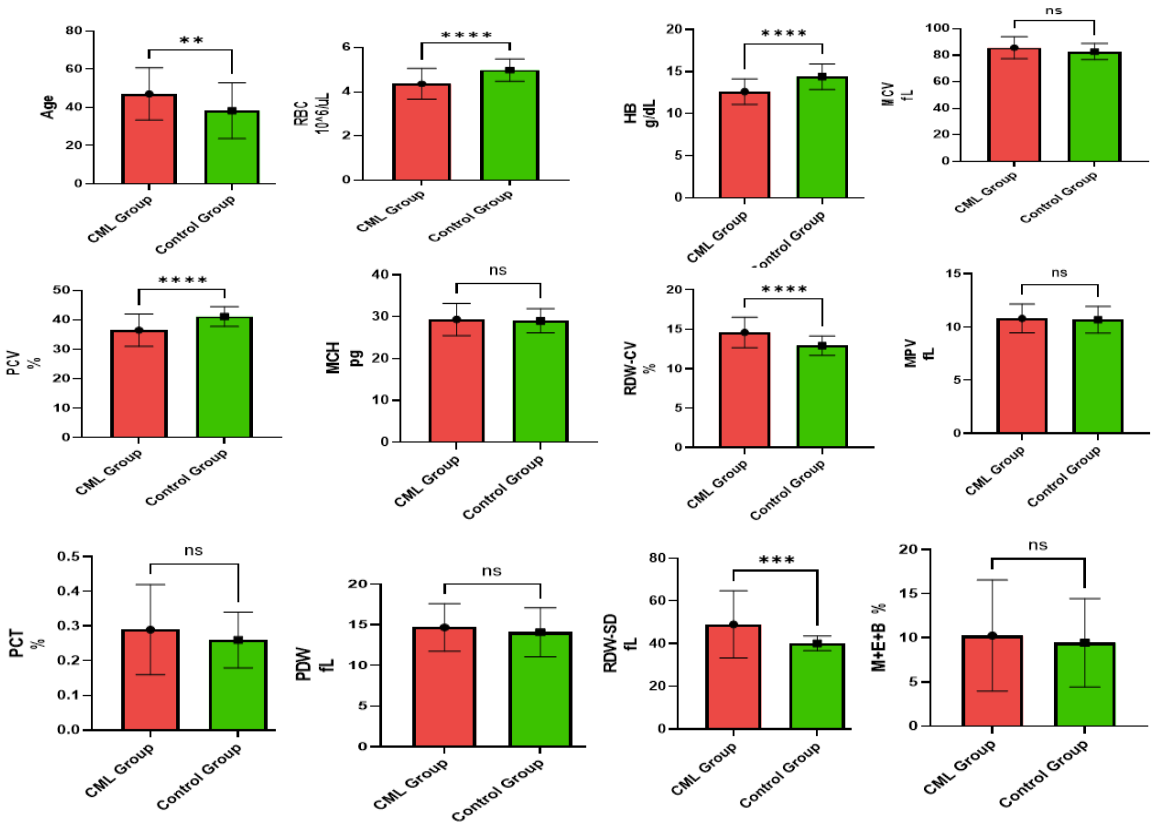

Figure 1. Demographic and hematological profiles of patients with CML and healthy controls.
The parameters included: red blood cell count (RBC), hemoglobin (Hb), hematocrit (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW-CV and SD), mean platelet volume (MPV), plateletcrit (PCT), platelet distribution width (PDW), and mixed cell population (MID%).
*Results were expressed as mean and standard deviation (SD). An independent-samples t-test was used for group comparisons. Significance was indicated by **p < 0.01, ***p < 0.001, and ****p < 0.0001, whereas non-significant findings were marked as ns.*

Table I. Distribution of smoking status among CML patients and healthy controls.

Group	Smoker n (%)	Non-smoker n (%)	p-value
CML (n = 50)	13 (26%)	37 (74%)	
Control (n = 50)	31 (62%)	19 (38%)	**0.0003**

Results shown as a number (percentage). For differences, significance was checked through the Chi-square method.

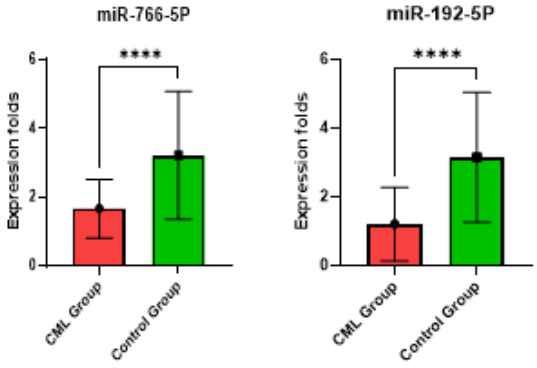

Figure 2. Relative expression levels of miR-766-5p and miR-192-5p in CML patients compared to healthy controls.
*Data are presented as mean ± SD. An independent samples t-test was used for statistical analysis. Significance was indicated by ****p < 0.0001.*

In conclusion, the status of smoking is still significantly associated with CML. However, the negative association might be due to cohort-specific bias. Therefore, the status of smoking is to be treated as a confounding factor.

The expression levels of miR-766-5P and miR-192-5P

Figure 2 demonstrates that the levels of miR-766-5p and miR-192-5p are significantly lower in CML patients compared to healthy controls. Specifically, miR-766-5p shows a marked reduction in CML patients, with a highly significant difference from controls ($p < 0.0001$). Likewise, miR-192-5p is also notably decreased in CML patients, with a significant difference versus controls ($p < 0.0001$). The decrease in miR-192-5p is more pronounced than that of miR-766-5p, suggesting it may be more closely associated with the pathophysiology of CML.

Overall, these findings indicate that the expression levels of miR-766-5p and miR-192-5p are substantially lower in CML patients than in normal controls, suggesting that these two miRNAs could act as tumor suppressor miRNAs.

Diagnostic performance of circulating miR-766-5p and miR-192-5p using ROC curve analysis

To determine the diagnostic value of miR-766-5p and miR-192-5p in distinguishing CML patients from healthy controls, receiver operating characteristic curve analysis was conducted, as shown in figure 3 and table II. Out of the two miRNAs, miR-192-5p was found to possess higher diagnostic potential compared to miR-766-5p. In this context, the area under the curve for miR-192-5p was 0.7958 (95% CI: 0.7094-0.8822, $p < 0.0001$), indicating greater diagnostic potential. At an optimal cutoff value of 1.74, the sensitivity and specificity of miR-192-5p were 74% and 72%, respectively. Furthermore, the diagnostic potential of miR-766-5p was found to be moderate, and the area under the curve for the same was 0.7164 (95% CI: 0.612-0.821, $p = 0.0002$). At a cutoff value of 2.64, the sensitivity and specificity of miR-766-5p were found to be 74 % and 64%, respectively.

In conclusion, microRNAs showed significant diagnostic potential. Nevertheless, miR-192-5p showed greater specificity and accuracy than miR-766-5p, suggesting it could serve as a more reliable biomarker for diagnosing CML.

Multivariate logistic regression analysis

In order to check the independent predictive ability of the biomarkers listed above, a multivariable logistic regression analysis was performed. The multivariable logistic regression analysis included four variables: miR-192-5p, miR-766-5p, smoking status, and age. The results, as indicated in table III confirmed the independent predictive ability of miR-192-5p for diagnosing chronic myeloid leukemia. The odds ratio for miR-192-5p was 0.48 (95% CI, 0.31-0.69). The independent predictive ability of miR-766-5p for diagnosing CML was not statistically significant, as indicated by an odds ratio of 0.81 (95% CI, 0.59-1.09).

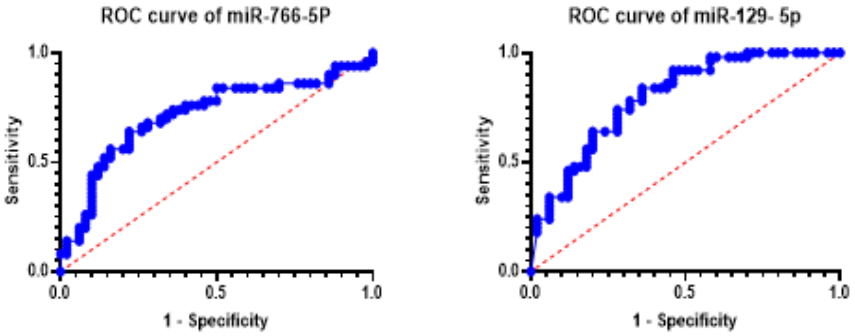

Figure 3. Receiver Operating Characteristic (ROC) Curve Analysis of miR-766-5p and miR-192-5p in CML.
The ROC curve indicates the potential of circulating miR-766-5p and miR-192-5p in distinguishing CML patients (n=50) from healthy controls (n=50). The diagonal dashed line indicates the reference line for no discrimination (AUC=0.5).

Table II. Diagnostic performance of miR-766-5p and miR-192-5p in CML patients.

Biomarker	AUC (95% CI)	Cutoff	Sensitivity	Specificity	p-value
miR-766-5P	0.7164 (0.612–0.821)	≈ 2.64	74%	64%	0.0002
miR-192-5P	0.7958 (0.7094–0.8822)	≈ 1.74	74%	72%	<0.0001

Summary of ROC Curve Analysis for miR-766-5p and miR-192-5p, covering AUC, 95% Confidence Intervals, optimal cut-off points, sensitivity, specificity, and p-values demonstrating statistical significance.

Table III. Multivariate logistic regression analysis of predictors for CML.

Variable	OR	95% CI	p-value
miR-192	0.48	0.31–0.69	<0.001
miR-766	0.81	0.59–1.09	NS
Smoking	0.23	0.07–0.68	<0.01
Age	1.037	1.00–1.079	~0.05

Multivariate logistic regression analysis illustrates the relationship between miR-192-5p, miR-766-5p, smoking status, and age with CML. Results are shown as odds ratios (OR) with 95% confidence intervals (CI). A p-value of less than 0.05 was considered statistically significant; NS indicates non-significance.

The multivariable model had better diagnostic performance than the individual markers when detecting chronic myeloid leukemia. In this case, the area under the receiver operating characteristic curve, which measures the diagnostic performance of the multivariable model, was 0.864, with a 95% confidence interval of 0.788 to 0.940. Moreover, the result was highly statistically significant, with a p-value < 0.0001. The multivariable model had an overall accuracy of 81%. In addition, the sensitivity of the multivariable model, which measures the proportion of the population actually suffering from the disease, was 86%. However, the specificity, which measures the proportion of the population free of the disease, was 76%. In terms of the model's calibration, the result indicated a good fit, as the Hosmer-Lemeshow test had a value of 0.57.

The diagnostic potential of the proposed integrated multivariate logistic regression model was further evaluated by ROC curve analysis (Figure 4). The proposed model, including miR-192-5p, miR-766-5p, smoking status, and age, had high discriminative potential in distinguishing CML patients from healthy controls. A significant increase in the ROC curve towards the upper left quadrant indicates high sensitivity and specificity for the proposed model.

The area under the curve for the proposed model was 0.864 (95% CI: 0.788-0.940, p < 0.0001), indicating high diagnostic performance. Compared with the diagnostic potential of individual miRNA markers, the proposed model shows a significant improvement. At the optimal classification threshold, the proposed model achieved an overall accuracy of 81%, sensitivity of 86%, and specificity of 76%.

The proposed model suggests that incorporating miRNA expression levels and clinical variables significantly enhances diagnostic accuracy. Thus, a multivariate approach for biomarker development is useful for diagnosing CML.

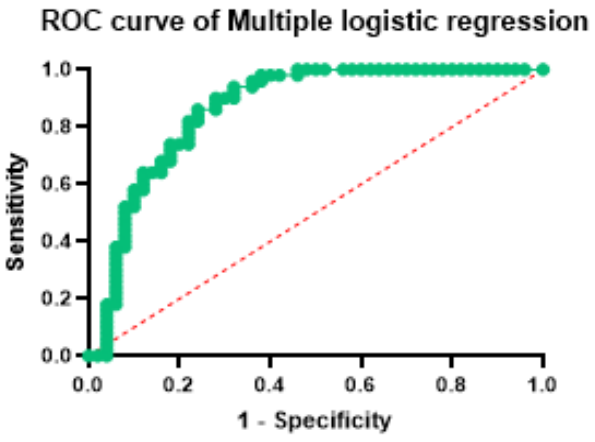

Figure 4. ROC curve of the multivariate logistic regression model for CML diagnosis.
The ROC curve shows the performance of the combined multivariable model, including miR-192-5p, miR-766-5p, smoking status, and age, for diagnosing CML.The performance of the model is excellent, as indicated by an area under the curve (AUC) of 0.864 (95% CI: 0.788–0.940, p < 0.0001). The diagonal dashed line is the reference line for no discrimination, where the AUC equals 0.5.

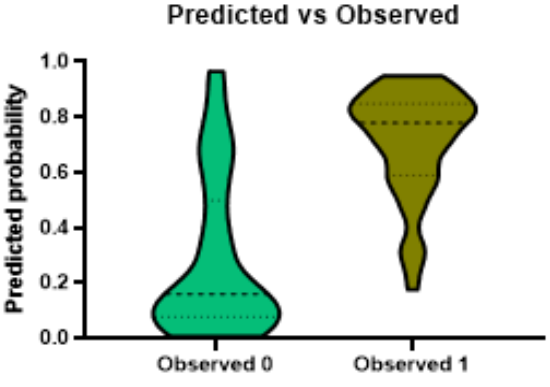

Figure 5. Predicted vs observed probabilities from the multivariate logistic regression model.
Predicted probabilities for CML, based on the multivariate logistic regression model including miR-192-5p, miR-766-5p, smoking status, and age, are shown for the observed group classification (Observed 0: healthy controls, Observed 1: CML patients). Violin plots are shown for the predicted probabilities. Dashed lines indicate the median and interquartile ranges. A clear distinction is observed between the groups, reflecting the model's high discriminative ability.

Predicted vs. Observed Probabilities

A more thorough evaluation of the multivariate logistic regression model's performance was carried out by comparing the predicted probabilities with the actual observed results. This is demonstrated in figure 5. From this figure, it is evident that the controls, or groups that were not CML patients (Observed 0), were likely to have low predicted probabilities, which were close to zero. In contrast, the groups that were CML patients (Observed 1) were likely to have higher predicted probabilities, which were concentrated on the higher end of the probability spectrum.

The probabilities clearly distinguish the two groups. There is a small amount of overlap, but this is expected. It is also important to note that the median for CML patients is significantly higher than that of the control group. It is also important to point out that the results obtained clearly indicate that the model has a high discriminative power. The results clearly show the model's strong discriminative ability, confirming its high power.

Discussion

It was observed that patients in the CML group had a significantly low red blood cell count, along with reduced hemoglobin levels and packed cell volume. There was also a notable increase in red cell distribution width. However, mean corpuscular volume and mean corpuscular hemoglobin remained relatively stable and showed no significant changes. This pattern of results is characteristic of anemia and anisocytosis and does not indicate a macrocytic or microcytic type of anemia, which is characteristic of a broader pattern of myeloproliferative syndromes and of inadequate erythropoiesis. In the current study, circulating miR-192-5p and miR-766-5p levels were significantly downregulated in patients with CML compared with healthy controls. It was also noted that miR-192-5p and miR-766-5p were capable of discriminating between patients and healthy controls in ROC curves. It was noted that miR-192-5p had higher discriminatory power as a biomarker than miR-766-5p, and only miR-192-5p showed independent predictive power in multivariable logistic regression analysis. It was also noted that the performance of miR-192-5p and miR-766-5p as biomarkers improved significantly when both were combined with age and smoking status variables. The AUC of the combined biomarkers, along with age and smoking status, was 0.864, with a sensitivity of 86% and a specificity of 76%. The results of the current study indicate the potential of miR-192-5p and miR-766-5p as biomarkers for CML, and also suggest that considering multiple parameters may yield much higher predictive and prognostic value for a particular outcome than a single parameter.

This is biologically plausible given our current understanding of the pathobiology of CML. It is characterized by the expression of the BCR:ABL1 oncogene [22]. This oncogene miRNA continuously activates a wide range of targets. So far, the conventional method of monitoring in CML relies on measuring the BCR:ABL1 fusion transcript, which is responsible for

the disease. However, there is currently growing interest in identifying specific blood markers that can be used to monitor the disease. Specifically, non-coding RNA molecules have already been miRNA CML. Several reviews have shown the potential of blood markers, also known as 'liquid biopsy' markers, for the diagnosis and possible monitoring of CML and other hematological malignancies, especially when used in combination with conventional 'clinicopathological' markers [22-24].

One of the main strengths of the current research is its focus on plasma miRNAs, a beneficial approach since they are relatively stable and accessible markers that can be easily measured following a standardized qRT-PCR protocol. Indeed, recent reviews of cancer biomarker methodology continue to support circulating miRNAs as a promising non-invasive approach. Nevertheless, these reviews have also emphasized the need for standardization and control of pre-analytical variables in assessing these biomarkers [23-25] . Thus, the current research provides primary data from a Jordanian cohort, and it is noteworthy that, in particular, circulating miRNA signatures in CML are significantly less well characterized than their counterparts in solid cancers.

Out of the two microRNAs under consideration, miR-192-5p was found to be a stronger biomarker. In the current dataset, the AUC of miR-192-5p was greater than that of miR-766-5p, and its association was confirmed after adjustment for potential confounders, including age and smoking status. This assertion can be explained by the general body of evidence suggesting that in most cases, miR-192-5p tends to play the role of a tumor suppressor in different types of cancer. A detailed review of the role of miR-192 -5p in cancer was conducted [26], which highlights the fact that in most cancer types, the downregulation of miR-192-5p tends to be related to uncontrolled cell growth, diminished apoptosis, and aggressive cancer phenotypes. According to the review, the role of miR-192 in carcinogenesis can be related to different pathways, such as MYC, XIAP, ZEB2, NFκB signaling, and other pathways related to cell growth [26].

The current results are consistent with existing hematologic data suggesting that low miR-192-5p expression levels may be linked to the development of malignant myeloid disorders. Indeed, recent reviews of existing data on miR-192-5p have identified this miR in pediatric acute myeloid leukemia and some lymphoid malignancies, supporting the possibility that downregulation of miR-192-5p is not limited to malignancies of epithelial origin [26]. Experimental data on leukemia have implicated miR-192-5p-regulated pathways in the regulation of proliferation and apoptosis. Although acute myeloid leukemia (AML) and CML are distinct disorders in their underlying biology, they both originate from myeloid cells and therefore might share common mechanisms of leukemogenesis. The current results, therefore, suggest that miR-192-5p might reflect an important aspect of CML biology and is not merely a nonspecific inflammatory marker [26].

In contrast, miR-766-5p showed a significant reduction in CML patients and moderate but significant performance in ROC analysis, although significance was not retained in the multivariable analysis. This is noteworthy and should not be interpreted as implying that miR-766-5p is not significant. Rather, it could suggest that miR-766-5p is conveying a similar message as other covariates and/or miR-192-5p, thereby losing significance when considered as a whole. The significance of miR-766 and its members, including miR-766-5p, is supported by existing literature [13]. A review published as recently as 2023 states that "miR-766 acts as a tumor suppressor or oncomiR depending on the cancer type, targets, and cellular context [13]. Low levels of miR-766-5p have been correlated with increased proliferative and migratory capabilities of cancer cells [13] suggest that these miRNAs could be used as potential targets for cancer therapy.

One mechanistic link that may have implications for the present study is inflammation. The pathogenesis of CML is not solely the result of BCR:ABL1 kinase activity and involves inflammatory and survival pathways, especially those involving NF-κB. miR-766-3p was found to play a role in anti-inflammatory responses through the indirect suppression of NF-κB pathways and, hence, may play a role in creating an environment for a more inflammatory state if it were downregulated [13,22]. Although the present study found miR-766-5p expression levels rather than miR-766-3p, and although these are not the same microRNAs, the overall literature regarding miR-766 microRNAs suggests a role for this microRNA family in pathways that may be important for overall survival, inflammation, and response to therapeutic agents [13, 22]. The current study demonstrated that miR-766-5p is biologically downregulated in CML patients, although it is not a useful standalone clinical marker in this cohort.

Another interesting observation is that the multivariable model clearly outperformed the individual miRNAs. This is consistent with the general rule of thumb that complex diseases like CML are rarely well characterized by a single biomarker. In fact, contemporary literature on the development of prediction models consistently supports integrating multiple variables to optimize discrimination accuracy [27,28]. This is because the various markers tend to represent different aspects of the disease. In the present investigation, the increase in AUC from the range of individual markers to 0.864 is consistent with the additive performance of the miRNA when interpreted in the context of the clinical covariates. In fact, the translational significance of the investigation is strengthened by the general rule of thumb that useful diagnostic tools are unlikely to be derived from individual markers [27,28]. However, caution is warranted when

interpreting the joint model concurrently [28,29]. In diagnostic modeling, the following caution is consistently offered by methodologists: "Estimates of model performance obtained from small development cohorts should be treated with caution, especially when the same data set is used for model development and evaluation. Internal metrics such as AUC, calibration, and apparent classification accuracy offer useful diagnostic information but should not be used in place of external validation [28,29]. Thus, the good performance of the current model should be viewed as 'promising but preliminary.' The subsequent step is validation in a separate cohort, preferably with pre-specified cutoffs and, if possible, bootstrapping and cross-validation.

The results for smoking require particularly close interpretation. About this cohort, it was found that the prevalence of smoking was higher in controls than in CML cases, and regression analysis indicated an inverse relationship between CML and smoking. However, this result is inconsistent with the overall epidemiologic literature [30,31]. A meta-analysis of epidemiologic studies suggested that CML risk is increased in a dose-response manner by smoking, and another study found that myeloid leukemia risk is increased in a dose-response manner by smoking [30,31]. It is therefore more likely that this inverse association is explained by the study design, sampling biases, and possibly by limitations of dichotomous categorizations of smokers and nonsmokers than by a protective effect of smoking.

Age had a borderline positive association with CML in the multivariable model, consistent with well-established epidemiological data. Current reviews of CML uniformly describe it as a condition in which diagnosis is more common in older individuals, although its presentation is heterogeneous across settings and health-care systems. The identification of age as a contributory factor in the joint model is consistent with expectations and reinforces the importance of biomarker studies controlling for basic demographic variables. A circulating biomarker that retains a significant association after controlling for these variables, such as was seen for miR-192-5p in this study, is stronger evidence for a biomarker than one for which the association is completely lost after controlling for basic demographic variables [23,32].

This current study provides evidence for the potential of using plasma-based miRNA testing as an auxiliary diagnostic tool in CML. However, as is evident from the existing literature on circulating miRNA-based tests, it is consistently emphasized that pre-analytical and analytical variability can significantly affect their results [24,25,33]. These include factors such as the choice of plasma vs. serum, anticoagulants, hemolysis, delay in analysis, RNA extraction method, and data normalization. These factors have recently been identified as key contributors to variability in results from different studies [24,25,33]. Although the present results look promising, for this study to be replicated in future studies, it is important that future studies include factors such as hemolysis and the choice of normalization.

The novelty of the study should also be emphasized. In fact, recent studies on miRNA screening in CML have identified other miRNAs that can act as potential biomarkers in CML, including miR-7-5p. However, to date, little is known regarding circulating levels of miR-192-5p and miR-766-5p in CML patients. Thus, the current study can be considered a valuable proof-of-concept study rather than a replication study. In fact, this study does not aim to validate a known biomarker but instead opens a new avenue in identifying potential biomarkers in CML, including two candidates that are considered to have high potential [23]. However, some limitations should be acknowledged. The study's small sample size and single setting may have restricted its scope. The second limitation is that the study used a cross-sectional design, which is useful for diagnosis but not for prognosis. Third, there was no external validation, and as such, the study remained at the development stage. The fourth limitation is that smoking status was collected in a simplistic, incomplete manner, which could have contributed to the findings. Lastly, although biological studies have shown that members of the miR-192 and miR-766 families have tumor-suppressor and inflammatory properties, there is no direct link between these miRNAs and BCR::ABL1 or NF-κB treatment, as shown by this study [13,25,26,29].

Conclusion

The present study aims to identify circulating miR-192-5p and miR-766-5p as plasma markers of CML, with the former being a more reliable independent marker, and the clinico-molecular model demonstrating superior performance. The results are consistent with current scientific understanding of CML, which indicates that its biology cannot be explained solely by BCR::ABL1 activity and that integrating liquid biopsy approaches may improve overall understanding of the disease. However, the study should be viewed as a landmark study that, given its current content, requires further validation, investigation, and standardization of assay approaches before any practical application.

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References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71:209-249. doi: 10.3322/caac.21660.
2. Rastogi S, Aldosary S, Saeedan AS, Ansari MN, Singh M, Kaithwas G. NF-κB mediated regulation of tumor cell proliferation in hypoxic microenvironment. Front Pharmacol. 2023;14:1108915. doi: 10.3389/fphar.2023.1108915.
3. Chaudhry GE, Md Akim A, Sung YY, Sifzizul TMT. Cancer and apoptosis: The apoptotic activity of plant and marine natural products and their potential as targeted cancer therapeutics. Front Pharmacol. 2022;13:842376. doi: 10.3389/fphar.2022.842376.
4. Kontomanolis EN, Koutras A, Syllaios A, Schizas D, Mastoraki A, Garmpis N, et al. Role of Oncogenes and Tumor-suppressor Genes in Carcinogenesis: A Review. Anticancer Res. 2020;40:6009-6015. doi: 10.21873/anticanres.14622.
5. Polychronakis I, Dounias G, Makropoulos V, Riza E, Linos A. Work-related leukemia: a systematic review. J Occup Med Toxicol. 2013;8:14. doi: 10.1186/1745-6673-8-14.
6. Zhao Y, Wang Y, Ma S. Racial Differences in Four Leukemia Subtypes: Comprehensive Descriptive Epidemiology. Sci Rep. 2018;8:548. doi: 10.1038/s41598-017-19081-4.
7. Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2020 update on diagnosis, therapy and monitoring. Am J Hematol. 2020;95:691-709. doi: 10.1002/ajh.25792.
8. Keohane EM, Otto CN, Walenga JM. Rodak's Hematology-E-BookElsevier Health Sciences, 2019.
9. Bosman MC, Schuringa JJ, Vellenga E. Constitutive NF-κB activation in AML: Causes and treatment strategies. Crit Rev Oncol Hematol. 2016;98:35-44. doi: 10.1016/j.critrevonc.2015.10.001.
10. Napetschnig J, Wu H. Molecular basis of NF-κB signaling. Annu Rev Biophys. 2013;42:443-68. doi: 10.1146/annurev-biophys-083012-130338.
11. Carrà G, Torti D, Crivellaro S, Panuzzo C, Taulli R, Cilloni D, et al. The BCR-ABL/NF-κB signal transduction network: a long lasting relationship in Philadelphia positive Leukemias. Oncotarget. 2016;7:66287-66298. doi: 10.18632/oncotarget.11507.
12. Peng Y, Croce CM. The role of MicroRNAs in human cancer. Signal Transduct Target Ther. 2016;1:15004. doi: 10.1038/sigtrans.2015.4.
13. Gupta J, Kareem Al-Hetty HRA, Aswood MS, Turki Jalil A, Azeez MD, Aminov Z, et al. The key role of microRNA-766 in the cancer development. Front Oncol. 2023 May 2;13:1173827. doi: 10.3389/fonc.2023.1173827.
14. You Y, Que K, Zhou Y, Zhang Z, Zhao X, Gong J, et al. MicroRNA-766-3p Inhibits Tumour Progression by Targeting Wnt3a in Hepatocellular Carcinoma. Mol Cells. 2018;41:830-841. doi: 10.14348/molcells.2018.0181.
15. Tian C, Zhang L, Li X, Zhang Y, Li J, Chen L. Low miR-192 expression predicts poor prognosis in pediatric acute myeloid leukemia. Cancer Biomark. 2018;22:209-215. doi: 10.3233/CBM-170657.
16. Hayakawa K, Kawasaki M, Hirai T, Yoshida Y, Tsushima H, Fujishiro M, et al. MicroRNA-766-3p Contributes to Anti-Inflammatory Responses through the Indirect Inhibition of NF-κB Signaling. Int J Mol Sci. 2019;20:809. doi: 10.3390/ijms20040809.
17. Li Q, Luan Q, Zhu H, Zhao Y, Ji J, Wu F, et al. Circular RNA circ_0005774 contributes to proliferation and suppresses apoptosis of acute myeloid leukemia cells via circ_0005774/miR-192-5p/ULK1 ceRNA pathway. Biochem Biophys Res Commun. 2021;551:78-85. doi: 10.1016/j.bbrc.2021.02.058.
18. Chen J, Wang J, Li H, Wang S, Xiang X, Zhang D. p53 activates miR-192-5p to mediate vancomycin induced AKI. Sci Rep. 2016 Dec 12;6:38868. doi: 10.1038/srep38868.
19. Chen P, Feng Y, Zhang H, Shi X, Li B, Ju W, et al. MicroRNA-192 inhibits cell proliferation and induces apoptosis in human breast cancer by targeting caveolin 1. Oncol Rep. 2019;42:1667-1676. doi: 10.3892/or.2019.7298.
20. Jin Y, Lu J, Wen J, Shen Y, Wen X. Regulation of growth of human bladder cancer by miR-192. Tumour Biol. 2015;36:3791-3797. doi: 10.1007/s13277-014-3020-8.
21. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 2001;29:e45. doi: 10.1093/nar/29.9.e45.
22. El-Tanani M, Nsairat H, Matalka II, Lee YF, Rizzo M, Aljabali AA, et al. The impact of the BCR-ABL oncogene in the pathology and treatment of chronic myeloid leukemia. Pathol Res Pract. 2024;254:155161. doi: 10.1016/j.prp.2024.155161.
23. Wosniaki DK, Marin AM, Oliveira RN, Koerich GM, Munhoz EC, Farias JSH, et al. The Screening of microRNAs in Chronic Myeloid Leukemia: A Clinical Evaluation. Int J Mol Sci. 2024;25:3363. doi: 10.3390/ijms25063363.
24. Naranbat D, Herdes E, Tapinos N, Tripathi A. Review of microRNA detection workflows from liquid biopsy for disease diagnostics. Expert Rev Mol Med. 2025;27:e11. doi: 10.1017/erm.2025.2.
25. Zendjabil M. Preanalytical, analytical and postanalytical considerations in circulating microRNAs measurement. Biochem Med (Zagreb). 2024;34:020501. doi: 10.11613/BM.2024.020501.
26. Yang Y, Razak SRA, Ismail IS, Ma Y, Yunus MA. Molecular mechanisms of miR-192 in cancer: a biomarker and therapeutic target. Cancer Cell Int. 2025;25:94. doi: 10.1186/s12935-025-03666-5.
27. Leerink JM, Feijen EAM, de Baat EC, Merkx R, van der Pal HJH, Tissing WJE, et al. A Biomarker-Based Diagnostic Model for Cardiac Dysfunction in Childhood Cancer Survivors. JACC CardioOncol. 2024;6:236-247. doi: 10.1016/j.jaccao.2024.02.008.
28. Collins GS, Moons KGM, Dhiman P, Riley RD, Beam AL, Van Calster B, et al. TRIPOD+AI statement: updated guidance for reporting clinical prediction models that use regression or machine learning methods. BMJ. 2024;385:e078378. doi: 10.1136/bmj-2023-078378. Erratum in: BMJ. 2024;385:q902. doi: 10.1136/bmj.q902.
29. Strandberg R, Jepsen P, Hagström H. Developing and

validating clinical prediction models in hepatology - An overview for clinicians. J Hepatol. 2024;81:149-162. doi: 10.1016/j.jhep.2024.03.030.

30. Qin L, Deng HY, Chen SJ, Wei W. Relationship between cigarette smoking and risk of chronic myeloid leukaemia: a meta-analysis of epidemiological studies. Hematology. 2017;22:193-200. doi: 10.1080/10245332.2016.1232011.
31. Musselman JR, Blair CK, Cerhan JR, Nguyen P, Hirsch B, Ross JA. Risk of adult acute and chronic myeloid leukemia with cigarette smoking and cessation. Cancer Epidemiol. 2013;37:410-416. doi: 10.1016/j.canep.2013.03.012.
32. Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2025 update on diagnosis, therapy, and monitoring. Am J Hematol. 2024;99:2191-2212. doi: 10.1002/ajh.27443.
33. Want A, Staniak K, Grabowska-Pyrzewicz W, Fesiuk A, Barczak A, Gabryelewicz T, et al. Optimized RT-qPCR and a novel normalization method for validating circulating miRNA biomarkers in ageing-related diseases. Sci Rep. 2023;13:20869. doi: 10.1038/s41598-023-47971-3.