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Original Article

Peripheral blood miR-24-3p level is inversely associated with CRP-defined systemic inflammation in carotid atherosclerotic stenosis: a retrospective study of 352 cases

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Abstract: Objectives: Circulating microRNAs have emerged as potential cardiovascular biomarkers, yet the clinical relevance of microRNA-24-3p (miR-24-3p) in carotid atherosclerotic stenosis (CAS) remains unclear. This present study evaluated the association between peripheral blood miR-24-3p levels and systemic inflammatory markers in patients with CAS. Methods: A total of 352 patients with imaging-confirmed CAS ≥ 50% were retrospectively enrolled. Peripheral blood miR-24-3p expression was quantified. Systemic inflammatory markers were assessed. Correlation and multivariable logistic regression analyses were performed. Receiver operating characteristic (ROC) curves were used to assess discriminatory performance. Results: miR-24-3p expression was inversely correlated with C-reactive protein (CRP) (r = -0.46) and interleukin-6 (IL-6) (r = -0.43), as well as tumor necrosis factor-alpha (TNF-α) (r = -0.31), interleukin-1 beta (IL-1β) (r = -0.28), neutrophil-to-lymphocyte ratio (NLR) (r = -0.34), and platelet-to-lymphocyte ratio (PLR) (r = -0.29) (all P < 0.001). Symptomatic patients showed lower miR-24-3p levels than asymptomatic patients (3.98 vs 4.87, P = 0.004), with a decreasing trend across stenosis severity (p for trend = 0.012). The low miR-24-3p group had higher CRP levels (4.12 vs 1.98 mg/L, P < 0.001). Multivariable analysis confirmed an independent association with elevated CRP (adjusted OR = 0.45, 95% CI: 0.37-0.55, P < 0.001). The area under the curve (AUC) for predicting systemic inflammation was 0.74 (95% CI: 0.69-0.79). Conclusions: Peripheral blood miR-24-3p levels are inversely associated with systemic inflammatory burden and clinical severity in CAS, suggesting potential use as a noninvasive biomarker of inflammatory activity.

Keywords: Carotid atherosclerotic stenosis, miR-24-3p, inflammation, C-reactive protein, cytokines, biomarkers

Introduction

Carotid atherosclerotic stenosis (CAS) is a major cause of ischemic stroke and transient ischemic attack, contributing substantially to global morbidity and mortality [1, 2]. Although the degree of luminal narrowing has traditionally guided clinical decision-making, evidence indicates that stroke risk cannot be fully explained by stenosis severity alone [3]. Plaque composition, inflammatory activity, and systemic biological responses play critical roles in determining plaque instability and thromboembolic potential [4, 5]. Therefore, identifying circulating biomarkers that reflect inflammatory status and disease activity remains an important unmet clinical need in patients with CAS.

Atherosclerosis is a chronic inflammatory disease characterized by endothelial dysfunction and sustained cytokine signaling [6]. In the carotid artery, inflammatory activation promotes plaque progression and destabilization, increasing the likelihood of rupture and downstream cerebrovascular events [7]. Circulating inflammatory markers such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and pro-inflammatory cytokines have been associated with cardiovascular risk; however, their clinical utility in CAS is limited by low specificity and substantial variability influenced

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by comorbid conditions [8, 9]. These limitations highlight the need for more stable and mechanistically informative biomarkers that better capture upstream regulatory processes in vascular inflammation.

MicroRNAs (miRs) are small, non-coding RNAs that regulate gene expression at the post-transcriptional level and participate in a wide range of cardiovascular and inflammatory pathways [10]. Notably, circulating miRNAs are detectable in peripheral blood and exhibit high stability due to their encapsulation in exosomes, microvesicles, or protein complexes [11]. These characteristics make circulating miRNAs attractive candidates as minimally invasive biomarkers. Circulating miRNA signatures have been associated with atherosclerosis, plaque vulnerability, and ischemic stroke [12-14]. Nevertheless, heterogeneity in study populations and miRNA targets has limited their translation into routine clinical practice. However, most prior studies have focused on composite miRNA panels or heterogeneous cardiovascular populations, whereas evidence regarding individual miRNAs with specific mechanistic relevance in CAS remains limited.

Among these candidates, miR-24-3p has attracted increasing attention due to its involvement in vascular biology and inflammatory regulation. Experimental studies indicate that miR-24 regulates endothelial function and vascular smooth muscle cell phenotypic switching [15, 16]. In addition, miR-24 has been shown to participate in inflammatory signaling by targeting genes involved in cytokine production, nuclear factor-κB activation, and immune cell responses [17, 18]. These biological functions suggest a role for miR-24-3p in atherosclerotic disease progression.

Clinical evidence regarding miR-24-3p remains limited. Altered circulating miR-24 levels have been reported in patients with coronary artery disease, metabolic disorders, and inflammatory conditions, although results are not entirely consistent across studies [19-21]. Importantly, data focusing specifically on carotid atherosclerotic stenosis are scarce, and the relationship between circulating miR-24-3p levels and systemic inflammatory markers in this population has not been systematically evaluated. Moreover, existing studies are often limited by small sample sizes and lack of detailed clinical stratification.

Based on these considerations, the present study aimed to investigate the association between peripheral blood miR-24-3p levels and systemic inflammatory status in a well-characterized cohort of patients with carotid atherosclerotic stenosis. Unlike previous studies focusing on heterogeneous miRNA profiles, this study specifically evaluates miR-24-3p as a functionally relevant regulator of vascular inflammation and integrates its expression with both conventional inflammatory markers and clinical severity indicators. By linking molecular regulation with clinically measurable inflammatory burden, this study provides novel evidence supporting a role of miR-24-3p as a noninvasive biomarker for risk stratification in CAS.

Materials and methods

Patient selection

In this retrospective observational study, a total of 352 eligible patients were included at The First Affiliated Hospital of Wenzhou Medical University between January 2025 and December 2025 (**Figure 1**). The index admission or outpatient visit, defined as the time at which carotid imaging and peripheral blood sampling were performed, was defined as baseline. Clinical, laboratory, and imaging data were retrieved from electronic medical records. The study protocol was approved by the institutional ethics committee of the First Affiliated Hospital of Wenzhou Medical University (approval. No. KY2026-026) and conducted in accordance with the Declaration of Helsinki, with informed consent obtained or waived, as appropriate due to the retrospective nature of the study. Eligible participants were adults aged ≥ 18 years with imaging-confirmed carotid atherosclerotic stenosis ≥ 50%. Peripheral blood samples had to be available at baseline. To further ensure cohort homogeneity and reduce potential sources of bias, additional inclusion criteria were applied. Patients were required to have complete baseline clinical records, including demographic characteristics, vascular risk factors, imaging reports, and laboratory data obtained at the index evaluation. Only patients with available high-sensitivi-

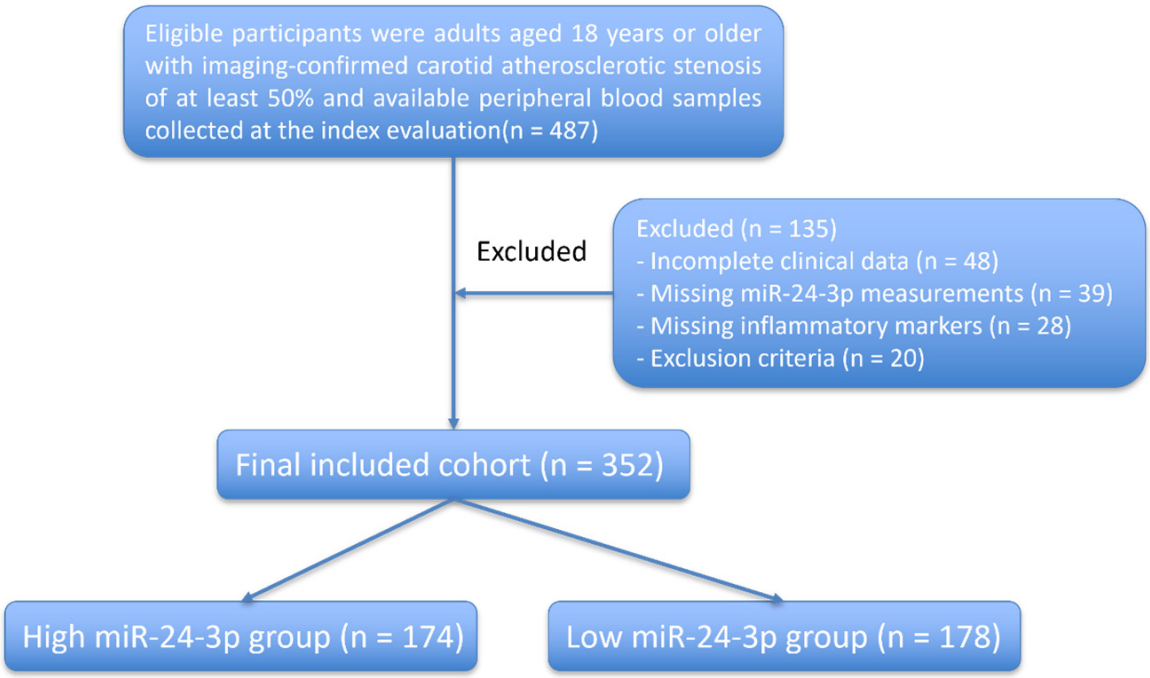

Figure 1. Flow diagram illustrating the patient screening and selection process. After applying the inclusion and exclusion criteria, 352 patients were included in the analysis.

ty CRP measurements and miR-24-3p expression data were included in the primary analysis. Expanded exclusion criteria were implemented to minimize confounding effects on systemic inflammatory status. Patients were excluded if they had evidence of acute infection within four weeks prior to blood sampling, chronic inflammatory or autoimmune diseases, active malignancy, or were receiving systemic immunosuppressive therapy. In addition, patients with severe hepatic dysfunction, advanced renal insufficiency, recent major surgery or trauma within 3 months, or hematologic disorders affecting leukocyte or platelet counts were excluded. Patients with incomplete or missing key variables required for correlation or regression analyses were also excluded. Patients with incomplete miR expression data or missing inflammatory marker measurements were also excluded. Application of these criteria directly informed the exclusion counts reported in the study flowchart (**Figure 1**).

All laboratory and miRNA measurements were obtained at the index visit (baseline). Systemic inflammation was primarily defined as elevated high-sensitivity CRP above the cohort median. To capture a broader inflammatory profile, circulating cytokines including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-1 beta (IL-1β) were considered, along with derived inflammatory indices such as the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR).

The primary outcome was CRP-defined systemic inflammation. Secondary outcomes included IL-6, TNF-α, IL-1β, NLR, and PLR. These indicators were used in all correlation, stratification, and regression analyses evaluating systemic inflammatory burden.

Data extraction

Clinical, laboratory, and molecular data were extracted from electronic medical records and laboratory databases using standardized protocols:

Clinical classification and definitions: The degree of CAS was extracted from formal clinical imaging reports and recorded as a continuous percentage into three predefined groups: 50-69%, 70-89%, and 90-99%. Disease laterality was classified as left-sided, right-sided, or bilateral involvement. Symptomatic status was defined based on the occurrence of transient ischemic attack, ischemic stroke, or amaurosis fugax attributable to the ipsilateral carotid artery within six months prior to the index eval-

uation. Patients without such events were classified as asymptomatic.

Clinical and demographic data collection: Demographic information, including age, sex, and body mass index (BMI), was collected from electronic medical records. Vascular risk factors such as hypertension, diabetes mellitus, dyslipidemia, coronary artery disease, atrial fibrillation, smoking status, and alcohol consumption were documented based on established diagnoses and clinical history. Medication use at the index date, including statins, antiplatelet agents, anticoagulants, antihypertensive drugs, and glucose-lowering therapies, was systematically recorded. All clinical data were extracted using a standardized case report form to ensure consistency and reproducibility.

Blood sample collection and processing: Peripheral venous blood samples were obtained at the admission or outpatient visit under routine clinical conditions. Fasting status at the time of blood collection was recorded when available. Serum or plasma was separated according to standardized laboratory protocols and stored under controlled temperature conditions until analysis. Pre-analytical variables relevant to circulating miRNA stability, including storage duration and freeze-thaw cycles, were documented where applicable to support analytical consistency.

Measurement of routine laboratory and inflammatory markers: Routine laboratory data were obtained from standard clinical testing and included lipid profiles, indices of glucose metabolism, renal function markers, and liver enzymes when available. Hematologic data were derived from complete blood counts, including total white blood cell count, neutrophil, lymphocyte, and monocyte counts, platelet count, and hemoglobin concentration. Systemic inflammation was primarily assessed using high-sensitivity C-reactive protein (CRP), with erythrocyte sedimentation rate (ESR) recorded when available. To capture a broader inflammatory profile, derived inflammatory indices, including the NLR, PLR, and systemic immune-inflammation index, were calculated *post hoc* from hematologic data and were not directly measured laboratory outputs.

Cytokine measurements: To further characterize systemic inflammation, circulating inflammatory cytokines, including IL-6, TNF-α, and IL-1β, were measured using standardized immunoassays in accordance with manufacturer instructions. Cytokine measurements were available for the full cohort or for predefined subsets, depending on sample availability, and were incorporated into exploratory analyses. Although cytokine measurements are not routine clinical tests, these data were obtained from patients who underwent extended laboratory evaluations during clinical care or stored sample analysis within the institutional biobank. No additional sampling was performed for research purposes, consistent with the retrospective design.

RNA extraction and quantification of miR-24-3p: Total RNA was extracted from serum or plasma samples using a commercially available miRNA extraction kit following the manufacturer's protocol. Reverse transcription was performed using miRNA-specific primers, and quantitative real-time polymerase chain reaction was conducted on a standardized platform. An endogenous or exogenous reference control, such as U6, was used for normalization. Cycle threshold values were recorded, and relative miR-24-3p expression was calculated using the ΔCt method and expressed as $2^{-\Delta Ct}$. Samples exceeding predefined thresholds were excluded to minimize analytical bias. Although miR measurements are not routine clinical tests, these data were obtained from patients who underwent extended laboratory evaluations during clinical care or stored sample analysis within the institutional biobank. No additional sampling was performed specifically for this study, consistent with the retrospective design.

Outcome measures

Exploratory clinical variables included carotid revascularization procedures, such as carotid endarterectomy or carotid artery stenting during hospitalization, as well as in-hospital cerebrovascular events and short-term follow-up outcomes when available. These variables were analyzed descriptively and were not considered primary endpoints of the study.

Statistical analysis

Continuous variables were summarized as mean ± standard deviation (SD) or median with interquartile range (IQR), and categorical vari-

Table 1. Baseline demographic and clinical characteristics of the study population

Variable	Total (n = 352)
Age (years)	66.0 ± 8.6
Male sex, n (%)	234 (66.5%)
Body mass index (kg/m^2)	25.3 ± 3.1
Hypertension, n (%)	245 (69.6%)
Diabetes mellitus, n (%)	116 (33.0%)
Dyslipidemia, n (%)	207 (58.8%)
Symptomatic carotid stenosis, n (%)	147 (41.8%)
Moderate stenosis (50-69%), n (%)	136 (38.6%)
Severe stenosis (70-89%), n (%)	144 (40.9%)
Near-occlusive stenosis (90-99%), n (%)	72 (20.5%)
C-reactive protein (mg/L)	1.78 (1.11-2.60)
Interleukin-6 (pg/mL)	1.40 (0.82-2.05)
Tumor necrosis factor-α (pg/mL)	0.96 (0.54-1.30)
Interleukin-1β (pg/mL)	0.68 (0.37-0.99)
Neutrophil-to-lymphocyte ratio	2.31 (1.80-3.00)
Platelet-to-lymphocyte ratio	122.60 (98.97-151.45)

ables were expressed as counts and percentages. Between-group comparisons were performed using Student's *t*-test or the Mann-Whitney *U* test for continuous variables and χ^2 test or Fisher's exact test for categorical variables. Correlation analyses were conducted to evaluate associations between miR-24-3p expression and inflammatory markers. Spearman correlation analysis was used to assess associations between continuous variables due to non-normal distributions. Multivariable logistic regression models were constructed to evaluate independent associations between miR-24-3p expression and systemic inflammation. Variables with $P < 0.10$ in univariate analysis were entered into the model and retained using a backward stepwise selection procedure with a retention criterion of $P < 0.05$. Model discrimination was evaluated using receiver operating characteristic (ROC) curves and the area under the curve (AUC). All statistical analyses were performed using standard statistical software, with a two-sided *p* value < 0.05 considered significant. Corresponding test statistics (t, Z, or χ^2) were reported where applicable.

Results

Study population and clinical characteristics

Among the 352 consecutive patients included in the final analysis, the mean age was 66.1 ± 9.0 years (range: approximately 42-85 years), and 222 patients (63.1%) were male (**Table 1**). The mean body mass index was 25.1 ± 3.2 kg/m^2 (**Table 1**). Vascular risk factors were common: 240 patients (68.2%) had a documented history of hypertension, 114 patients (32.4%) had diabetes mellitus, and 204 patients (58.0%) had dyslipidemia (**Table 1**). A total of 145 patients (41.2%) were classified as symptomatic, having experienced a transient ischemic attack, ischemic stroke, or amaurosis fugax attributable to the ipsilateral carotid artery within six months prior to the index evaluation (**Table 1**). Regarding carotid lesion severity, 135 patients (38.4%) had moderate stenosis (50-69%), 147 patients (41.8%) had severe stenosis (70-89%), and 70 patients (19.8%) had near-occlusive disease (90-99%) (**Table 1**).

Baseline systemic inflammatory and hematologic characteristics

Baseline inflammatory and hematologic data are summarized in **Table 1**. Serum CRP levels exhibited a right-skewed distribution, with a median value of 1.78 mg/L (IQR: 1.11-2.60) (**Table 1**). The median ESR was 18 mm/h (IQR: 10-31). Among measured cytokines, median circulating IL-6 levels were 1.87 pg/mL (IQR: 1.14-2.93), while TNF-α and IL-1β levels were 1.29 pg/mL (IQR: 0.82-1.96) and 0.96 pg/mL (IQR: 0.61-1.42), respectively (**Table 1**). Hematologic indices derived from complete blood counts demonstrated a median neutrophil-to-lymphocyte ratio (NLR) of 2.39 (IQR: 1.74-3.22) and a median platelet-to-lymphocyte ratio (PLR) of 122.6 (IQR: 97.4-158.3) (**Table 1**). These indices were then included in correlation, stratified, and multivariable analyses evaluating systemic inflammatory burden.

Distribution and stratification of peripheral blood miR-24-3p expression

Peripheral blood miR-24-3p expression values demonstrated a non-Gaussian, right-skewed distribution, with a median relative expression level of 4.53 (IQR: 3.21-6.34). The full distribu-

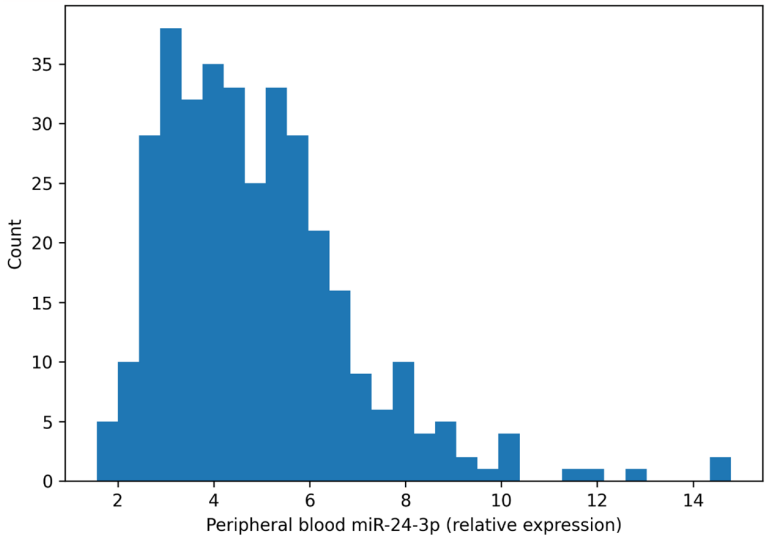

Figure 2. Distribution of peripheral blood miR-24-3p expression in patients with carotid atherosclerotic stenosis (CAS). Histogram showing the distribution of relative peripheral blood miR-24-3p expression levels among 352 patients with CAS.

Table 2. Correlation between peripheral blood miR-24-3p expression and inflammatory markers

Inflammatory marker	Correlation coefficient (r)
C-reactive protein	-0.46
Interleukin-6	-0.43
Tumor necrosis factor-α	-0.31
Interleukin-1β	-0.28
Neutrophil-to-lymphocyte ratio	-0.34
Platelet-to-lymphocyte ratio	-0.29

tion of miR-24-3p expression across the cohort is shown in **Figure 2**, illustrating substantial interindividual variability. For analytical purposes, patients were stratified into low and high miR-24-3p expression groups based on the cohort median. Specifically, the cohort median value of 4.53 was used as the cutoff: patients with miR-24-3p expression above the median were categorized as the high-expression group, and those below or equal to the median were categorized as the low-expression group. This stratification guided all subsequent comparative, correlation, and regression analyses. Comparison of baseline demographic and clinical variables between these two groups revealed no significant differences in age, sex distribution, body mass index, or prevalence of major vascular risk factors (all P > 0.05), indicating reasonable baseline comparability between expression groups (**Figure 2**).

Correlation between miR-24-3p expression and systemic inflammatory markers

Correlation analyses demonstrated consistent inverse associations between circulating miR-24-3p expression and multiple markers of systemic inflammation (**Table 2**). Specifically, miR-24-3p levels were moderately negatively correlated with CRP ($r = -0.46$, $P < 0.001$) and IL-6 ($r = -0.43$, $P < 0.001$). Additional significant inverse correlations were observed between miR-24-3p and TNF-α ($r = -0.31$, $P < 0.001$), IL-1β ($r = -0.28$, $P < 0.001$), NLR ($r = -0.34$, $P < 0.001$), and PLR ($r = -0.29$, $P < 0.001$). Scatter plots depicting these relationships are shown in **Figure 3**. These plots demonstrated a broadly linear inverse trend across the observed range of miR-24-3p values, without evidence of marked threshold effects or extreme outliers driving the associations.

Inflammatory profiles according to miR-24-3p expression level

Comparative analyses revealed clear differences in inflammatory burden between patients stratified by miR-24-3p expression level (**Table 3**). Patients in the low miR-24-3p expression group exhibited significantly higher systemic inflammatory markers than those in the high-expression group. Median CRP levels were 4.12 mg/L in the low-expression group compared with 1.98 mg/L in the high-expression group ($P < 0.001$). Similarly, IL-6, TNF-α, and IL-1β concentrations were all significantly elevated among patients with lower miR-24-3p expression. No significant differences were observed for NLR or PLR between groups (both $P > 0.05$), and trends were less consistent compared to those of CRP and cytokine markers.

MiR-24-3p expression across clinical subgroups of CAS

Subgroup analyses demonstrated that miR-24-3p expression differed according to clinical pre-

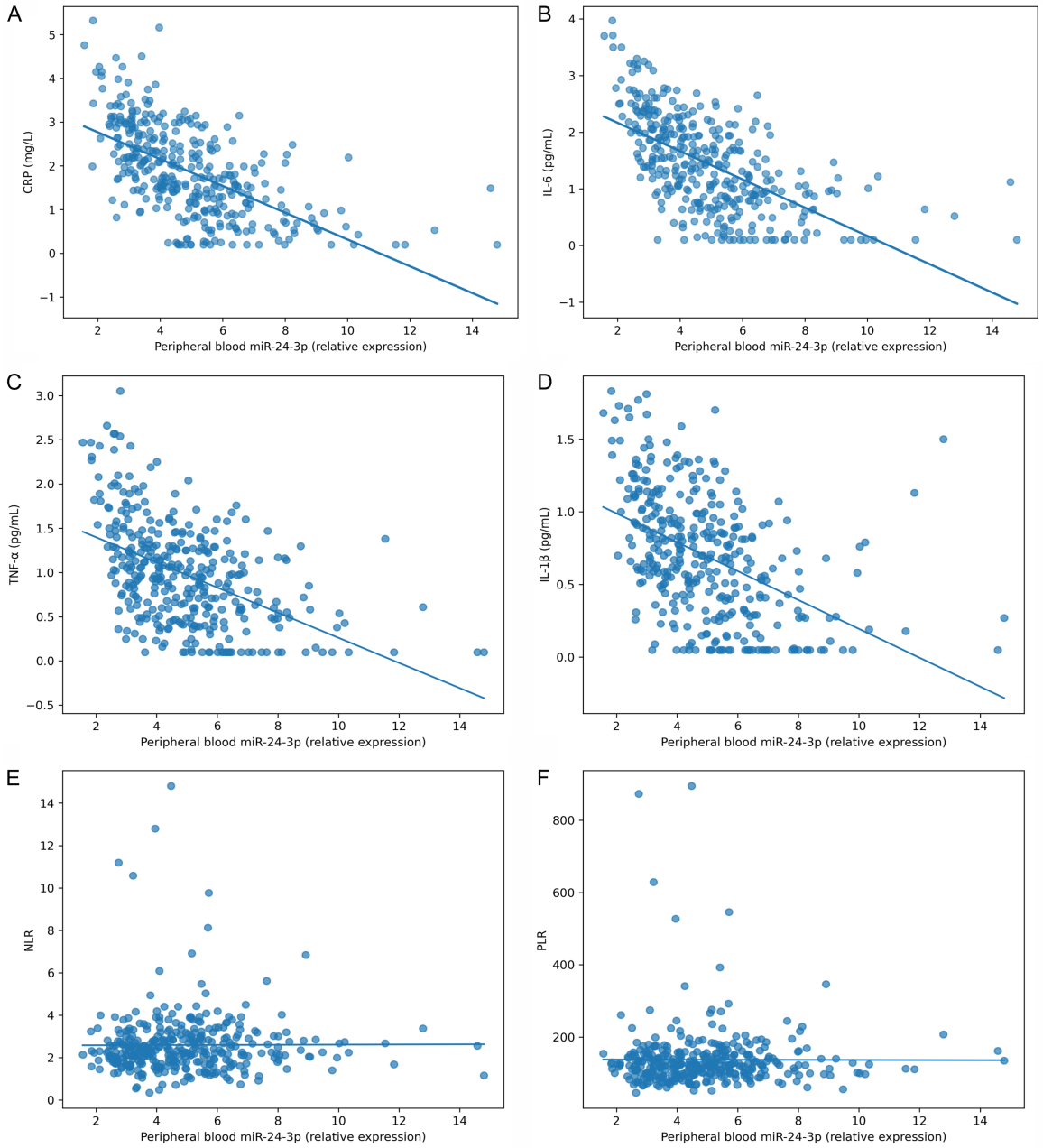

Figure 3. Association between peripheral blood miR-24-3p expression and systemic inflammatory markers. A. Scatter plot illustrating the relationship between miR-24-3p expression and serum C-reactive protein (CRP) levels. B. Scatter plot illustrating the relationship between miR-24-3p expression and circulating interleukin-6 (IL-6) levels. C. Scatter plot illustrating the relationship between miR-24-3p expression and tumor necrosis factor-alpha (TNF-α) levels. D. Scatter plot illustrating the relationship between miR-24-3p expression and circulating interleukin-1 beta (IL-1β) levels. E. Scatter plot illustrating the relationship between miR-24-3p expression and neutrophil-to-lymphocyte ratio (NLR) levels. F. Scatter plot illustrating the relationship between miR-24-3p expression and platelet-to-lymphocyte ratio (PLR) levels.

sentation and disease severity (**Figure 4**). Patients with symptomatic carotid atherosclerotic stenosis exhibited significantly lower miR-24-3p expression compared to asymptomatic patients (median 3.98 vs 4.87, P = 0.004). In addition, miR-24-3p expression progressively declined with increasing stenosis severity. Patients with moderate stenosis (50-69%) had the highest median miR-24-3p levels, while those with near-occlusive disease (90-99%) exhibited the lowest levels (p for trend = 0.012).

Table 3. Comparison of inflammatory markers by miR-24-3p expression level

Marker	High miR-24-3p (n = 174)	Low miR-24-3p (n = 178)	Test statistic	*p* value
C-reactive protein (mg/L)	1.25 (0.66-1.85)	2.35 (1.73-3.01)	Z = -6.12	< 0.001
Interleukin-6 (pg/mL)	0.92 (0.51-1.42)	1.92 (1.38-2.38)	Z = -5.87	< 0.001
Tumor necrosis factor-α (pg/mL)	0.71 (0.39-1.12)	1.16 (0.84-1.57)	Z = -4.95	< 0.001
Interleukin-1β (pg/mL)	0.51 (0.16-0.82)	0.83 (0.59-1.15)	Z = -4.32	< 0.001
Neutrophil-to-lymphocyte ratio	2.37 (1.81-3.13)	2.25 (1.79-2.82)	Z = -1.21	0.226
Platelet-to-lymphocyte ratio	123.75 (101.52-155.75)	120.40 (94.05-148.45)	Z = -0.98	0.321

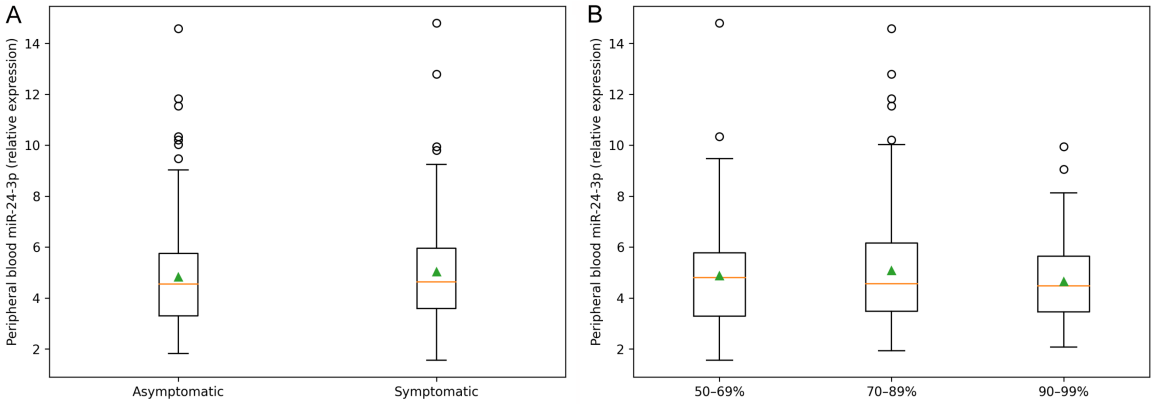

Figure 4. Peripheral blood miR-24-3p expression across clinical subgroups of carotid atherosclerotic stenosis (CAS). A. Comparison of miR-24-3p expression between asymptomatic and symptomatic patients. B. Comparison of miR-24-3p expression across stenosis severity categories (50-69%, 70-89%, and 90-99%).

Diagnostic performance of miR-24-3p for elevated systemic inflammation

Receiver operating characteristic analysis was conducted to evaluate the discriminatory ability of miR-24-3p expression for identifying patients with elevated systemic inflammation, defined as CRP levels above the cohort median. CRP was chosen as the primary endpoint for ROC analysis because it is a standardized and widely accepted marker of systemic inflammation in clinical practice. Other systemic inflammation indices, including IL-6, TNF-α, IL-1β, NLR, and PLR, were analyzed in exploratory and correlation analyses. These secondary markers showed consistent trends with CRP, confirming the robustness of the association between miR-24-3p expression and systemic inflammatory burden. MiR-24-3p yielded an area under the curve (AUC) of 0.74 (95% CI: 0.69-0.79), indicating moderate discrimination. The ROC curve is shown in **Figure 5A**, with sensitivity and specificity values corresponding to selected cut-off points. To further explore potential gradients, inflammatory markers were examined across miR-24-3p tertiles. As shown in **Figure 5B**, a stepwise increase in CRP, IL-6, and NLR levels was observed with decreasing miR-24-3p expression (p for trend < 0.001 for all comparisons), suggesting a dose-dependent association.

Multivariable association between miR-24-3p expression and elevated systemic inflammation

Multivariable logistic regression analyses were performed to assess whether the observed associations between miR-24-3p expression and systemic inflammation persisted after adjustment for potential confounders (**Tables 4** and **5**). After adjustment for age, sex, body mass index, hypertension, diabetes mellitus, dyslipidemia, smoking status, and statin use, lower miR-24-3p expression remained independently associated with elevated CRP levels (adjusted OR per unit decrease = 0.45, 95% CI: 0.37-0.55, P < 0.001). Consistent results were observed when IL-6 and NLR were used as alternative inflammatory outcomes. Subgroup analyses demonstrated that the inverse association between miR-24-3p expression and

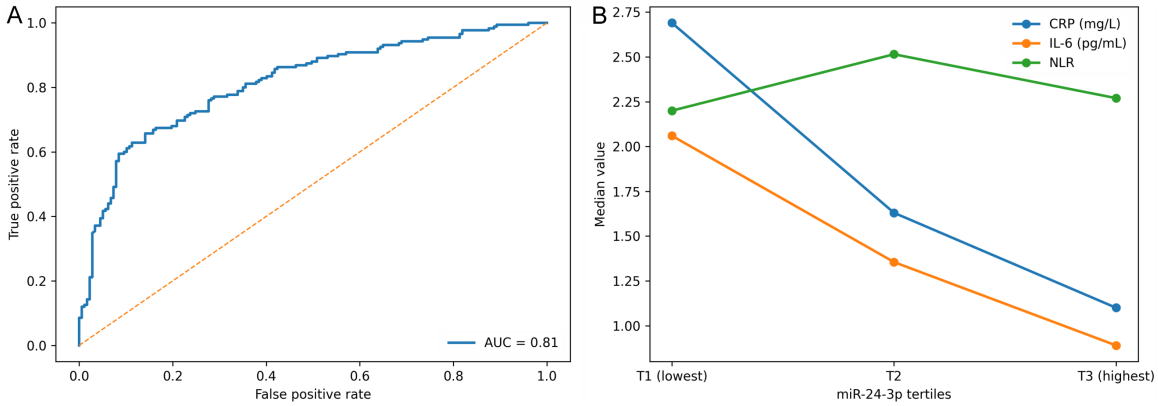

Figure 5. Receiver operating characteristic (ROC) curve of miR-24-3p expression for elevated systemic inflammation, and dose-response relationship between miR-24-3p expression tertiles and inflammatory markers. A. ROC curve evaluating the discriminatory performance of peripheral blood miR-24-3p expression for identifying elevated systemic inflammation, defined as serum CRP levels above the cohort median. B. A stepwise increase in inflammatory marker levels is observed with decreasing miR-24-3p expression.

Table 4. Univariate logistic regression analyses of factors associated with elevated systemic inflammation

Variable	Univariate OR	95% CI	*p* value
miR-24-3p (per unit increase)	0.45	0.38-0.55	< 0.001
Age (years)	1	0.97-1.02	0.824
Male sex	1.21	0.77-1.88	0.408
Body mass index (kg/m^2)	1.03	0.96-1.10	0.446
Hypertension	0.82	0.52-1.28	0.378
Diabetes mellitus	0.71	0.45-1.11	0.131
Dyslipidemia	1	0.66-1.54	0.985

Table 5. Multivariable logistic regression analyses of factors associated with elevated systemic inflammation

Variable	Adjusted OR	95% CI	*p* value
miR-24-3p (per unit increase)	0.45	0.37-0.55	< 0.001
Age (years)	1	0.97-1.03	0.953
Male sex	1.24	0.73-2.10	0.433
Body mass index (kg/m^2)	1.03	0.95-1.11	0.521
Hypertension	0.83	0.48-1.43	0.504
Diabetes mellitus	0.61	0.36-1.05	0.072
Dyslipidemia	1.1	0.66-1.82	0.713

elevated systemic inflammation was consistent across symptomatic status and stenosis severity categories, with similar effect sizes observed in both univariate and multivariable models (**Tables 6** and **7**). The adjusted effect estimates for these models are summarized graphically in **Figure 6**, demonstrating consistent direction and magnitude of association across inflammatory endpoints.

Discussion

This study retrospectively included 352 patients with imaging-confirmed CAS. A consistent inverse association was observed between circulating miR-24-3p levels and systemic inflammatory burden. Lower miR-24-3p expression correlated significantly with CRP, IL-6, TNF-α, IL-1β, and hematologic inflammatory indices, and these relationships persisted after multivariable adjustment. In addition, reduced miR-24-3p levels were observed in symptomatic patients and in those with more advanced stenosis. These findings suggest that circulating miR-24-3p reflects inflammatory activity in CAS and may provide clinically relevant information beyond stenosis severity alone. Mechanistically, miR-24-3p has been implicated in the regulation of inflammatory signaling pathways, particularly through modulation of NF-κB activation and downstream cytokine production [18, 19]. Reduced miR-24-3p expression has been associated with enhanced expression of pro-inflammatory mediators, including IL-6, TNF-α, and IL-1β, suggesting that miR-24-3p may function as a negative regulator of vascular inflammation. The inverse correlations observed in the present study are therefore consistent with these experimental findings and support a biologically plausible

Table 6. Subgroup univariate logistic regression analyses of miR-24-3p and elevated systemic inflammation

Subgroup	Univariate OR	95% CI	*p* value
Symptomatic CAS	0.43	0.32-0.59	< 0.001
Asymptomatic CAS	0.47	0.37-0.60	< 0.001
Moderate stenosis (50-69%)	0.46	0.34-0.61	< 0.001
Severe/near-occlusive stenosis (≥ 70%)	0.45	0.35-0.57	< 0.001

CAS, carotid atherosclerotic stenosis.

Table 7. Subgroup multivariable logistic regression analyses of miR-24-3p and elevated systemic inflammation

Subgroup	Adjusted OR	95% CI	*p* value
Symptomatic CAS	0.42	0.31-0.57	< 0.001
Asymptomatic CAS	0.45	0.35-0.58	< 0.001
Moderate stenosis (50-69%)	0.43	0.32-0.59	< 0.001
Severe/near-occlusive stenosis (≥ 70%)	0.44	0.34-0.57	< 0.001

CAS, carotid atherosclerotic stenosis.

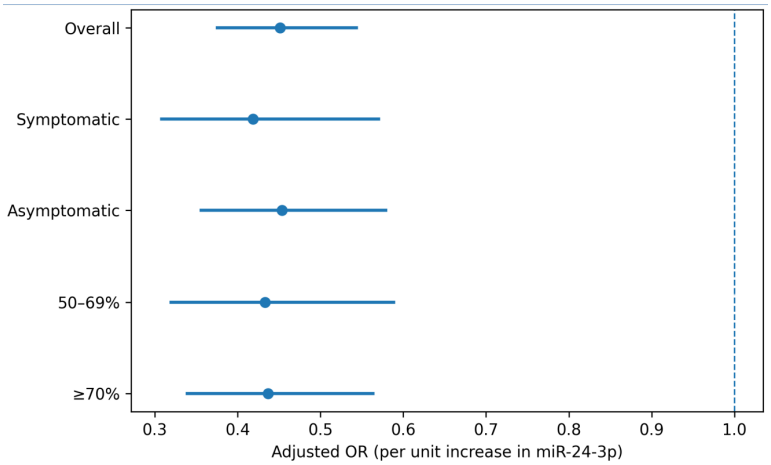

Figure 6. Forest plot of adjusted association between miR-24-3p expression and elevated systemic inflammation. Forest plot displaying adjusted odds ratios (ORs) with 95% confidence intervals for the association between miR-24-3p expression and elevated CRP levels in the overall cohort and predefined subgroups (symptomatic status and stenosis severity categories). Multivariable models were adjusted for age, sex, body mass index, hypertension, diabetes mellitus, and dyslipidemia.

link between miR-24-3p and systemic inflammatory activity.

Atherosclerosis is widely recognized as a chronic inflammatory disease characterized by immune activation and cytokine signaling throughout plaque development and destabilization [5, 6, 22]. In carotid diseases, inflammatory activity within plaques contributes to fibrous cap thinning, thrombogenicity, and embolic risk [3]. Traditional inflammatory markers such as CRP have prognostic value but limited specificity, as they reflect systemic acute-phase responses rather than vascular-specific processes [8, 9]. Therefore, molecular biomarkers capable of capturing upstream regulatory signals are of considerable interest. Circulating miRNAs have emerged as promising biomarkers in vascular disease. Their stability in peripheral blood, conferred by packaging in extracellular vesicles or association with RNA-binding proteins, allows reliable quantification in clinical samples [10, 11, 23]. Several studies have demonstrated altered circulating miRNA signatures in coronary artery disease and acute myocardial infarction [12, 13]. In cerebrovascular diseases, distinct plasma miR patterns have been reported in ischemic stroke, with potential diagnostic and prognostic implications [14, 21]. However, research specifically focused on carotid stenosis remains comparatively limited.

Importantly, human studies examining miRs in carotid atherosclerosis have demonstrated differential expression between symptomatic and asymptomatic patients. Zampetaki et al. reported endothelial-related miR alterations in vascular disease populations [13], while other profiling studies identified circulating miRs associated with plaque instability and cerebrovascular events [24]. A recent review on miRs in carotid plaque biology highlighted their potential role as noninvasive biomarkers reflecting plaque vulnerability rather than luminal narrowing

alone [25]. Our observation that symptomatic CAS patients exhibited lower miR-24-3p levels is consistent with these reports and suggests that miR-24-3p may align with inflammatory plaque activity. Notably, differences between studies may arise from heterogeneity in study populations, disease stages, and biological sample type, as well as variations in miR detection platforms and normalization strategies [11, 12, 25]. Previous investigations of circulating miRs in cardiovascular and cerebrovascular diseases have reported inconsistent expression patterns across cohorts, partly due to differences in patient selection, comorbidity burden, and analytical methodologies [11, 12]. In addition, many earlier studies were limited by relatively small sample sizes or included heterogeneous cardiovascular conditions, which may reduce the robustness and generalizability of their findings [25]. Compared to these studies, the present study provides more reliable evidence by focusing on a relatively large and clinically well-characterized cohort of patients with carotid atherosclerotic stenosis, thereby reducing population heterogeneity and enhancing internal validity.

Although most carotid-focused miR studies have evaluated broader panels rather than individual targets, recent clinical data implicate miR-24 in cardiovascular conditions characterized by inflammatory activation. Circulating miR-24 levels have been reported to differ in coronary heart disease and metabolic disorders [15, 19]. In ischemic stroke cohorts, dysregulated miR profiles including inflammation-associated miRNAs have been described [21]. While miR-24 has not been as extensively characterized as miR-126 or miR-21 in stroke populations, its inclusion in inflammatory miRNA networks supports biological plausibility. Our findings extend this literature by providing quantitative evidence linking circulating miR-24-3p levels to inflammatory markers across a relatively large CAS cohort. The stepwise decline of miR-24-3p across stenosis severity categories suggests a graded association with disease activity. Moreover, the persistence of associations after adjusting for age, diabetes, hypertension, dyslipidemia, and statin use indicates that the relationship is not merely a reflection of conventional vascular risk factors.

Clinically, the moderate discriminatory performance suggests that miR-24-3p should not be considered a standalone diagnostic marker. However, previous research indicates that multimarker strategies integrating imaging findings with molecular signatures may improve risk prediction in carotid disease [25]. Given that classical surgical trials such as NASCET demonstrated that stroke risk is influenced by factors beyond stenosis percentage alone, incorporation of inflammatory biomarkers may enhance individualized stratification.

Several limitations of the current study should be acknowledged. First, the retrospective design limits causal inference, and the observed associations cannot establish a direct mechanistic role of miR-24-3p in inflammatory regulation. Second, inflammatory markers and miRNA levels were measured at a single time point, which may not have captured temporal variability or dynamic changes in inflammatory status, possibly leading to misclassification bias. Third, although major vascular risk factors were adjusted for, residual confounding from unmeasured variables, such as medication adherence, lifestyle factors, or subclinical inflammatory conditions, cannot be excluded. Fourth, the lack of direct plaque characterization, including imaging-based assessment of plaque vulnerability or histopathological validation, limits the ability to link circulating miR-24-3p levels with local vascular inflammation. Finally, the absence of external validation restricts the generalizability of the findings, and independent prospective cohorts are required to confirm these results. Despite these limitations, the study benefits from a relatively large sample size, standardized imaging classification, and comprehensive inflammatory profiling. The consistent inverse correlations across multiple inflammatory endpoints reinforce the robustness of the findings.

Collectively, these findings demonstrate that circulating miR-24-3p levels are inversely associated with systemic inflammatory burden and correlate with clinical severity in CAS. These data support further investigation of miR-24-3p as a noninvasive biomarker reflecting inflammatory activity in related disorders. While miR-24-3p alone may not be sufficient as a standalone diagnostic tool, its integration with established inflammatory markers and imaging findings may improve risk stratification and provide additional insight into disease activity. However, given the limitations associated with the retro-

spective design, prospective validation and integration with imaging findings may clarify its role in future risk stratification frameworks.

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Disclosure of conflict of interest

None.

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