

Diagnostic values of NLR and miR-141 in patients with osteoarthritis and their association with severity of knee osteoarthritis

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Abstract. The present study aimed to explore the diagnostic values of neutrophil-lymphocyte ratio (NLR) and microRNA (miR)-141 in patients with osteoarthritis and their association with the severity of knee osteoarthritis. In total 142 patients with osteoarthritis (the study group) admitted to Shanghai TCM-Integrated Hospital, Shanghai University of TCM from January 2017 to January 2019 and 150 healthy controls (the control group) were enrolled in the present study. NLR and miR-141 in peripheral blood and their diagnostic values for osteoarthritis were compared between the two groups. The two indicators in the study group were significantly increased ($P < 0.001$), and their combined detection had a better diagnostic value for the disease ($P < 0.001$). Moreover, they were closely associated to the progression of the disease and were independent risk factors ($P < 0.001$). To sum up, NLR and miR-141 were significantly increased in the peripheral blood of patients with osteoarthritis. Their combined detection exhibited a good diagnostic value for the disease and may become a potential therapeutic target osteoarthritis in the future.

Introduction

Osteoarthritis is a non-inflammatory and degenerative joint disease that is mainly characterized by arthralgia and ankylosis (1). At present, the disease mostly occurs in women, and its prevalence increases with age (2). Statistics have revealed that it affected approximately 240 million people around the world in 2016 (3), with its incidence having increased from 13.8 to 21.6% in 2018 (4). Although the cause of the disease

remains unclear, studies at home and abroad suggest that the cause is closely related to age and obesity, and possibly correlated with excessive joint activity, arthrotrauma, heredity, and osteoporosis (5,6). Osteoarthritis mainly occurs in cartilage. The original and normal cartilage tissue undergoes morphological changes and destruction, and then forms new spurs at the articular margin (7). Moreover, the disease may also involve ligaments, joint capsules, synovium, and surrounding muscles during its course. Patients with slight conditions suffer from arthralgia and limited daily activities, while those with serious conditions suffer from joint dysfunction and paralysis (8,9). Currently, osteoarthritis is diagnosed by complex joint examinations, which include erythrocyte sedimentation rate, mucin, rheumatoid factors, X-ray, CT, and MRI; however, the disease has generally progressed to the advanced stage when detected by bone scanning and arthroscopy (10).

The challenges produced by osteoarthritis have become increasingly serious, thus researchers at home and abroad are striving to find an early screening program that is more effective, in order to improve the rehabilitation effect on patients. With the advancement of research, it has been revealed that as non-coding short-stranded RNAs with a length of approximately 22 nt, microRNAs (miRs) inhibit the translation and transcription of target genes by binding to the 3' untranslated region (3'UTR) of downstream target gene mRNAs, thus altering the expression of the target gene (11). microRNAs play an important role in numerous diseases (12). It has been confirmed that microRNA-141 (miR-141) inhibits bone resorption and has aberrant expression in osteoporosis rats, which is closely related to changes in normal bone morphology (13,14). However, its role in osteoarthritis remains unclear. It is theorized that this miR may be closely correlated with osteoarthritis, and thus may be particularly significant to the future diagnosis and treatment of the disease. Neutrophil-lymphocyte ratio (NLR) has a great predictive value for coronary artery disease (15), and a significant monitoring value for inflammation development (16). Therefore, miR-141 and NLR in patients with osteoarthritis were analyzed in the present study, to explore their significance and provide new insights for the clinical diagnosis and treatment of the disease.

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Key words: neutrophil-lymphocyte ratio, miR-141, osteoarthritis, related factors

Materials and methods

General information. One hundred and forty-two patients with osteoarthritis (the study group) admitted to Shanghai TCM-Integrated Hospital, Shanghai University of TCM from January 2017 to January 2019 and 150 healthy controls (the control group) were enrolled in this study for a prospective analysis. The present study was approved by the Ethics Committee of Shanghai TCM-Integrated Hospital, Shanghai University of TCM. All research subjects or their immediate families signed the informed consent form.

Inclusion and exclusion criteria. Inclusion criteria were as follows: i) Patients who met clinical manifestations of knee osteoarthritis (17); ii) patients confirmed with knee osteoarthritis by a series of examinations at Shanghai TCM-Integrated Hospital, Shanghai University of TCM; iii) patients with complete medical records; iv) patients who had not received any antibiotic treatment within 3 months before admission; iv) patients willing to cooperate and participate in the investigation of medical staff at Shanghai TCM-Integrated Hospital, Shanghai University of TCM. Exclusion criteria were as follows: i) Patients complicated with tumors, cardiovascular and cerebrovascular diseases, autoimmune diseases, organ dysfunction, infectious diseases, or neurological disorders; ii) patients allergic to drugs used in the present study; iii) patients with physical disabilities and who could not take care of themselves; iv) patients who transferred to other hospitals.

Methods. After admission, the patients were orally administered loxoprofen sodium tablets (Daiichi Sankyo Pharmaceutical (Shanghai) Co., Ltd.; State Food and Drug Administration (China) Approval no. H20030769), 60 mg x3 times/day, and Gukang capsules (Guizhou Weikang Zifan Pharmaceutical Co., Ltd.; SFDA Approval no. Z20025657), 0.4 g/capsule, 3 capsules x3 times/day. The course of treatment was one month in total. Before treatment, the fasting venous blood (5 ml) of patients was extracted and placed into EDTA anticoagulant tubes (2 ml) and blood collection tubes (3 ml) containing inert separation gel and coagulants. NLR expression in the venous blood of the EDTA anticoagulant tubes was detected by a flow cytometer, whereas the venous blood in the blood collection tubes was centrifuged at 1,505 x g for 10 min at 24°C, to collect the serum. Part of the serum was used for subsequent experiments, while the rest was placed in an RNase-free EP tube and stored at -80°C for later use.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR) detection. An EasyPure miRNA kit (cat. no. ER601-01; Beijing TransGen Biotech Co., Ltd.) was used to extract total RNA from the collected serum. An ultraviolet spectrophotometer and agarose gel electrophoresis were used to detect its purity, concentration, and integrity. TransScript® miRNA RT Enzyme Mix and 2xTS miRNA Reaction Mix (cat. no. AT351-01; Beijing TransGen Biotech Co., Ltd.) were used to reversely transcribe the total RNA, with the steps in strict accordance with the manufacturer's kit. Then, PCR amplification was carried out. The system

consisted of 1 µl of cDNA, each 0.4 µl of upstream and downstream primers, 10 µl of TranStart® Tip Green qPCR SuperMix (cat. no. AQ141-01; Beijing TransGen Biotech Co., Ltd.), 0.4 µl of Passive Reference Dye (50X) (cat. no. 600536; Beijing Biomars-Technology Co., Ltd.), and ddH₂O finally added to make up to 20 µl. The conditions were as follows: Pre-denaturation at 94°C for 30 sec, denaturation at 94°C for 5 sec, annealing and extension at 60°C for 30 sec, for a total of 40 cycles. Each sample was provided with 3 repeated wells, and the experiment was conducted in triplicate. In the present study, U6 was used as an internal reference and 2^{-ΔΔCq} was used to analyze the data (18). Primer sequences are presented in Table I.

Outcome measures. Assessments included: i) Differences in miR-141 and NLR expression; ii) diagnostic values of miR-141 and NLR for osteoarthritis; iii) correlations of miR-141 and NLR with the clinical pathology of osteoarthritis; iv) correlations of miR-141 and NLR with Lysholm Knee Scoring Scale (LKSS) (19), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) score (20), and visual analogue scale (VAS) score (21) in the study group; v) correlation of miR-141 with NLR in the study group; and iv) risk factors for osteoarthritis.

Statistical methods. SPSS 24.0 (Shanghai Yuchuang Network Technology Co., Ltd.) was used for statistical calculation. Graphpad 8 (SOFTEAD, Inc.) was used to plot figures and verify the results. Count data including sex and dwelling environment were expressed by rate, and chi-square test was used for their comparison between groups. Measurement data for miR-141 and NLR were expressed by the mean ± standard deviation. Independent sample t-test was used for comparison between groups, and paired t-test was used for comparison before and after treatment. One-way ANOVA and LSD post hoc test were used for the comparison among multiple groups. Receiver operating characteristic (ROC) curves were plotted to analyze the diagnostic and predictive values. Binary Logistic regression analysis was used for the combined detection, to obtain the constants and coefficients of the regression equation, and then the ROC curves were analyzed. Pearson correlation coefficient was used for correlation analysis. Multivariate Logistic regression was used to analyze risk factors. A P<0.05, was considered to indicate a statistically significant difference.

Results

Comparison of general information. There were significant differences between the study and control groups in past medical history and exercise habits (P<0.001), not in age, body mass index (BMI), total cholesterol, triglyceride, low density lipoprotein, creatinine, urine nitrogen, sex, smoking, dwelling environment, and nationality (P>0.050; Table II).

Comparison of miR-141 and NLR. The expression level of miR-141 in the study group was significantly higher than that in the control group (17.52±4.58 vs. 13.24±3.09) (P<0.001). NLR in the study group (2.81±1.14%) was significantly higher than in the control group (1.42±0.62%) (P<0.001; Fig. 1).

Table I. Primer sequences of miR-141 and internal reference.

Gene	Forward	Reverse
miR-141	5'-CTCAAGGCAACCTACCGAAAAG-3'	5'-TATCGGACCCATCACGGAGTGG-3'
U6	5'-GATTAGAACCGTCGGTAACGGAA-3'	5'-AGCGATCTCGTTGGCCTTTCTACC-3'
miR, microRNA.		

Table II. Comparison of general information [n (%)].

Characteristics	Study group (n=142)	Control group (n=150)	t or χ^2	P-value
Age (years)	59.4±9.1	60.1±10.2	0.618	0.537
BMI (kg/cm ²)	23.15±1.89	23.06±1.98	0.397	0.692
Total cholesterol (mmol/l)	4.42±1.20	4.52±1.09	0.746	0.456
Triglyceride (mmol/l)	1.85±0.87	1.90±0.77	0.521	0.603
Low density lipoprotein (mmol/l)	2.67±0.62	2.58±0.72	1.142	0.255
Creatinine (μ mol/l)	72.85±12.52	71.19±11.96	1.159	0.248
Urea nitrogen (mmol/l)	6.24±1.63	6.42±1.82	0.889	0.375
Course of disease (years)	3.54±1.54			
Sex			0.070	0.791
Male	35 (24.65)	39 (26.00)		
Female	107 (75.35)	111 (74.00)		
Past medical history			49.172	<0.001
Diabetes	56 (39.44)	24 (16.00)		
Hypertension	42 (29.58)	18 (12.00)		
None	44 (30.99)	108 (72.00)		
Smoking			0.024	0.876
Yes	48 (33.80)	52 (34.67)		
No	94 (66.20)	98 (65.33)		
Dwelling environment			1.417	0.233
City	92 (64.79)	87 (58.00)		
Countryside	50 (35.21)	63 (42.00)		
Nationality			0.372	0.798
Han	138 (97.18)	148 (98.67)		
Ethnic minorities	4 (2.82)	2 (1.33)		
Exercise habits			42.642	<0.001
Yes	16 (11.27)	69 (46.00)		
No	126 (88.73)	81 (54.00)		
Severity				
Early stage	38 (26.76)			
Middle and advanced stages	104 (86.62)			
Classification				
Primary	104 (73.24)			
Secondary	38 (26.76)			

BMI, body mass index.

Diagnostic values of miR-141 and NLR. According to the ROC curves, when the cut-off value was 14.85, the sensitivity and specificity of miR-141 for osteoarthritis diagnosis were 72.00 and 69.01%, respectively. When the

cut-off value was 2.51, the sensitivity and specificity of NLR were 96.67 and 67.61%, respectively. With miR-141 and NLR as independent variables, binary logistic regression analysis was performed to obtain the logistic

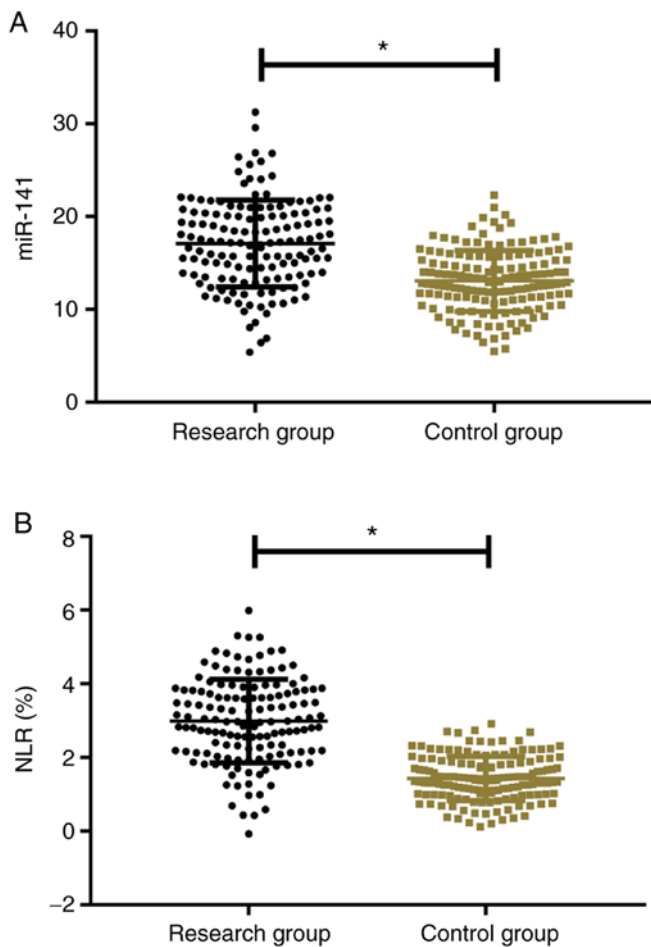


Figure 1. Comparison of miR-141 and NLR. (A) The expression level of miR-141 in the study group was significantly higher than that in the control group. * $P < 0.001$. (B) NLR in the study group was significantly higher than that in the control group. * $P < 0.001$. miR, microRNA; NLR, neutrophil-lymphocyte ratio.

regression model: $\text{Logit}(P) = -8.688 + 0.288 \text{ miR-141} + 2.088 \text{ NLR}$. When the cut-off value was 0.63, the sensitivity and specificity of the model were 95.33 and 78.87%, respectively (Tables III and IV and Fig. 2).

Clinicopathological associations between miR-141, NLR and osteoarthritis. miR-141 was closely associated to the course of disease, severity, and classification of diseases of patients with osteoarthritis ($P < 0.001$), not significantly associated with their age, BMI, total cholesterol, triglyceride, low density lipoprotein, creatinine, urine nitrogen, sex, past medical history, smoking, dwelling environment, nationality, and exercise habits ($P > 0.05$). NLR was closely associated to the age, course of disease, and severity of patients with osteoarthritis ($P < 0.001$), not significantly associated with their BMI, total cholesterol, triglyceride, low density lipoprotein, creatinine, urine nitrogen, sex, past medical history, smoking, dwelling environment, nationality, exercise habits, and classification of diseases ($P > 0.05$; Table V).

Correlations of miR-141 and NLR with LKSS, WOMAC, and VAS scores. Before treatment, LKSS, WOMAC, and VAS scores in the study group were (52.62 ± 6.21) , (63.35 ± 7.21) , and (8.26 ± 2.62) points, respectively. According to the

Table III. Analysis of ROC curves.

Parameters	miR-141	NLR	miR-141+NLR
Cut-off value	14.85	2.51	0.63
AUC	0.753	0.887	0.927
SE	0.029	0.020	0.016
95% CI	0.697-0.809	0.847-0.927	0.895-0.958
P-value	<0.001	<0.001	<0.001
Sensitivity (%)	72.00	96.67	95.33
Specificity (%)	69.01	67.61	78.87

ROC, receiver operating characteristic; miR, microRNA; NLR, neutrophil-lymphocyte ratio; AUC, area under the curve; SE, standard error; CI, confidence interval.

Table IV. Binary logistic regression analysis.

Factors	B	SE	Wald	Df	Sig	Exp (B)
miR-141	0.288	0.051	32.195	1	$P < 0.001$	1.334
NLR	2.088	0.262	63.571	1	$P < 0.001$	8.070

miR, microRNA; NLR, neutrophil-lymphocyte ratio; B, regression coefficient; SE, standard error of regression; Wald, Wald statistics; df, degrees of freedom; Sig, P-value; Exp (B), exponentiation of the B coefficient, which is an odds ratio.

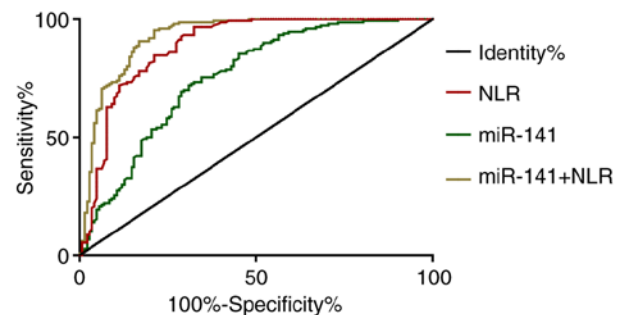


Figure 2. ROC curves of miR-141 and NLR. ROC, receiver operating characteristic; miR, microRNA; NLR, neutrophil-lymphocyte ratio.

analysis with Pearson correlation coefficient, miR-141 was negatively correlated with LKSS score ($r = -0.671$, $P < 0.001$), positively correlated with WOMAC score ($r = 0.778$, $P < 0.001$), and positively correlated with VAS score ($r = 0.846$, $P < 0.001$). NLR was negatively correlated with LKSS score ($r = -0.649$, $P < 0.001$), positively correlated with WOMAC score ($r = 0.722$, $P < 0.001$), and positively correlated with VAS score ($r = 0.825$, $P < 0.001$). In the study group, miR-141 was positively correlated with NLR ($r = 0.889$, $P < 0.001$) (Fig. 3).

Analysis of related risk factors. With the comparison of general information between the two groups as the results of univariate analysis, the indicators with differences were assigned values (Table VI). Then, forward logistic regression

Table V. Clinicopathological associations between miR-141, NLR and osteoarthritis.

Characteristics	n	miR-141	t or F-value	P-value	NLR	t or F-value	P-value
Age (years)			0.239	0.811		4.881	<0.001
<59.4	42	17.34±4.14			2.12±0.98		
≥59.4	100	17.55±5.01			3.09±1.12		
BMI (kg/cm ²)			0.408	0.684		0.245	0.807
<23.15	38	17.73±4.66			2.92±0.84		
≥23.15	104	18.14±5.52			2.97±1.15		
Total cholesterol (mmol/l)			0.666	0.506		1.034	0.303
<4.42	80	17.45±3.63			2.71±0.92		
≥4.42	62	17.88±4.04			2.90±1.27		
Triglyceride (mmol/l)			0.194	0.846		0.167	0.868
<1.85	83	17.85±3.75			2.71±0.98		
≥1.85	59	17.72±4.17			2.68±1.16		
Low density lipoprotein (mmol/l)			0.186	0.852		1.719	0.088
<2.67	79	17.57±4.87			2.99±1.12		
≥2.67	63	17.72±4.63			2.58±1.71		
Creatinine (μmol/l)			0.519	0.605		1.112	0.268
<72.85	68	17.14±4.59			3.13±1.43		
≥72.85	74	17.58±5.43			2.86±1.46		
Urea nitrogen (mmol/l)			0.180	0.857		1.131	0.260
<6.24	70	17.59±4.72			2.75±1.10		
≥6.24	72	17.73±4.54			2.52±1.31		
Course of disease (years)			7.216	<0.001		5.227	<0.001
<3.54	49	14.57±5.63			2.50±1.24		
≥3.54	93	20.24±3.69			3.62±1.20		
Sex			0.164	0.870		0.612	0.542
Male	35	17.85±3.75			2.80±1.24		
Female	107	17.72±4.17			2.92±0.92		
Past medical history			0.756	0.472		10.332	<0.001
Diabetes	56	17.73±4.54			2.85±1.11		
Hypertension	42	17.59±4.58			2.72±0.92		
None	44	18.62±3.64			2.04±0.62		
Smoking			0.281	0.779		0.559	0.577
Yes	48	18.08±3.95			2.98±1.14		
No	94	17.86±4.62			3.12±1.53		
Dwelling environment			0.024	0.981		0.779	0.437
City	92	17.16±4.59			3.12±1.20		
Countryside	50	17.14±4.86			2.96±1.11		
Nationality			0.080	0.936		0.138	0.890
Han	138	16.93±4.68			2.98±1.14		
Ethnic minorities	4	17.12±4.25			3.06±1.13		
Exercise habits			0.188	0.851		0.408	0.684
Yes	16	17.63±4.58			2.85±1.24		
No	126	17.86±4.62			2.71±1.30		
Severity			4.152	<0.001		3.628	<0.001
Early stage	19	14.62±5.86			2.12±1.63		
Middle and advanced stages	123	21.21±6.52			3.25±1.20		
Classification			12.321	<0.001		0.419	0.676
Primary	104	15.12±2.85			2.75±1.31		
Secondary	38	22.62±4.05			2.85±1.10		

miR, microRNA; NLR, neutrophil-lymphocyte ratio; BMI, body mass index.

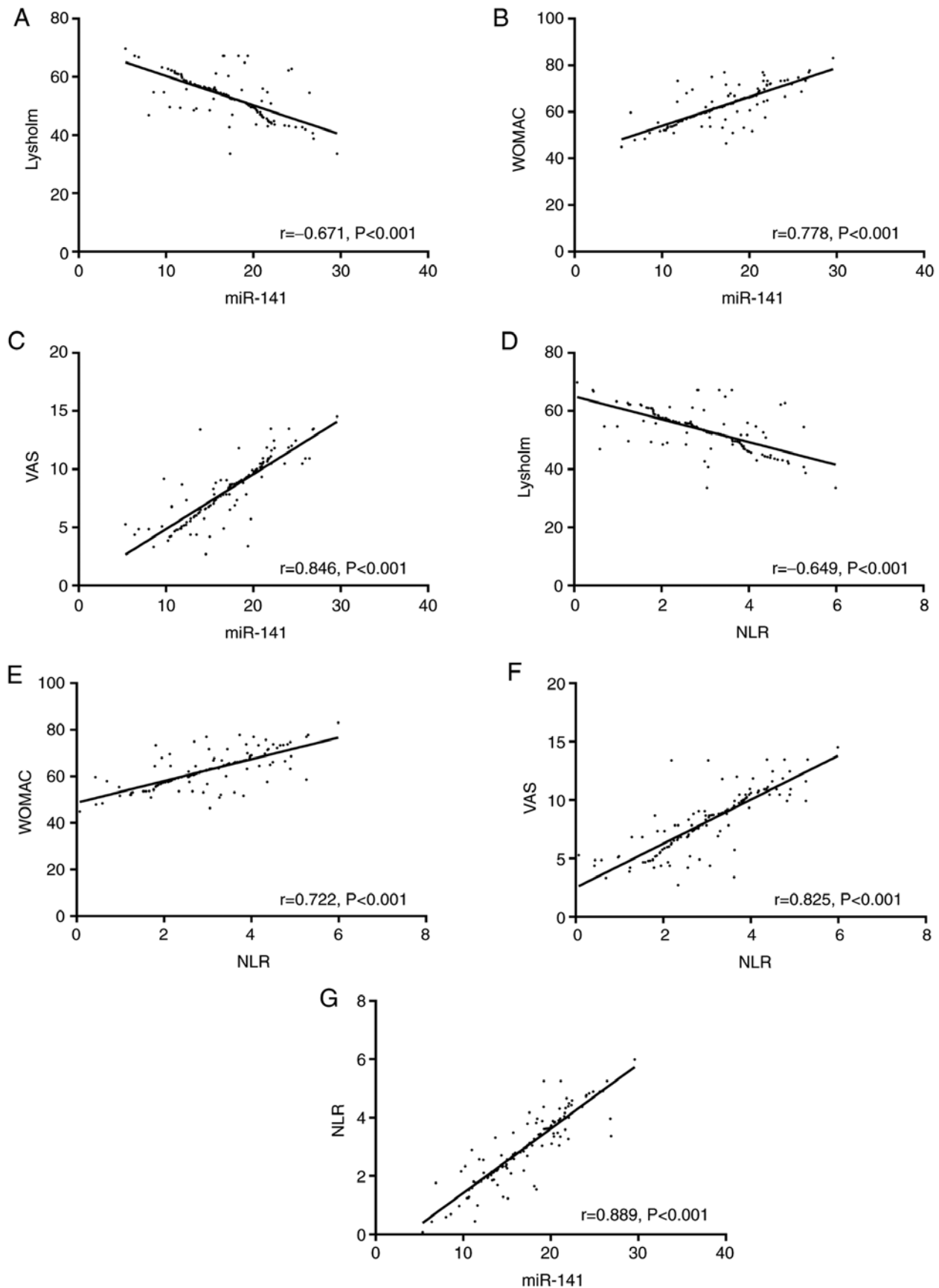


Figure 3. Correlations of miR-141 and NLR with LKSS, WOMAC, and VAS scores. (A) miR-141 was negatively correlated with LKSS score ($r = -0.671$, $P < 0.001$). (B) miR-141 was positively correlated with WOMAC score ($r = 0.778$, $P < 0.001$). (C) miR-141 was positively correlated with VAS score ($r = 0.846$, $P < 0.001$). (D) NLR was negatively correlated with LKSS score ($r = -0.649$, $P < 0.001$). (E) NLR was positively correlated with WOMAC score ($r = 0.722$, $P < 0.001$). (F) NLR was positively correlated with VAS score ($r = 0.825$, $P < 0.001$). (G) miR-141 was positively correlated with NLR ($r = 0.889$, $P < 0.001$). miR, microRNA; NLR, neutrophil-lymphocyte ratio; LKSS, Lysholm Knee Scoring Scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; VAS, visual analogue scale.

Table VI. Assignment.

Factors	Assignment
Past medical history	None=0; diabetes=1; hypertension=2
Exercise habits	Yes=0; no=1
Course of disease	A continuous variable analyzed by original data
Severity	Early stage=0; middle and advanced stages=1
Classification of diseases	Primary=0; secondary=1
miR-141	A continuous variable analyzed by original data
NLR	A continuous variable analyzed by original data

miR, microRNA; NLR, neutrophil-lymphocyte ratio.

(LR) was selected for multivariate logistic regression analysis. The results showed that the course of disease, severity, and the classification of diseases were not independent risk factors for osteoarthritis. However, past medical history (OR: 12.245; 95% CI: 2.652-54.262), miR-141 (OR: 1.256; 95% CI: 1.154-1.452), and NLR (OR: 25.627; 95% CI: 1.953-86.621) were the independent risk factors. Exercise habits (OR: 0.497; 95% CI: 0.085-3.215) was the independent protective factor for the disease (Table VII).

Discussion

As a chronic inflammation with articular cartilage degeneration as the core, osteoarthritis is mainly manifested as multi-directional and multi-level bone structural lesions (22). It causes arthralgia, joint dysfunction, and joint deformity, affecting the normal life and actions of patients (23). Therefore, a new clinical index for its early screening is being explored to effectively diagnose and treat this disease. For example, a study by Li *et al* suggested that ADAMTS-4 may be a potential marker for osteoarthritis (24). Park *et al* revealed that the detection of CTX-II in serum and urine by BeadChip had a markedly high diagnostic value for the disease (25). Previous studies have revealed that differences in miR expression are closely associated with cartilage destruction, chondrocyte apoptosis, and synovitis during the development and progression of certain osteoarticular diseases (26,27). miR-141, which belongs to the miR-200 family and is located at 12p 13.31 in the human body, was revealed to be closely related to gastric and non-small cell lung cancer (28,29), however, its role in osteoarthritis is still unclear. In the present study, the roles of miR-141 and NLR in osteoarthritis were explored, and the findings indicated that they are essential for the diagnosis and treatment of the disease.

In the present study, miR-141 and NLR were significantly increased in the peripheral blood of patients with osteo-

arthritis, suggesting that they may be involved in the development and progression of the disease. This is consistent with the findings of Dong *et al* (miR-141 in endometrial cancer) and Gundogdu *et al* (NLR in osteoarthritis) (30,31), which support the results of the present study. The mechanism of action of miR-141 in osteoarthritis remains unclear. As an osteoblastic negative regulatory factor, miR-141 inhibits the cascade action of Dlx5/Runx2/Msx2 axis after transcription, thus reducing osteogenic activities (32). Karlsson *et al* revealed that the Msx2 gene is involved in the bone formation of patients with osteoarthritis (33). Therefore, it is speculated that miR-141 may inhibit Msx2 and then inhibit the activation of osteoblasts, thereby deteriorating osteoarthritis. NLR, a blood inflammatory marker obtained through the ratio of neutrophils to lymphocytes, has a better predictive value for inflammation than traditional and single inflammatory markers (34). In our study, NLR in patients with osteoarthritis was significantly increased, suggesting that adverse conditions caused by inflammatory stimulation significantly reduce the lymphocytes of patients. The mechanism of NLR affecting the disease may be achieved through IL-6, IL-8, and TNF- α (35). A study by Goldring and Otero *et al* revealed the significant increase of the inflammatory cytokines in patients with osteoarthritis (36), which also confirms our theory. Inflammatory cytokines can enhance the cellular activity of the synovial tissue, thus neutrophils, platelets, or lymphocytes in peripheral blood significantly increase, while other indices are stable in patients with tumors and immune diseases (37). Therefore, NLR is commonly used as an indicator to evaluate the health of patients, however its diagnostic specificity for a certain disease is poor (38). In the present study, the combined detection of miR-141 and NLR had better sensitivity and specificity for predicting osteoarthritis. This reveals that the combined detection can be used for the early screening of osteoarthritis in clinical practice, and can improve its early diagnostic rate, which is important for the early treatment of the patients. At present, the diagnosis of osteoarthritis depends on imaging technology. The detection of miR-141 combined with NLR has several advantages. Firstly, the evaluation is objective. The assessment of results is not affected by the previous experience and subjective consciousness of doctors. Secondly, detection methods are simple. The peripheral blood is only needed for detection. Thirdly, the preservation time is long. The preservation time of peripheral blood in the low-temperature environment is long and the detection is less affected by environmental factors (39).

According to the analysis of the correlations of miR-141 and NLR with clinical pathology, miR-141 was closely associated to the course of disease, severity, and the classification of diseases. NLR was closely associated to age (40), past medical history, the course of disease, and severity. These results indicated that miR-141 and NLR are closely associated with osteoarthritis progression. There were differences in NLR between patients of different ages. This may be because the immune and metabolic functions of older patients decline, which makes them more vulnerable to inflammatory responses and diseases. Similarly, the findings of Soysal *et al* (41), support this theory. There were significant differences in the two indicators between patients with different classification

Table VII. Multivariate logistic analysis.

Factors	B	SE	Wald	Sig	OR	95% CI	
						Lower	Upper
Past medical history	2.524	0.862	9.412	0.002	12.245	2.652	54.262
Exercise habits	1.168	0.983	1.512	0.026	0.497	0.085	3.215
miR-141	0.225	0.035	54.216	P<0.001	1.256	1.154	1.452
NLR	3.542	1.236	5.921	0.012	25.627	1.953	86.621

miR, microRNA; NLR, neutrophil-lymphocyte ratio; B, regression coefficient; SE, standard error of regression; Wald, Wald statistics; df, degrees of freedom; Sig, P-value; OR, odds ratio.

of diseases, which may be due to the fact that patients with primary osteoarthritis usually have no trauma, infection, congenital malformation, genetic defects, and abnormalities in systemic metabolism and endocrine, with lighter conditions and lower treatment difficulty compared with those of patients with secondary arthritis (osteoarthritis occurring based on local original joint lesions). This is similar to the experimental results of Lunebourg *et al* (42). According to the correlations of miR-141 and NLR with LKSS, WOMAC, and VAS scores, miR-141 and NLR were negatively correlated with LKSS score, but positively correlated with WOMAC and VAS scores. This suggests that the two indicators are closely related to the conditions of patients with osteoarthritis. The two were also significantly correlated, which indicates that they have consistent synergy and may be closely related to the inflammatory responses of patients. According to the multivariate logistic regression analysis, past medical history, miR-141, and NLR were independent risk factors for osteoarthritis, demonstrating that miR-141 and NLR may be potential therapeutic targets for the disease. Exercise habits were the independent protective factor for the disease, suggesting that proper exercise can prevent its development, thus the importance of regular exercise should be emphasized for middle-aged people.

The present study aimed to explore the roles of miR-141 and NLR in osteoarthritis, but it has limitations due to the limited experimental conditions. For example, the underlying mechanisms of the effects of miR-141 and NLR in osteoarthritis are still conjecture and lack the support of basic experiments. We hope in future that relevant experimental analysis can be conducted according to our conjecture. In addition, the age range of the subjects included in the present study was small and the differences in regions and ethnicities was limited, therefore the results may be biased. Moreover, sequences of the miR family are numerous, thus there may be other miRs especially expressed in osteoarthritis, which require further analysis and discussion. In future, this study will be improved in order to achieve optimal experimental results.

In summary, miR-141 and NLR were significantly increased in the peripheral blood of patients with osteoarthritis. Their combined detection has a good diagnostic value for the disease and may become a potential therapeutic target for it in the future.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

RZ conceived the study and wrote the manuscript. CC performed the PCR experiments. XX analyzed and interpreted the data of patients. GL and HG helped with the statistical analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Shanghai TCM-Integrated Hospital, Shanghai University of TCM. Patients who participated in the present research, signed the informed consent and had complete clinical data. Signed written informed consents were obtained from the patients and/or guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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