

The diagnostic and prognostic values of microRNA-21 in patients with gastric cancer: a meta-analysis

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Abstract. – **OBJECTIVE:** Previous studies demonstrated the pivotal role of miR-21 in the pathogenesis and progression of gastric cancer. This meta-analysis was designed to confirm the diagnostic and prognostic value of microRNA-21 (miR-21) in gastric cancer and to investigate the influence of samples on the results of miR-21 detection.

MATERIALS AND METHODS: A literature search was conducted in PubMed and Embase databases up to August 2016. The pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and area under the summary receiver-operating characteristic (AUC) were calculated by Meta-Disc (version 14.0) or RevMan 5.3 software.

RESULTS: Ten studies including 516 patients with gastric cancer and 239 healthy controls were selected. Pooled sensitivity (0.74, 95% CI: 0.69-0.79), specificity (0.81, 95% CI: 0.76-0.86), PLR (3.85, 95% CI: 3.00-4.94), NLR (0.22, 95% CI: 0.31-0.45), and DOR (13.07, 95% CI: 8.81-19.39) as well as AUC (0.8561 ± 0.0204) indicated the good diagnostic accuracy of miR-21 in detecting gastric cancer. The prognostic value of miR-21 for lymph node metastasis in gastric cancer was also demonstrated by the pooled sensitivity (0.56, 95% CI: 0.48-0.64), specificity (0.62, 95% CI: 0.53-0.71), PLR (2.02, 95% CI: 0.90-4.54), NLR (0.58, 95% CI: 0.45-0.75), and DOR (3.50, 95% CI: 1.04-11.83) as well as AUC (0.6673 ± 0.0469). Subgroup analyses showed that the samples used to detect miR-21 were the source of heterogeneity could affect the diagnostic or prognostic value of miR-21 in gastric cancer.

CONCLUSIONS: miR-21 can be used for the diagnosis of gastric cancer and prognosis of lymph node metastasis in gastric cancer.

Key Words:

MicroRNA-21, Gastric cancer, Diagnosis, Prognosis, Meta-analysis.

Introduction

Although the incidence of gastric cancer has been decreasing over the years, it still ranks as the 4th most common malignancy and is one of the leading causes of cancer death in recent statistics, especially in less developed countries¹⁻⁴. Currently, gastroscopy is still the standard procedure for the clinical diagnosis of gastric cancer⁵. However, its invasive nature influences the wide application of gastroscopy in the early diagnosis of gastric cancer. Moreover, the clinical symptoms of an early stage gastric cancer are not specific enough for an early diagnosis of gastric cancer. Thus, almost one-third of gastric cancer cases are diagnosed at late stages. Moreover, the survival of patients with late stage (stage III or IV) gastric cancer is still poor despite the progress in medical technology in recent years⁶⁻⁸. Therefore, it is urgent to further explore new methods for an early diagnosis of gastric cancer and to improve its poor prognosis.

Currently, some biomarkers are used for the diagnosis or prognosis of gastric cancer such as microRNAs (miRNAs), which are related to the generation and development of cancer⁹⁻¹². Evidence shows that patients with gastric cancer present high levels of serum microRNA-21 (miR-21)¹³, which plays a pivotal role in the pathogenesis and progression of gastric cancer¹⁴. A previous meta-analysis¹⁵ indicated the good diagnostic accuracy of miR-21 in detecting gastric cancer. Another meta-analysis showed the potential of miR-21 as a prognostic factor for gastric cancer¹⁶. However, subgroup analyses should be performed to explore the sources of

heterogeneity and controversies still exist based on recent studies¹⁷⁻¹⁹. Therefore, we performed this updated meta-analysis to further confirm the diagnostic and prognostic values of miR-21 and explored the sources of heterogeneity by subgroup analyses. Also, we specifically assessed the predictive accuracy of miR-21 for lymph node metastasis in gastric cancer.

Materials and Methods

Search Strategy

A literature search was conducted in PubMed and Embase databases up to August 2016. The following key words were used: (“gastric cancer” or “gastric tumor” or “gastric carcinoma”) and (“microRNA-21” or “miRNA-21” or “miR-21”). Besides, we manually scanned the reference lists of some relevant reviews to select additional studies.

Inclusion and Exclusion Criteria

All included studies should meet the following criteria: (1) participants were patients with gastric cancer; (2) the level of miR-21 in tissue or blood was detected; (3) the diagnostic value or prognostic significance of miR-21 in gastric cancer was assessed; (4) the false positive (FP), false negative (FN), true positive (TP), and true negative (TN) values were reported or could be obtained by calculation.

The studies were excluded when they were: (1) duplicated publications; (2) letters, comments, or reviews, (3) studies without available data.

Data Extraction and Quality Assessment

Two investigators independently reviewed the full texts of included studies and assessed their quality. Differences were resolved by discussion to ensure consistency. The following data should be recorded in a predesigned form: first author, year of publication, country, ethnicity, sample size, age, sex, TNM stage of gastric cancer, method of detecting miR-21, cut-off value, and outcomes (TP, FP, FN, and TN).

The revised Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) criteria were used to assess the quality of these included studies²⁰, which include 4 key domains and

seven questions. Each question is answered by “yes,” “no,” or “unclear.” A “yes” answer represents the low-risk of bias and is assigned a score of 1, while a “no” or “unclear” answer represents a high-risk of bias and is assigned a score of 0. The studies were considered as low-quality studies when less than 4 scores were obtained.

Statistical Analysis

All analyses were performed using Meta-Disc (version 14.0) or RevMan 5.3 software. The X^2 and I^2 tests were used to assess the heterogeneity among studies. A p -value < 0.1 or $I^2 > 50\%$ indicated the existence of significant heterogeneity. An appropriate statistical model (random or fixed effects model) was used to calculate the pooled odds ratio (OR), sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR) as well as the corresponding 95% confidence interval (CI), based on the results of heterogeneity testing. In addition, the summary receiver operating characteristic (SROC) curves were also plotted to synthetically assess the diagnostic accuracy of miR-21 in detecting gastric cancer and its predictive accuracy for lymph node metastasis in patients with gastric cancer. Also, subgroup analyses were performed based on the source of miR-21.

Results

Characteristics of Included Studies

The flow chart of the literature search and study selection is presented in Figure 1. The initial literature search identified 439 articles (331 from PubMed and 108 from Embase). No additional studies were selected by manual search. After excluding duplicates, 172 potentially relevant articles remained. Among them, 147 articles were removed by scanning the titles and abstracts based on the inclusion and exclusion criteria. After reading the full-texts, 15 articles were excluded. Finally, 10 articles were included in this meta-analysis^{13,17-19,21-26}.

The main characteristics of the included articles are listed in Table I. Among them, four studies reported the diagnostic value of miR-21 for gastric cancer^{17,18,24,26}, five studies analyzed the prognostic role of miR-21 in patients with

gastric cancer^{13,21-23,25}, and one study assessed both the diagnostic and prognostic values of miR-21 in gastric cancer¹⁹. A total of 516 gastric cancer patients and 239 healthy controls were reanalyzed in the present meta-analysis. The publication years of these included articles ranged from 2008 to 2015. In all included studies, gastric cancer was diagnosed based on gastroscopy screening, which is considered the gold standard for gastric cancer diagnosis. The detection of miR-21 was performed using a quantitative real-time reverse transcription-PCR (qRT-PCR) method in all studies. The sources of miR-21 were plasma (n = 2)^{17,21}, serum (n = 4)^{18,19,24}, peripheral blood mononuclear cells (PBMC) (n = 1)¹⁹, and blood or tissue (n = 4)^{13,22,23,25} in these studies. The levels of miR-21 in both serum and PBMC were investigated by Wu et al¹⁹. The cut-off values of miR-21 were different among these included studies. Also, based on the revised QUADAS-2, no low-quality studies were included in this meta-analysis (Table I).

Diagnostic Accuracy of miR-21 for Gastric Cancer

As shown in Figure 2, significant heterogeneity ($I^2 > 50\%$, $p < 0.10$) among studies was observed when pooling sensitivity, specificity, and NLR. Thus, the random-effects model was used. Meanwhile, the fixed effects model was applied when pooling the PLR and DOR because no significant heterogeneity was detected among the included studies ($I^2 < 50\%$, $p > 0.10$). The pooled sensitivity (0.74, 95% CI: 0.69-0.79), specificity (0.81, 95% CI: 0.76-0.86), PLR (3.85, 95% CI: 3.00-4.94), NLR (0.22, 95% CI: 0.31-0.45), and DOR (13.07, 95% CI: 8.81-19.39) as well as the area under SROC ($AUC = 0.8561 \pm 0.0204$) indicated the good diagnostic accuracy of miR-21 in detecting gastric cancer.

In subgroup analyses, we reanalyzed the diagnostic accuracy of serum miR-21 for gastric cancer, which showed consistent results with the overall analyses. A significant heterogeneity among studies still existed when pooling sensitivity, specificity, and NLR in subgroup analyses, indicating that there were other sources of heterogeneity among works (Figure 3).

The subgroup analyses for diagnostic accuracy of the blood, plasma, PBMC, and tissue miR-21 could not be performed due to the limited number of papers.

Table I. Characteristics of the studies selected for the meta-analysis.

First author, year	Gastric cancer patients				Healthy controls			TNM Stage	Sample	Cut-off value	QUADAS-2
	Country	No.	Age	Male ratio	No.	Age	Male ratio				
Li BS, 2012	China	60	54	0.70	60	51	0.63	Stage I-IV	Plasma	$2^{-\Delta\Delta Ct}=0.5$	4
Shiotani A, 2013	Japan	62	67.8	0.71	70	66.7	0.67	Stage I-II	Serum	Ct=0.04	5
Wang B, 2012	China	30	58	0.73	39	46	0.77	Stage I-IV	Serum	$2^{-\Delta\Delta Ct}=5.63$	4
Zheng Y, 2011	China	53	60	0.66	20	60	0.66	Stage I-IV	Blood	$-\Delta Ct=7.73$	5
Wu J, 2015 (1)*	China	50	NA	0.48	50	NA	NA	Stage I-IV	Serum	$2^{-\Delta\Delta Ct}=2.78$	5
Wu J, 2015 (2)*	China	50	NA	0.48	50	NA	NA	Stage I-IV	PBMC	$2^{-\Delta\Delta Ct}=3.02$	5
Chan SH, 2008	China	37	NA	0.73	-	-	-	Stage I-IV	Tissue	2 fold increase	4
Komatsu S, 2013	Japan	69	NA	0.62	-	-	-	Stage I-IV	Plasma	0.0326amol/μL	4
Motoyama K, 2010	Japan	49	NA	NA	-	-	-	Stage I-IV	Tissue	NA	4
Tchernitsa O, 2010	Germany	20	NA	NA	-	-	-	NA	Tissue	1.5 fold median	4
Xu Y, 2012	China	86	NA	0.64	-	-	-	Stage I-IV	Tissue	$-\Delta Ct=5.12$	4

*: The study of Wu J et al reported two sources of microRNA-21 and we analyzed them as two trials in this meta-analysis. -: No healthy controls in these studies. NA: not reported; PBMC: peripheral blood mononuclear cells.

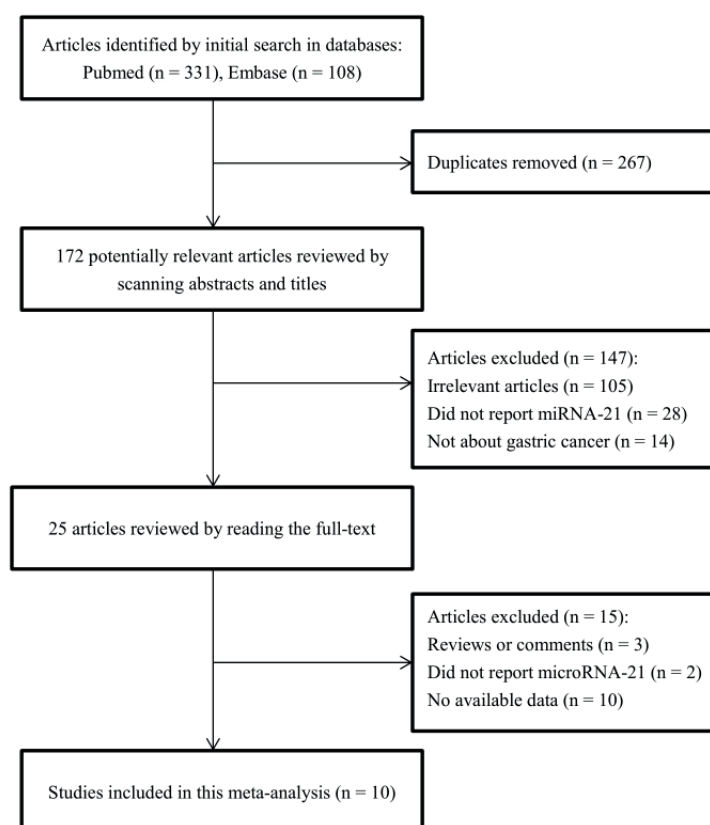


Figure 1.

Prognostic Value of miR-21 in Gastric Cancer

We also assessed the prognosis value of miR-21 in gastric cancer by meta-analyses (Figure 4).

The test of heterogeneity showed that there was a significant heterogeneity among studies in the overall analysis for lymph node metastasis ($I^2=70\%$, $p=0.01$). Thus, the random-effects model was used. However, in subgroup analysis, the significant heterogeneity was absent ($I^2=25\%$, $p=0.26$) after excluding the studies that did not report the miR-21 levels in tissue. Thus, the fixed effects model was applied when pooling data. Results indicated that patients with high-levels of miR-21 showed higher-risk of lymph node metastasis in the overall analysis (OR=1.50, 95% CI=1.04-11.83, $p=0.04$, Figure 4A(a)) and the subgroup analysis (OR=6.34, 95% CI=3.03-13.25, $p<0.00001$, Figure 4A(b), which only included the reports that investiga-

ted serum miR-21), suggesting that miR-21 might be associated with the occurrence of lymph node metastasis.

In addition, results showed a significant heterogeneity in the subgroup analysis for lymphatic invasion ($I^2=62\%$, $p=0.10$, Figure 4C(b)). Thus, the random-effects model was applied. The fixed effects model was used in the other analyses because no significant heterogeneity ($I^2<50\%$, $p>0.10$) was detected. No association was observed between miR-21 level and TNM stage, lymphatic invasion, liver metastasis, peritoneal dissemination, and venous invasion in gastric cancer in this meta-analysis ($p>0.05$, Figure 4B-F).

The subgroup analyses for diagnostic accuracy of serum miR-21 could not be performed due to the limited number of researches.

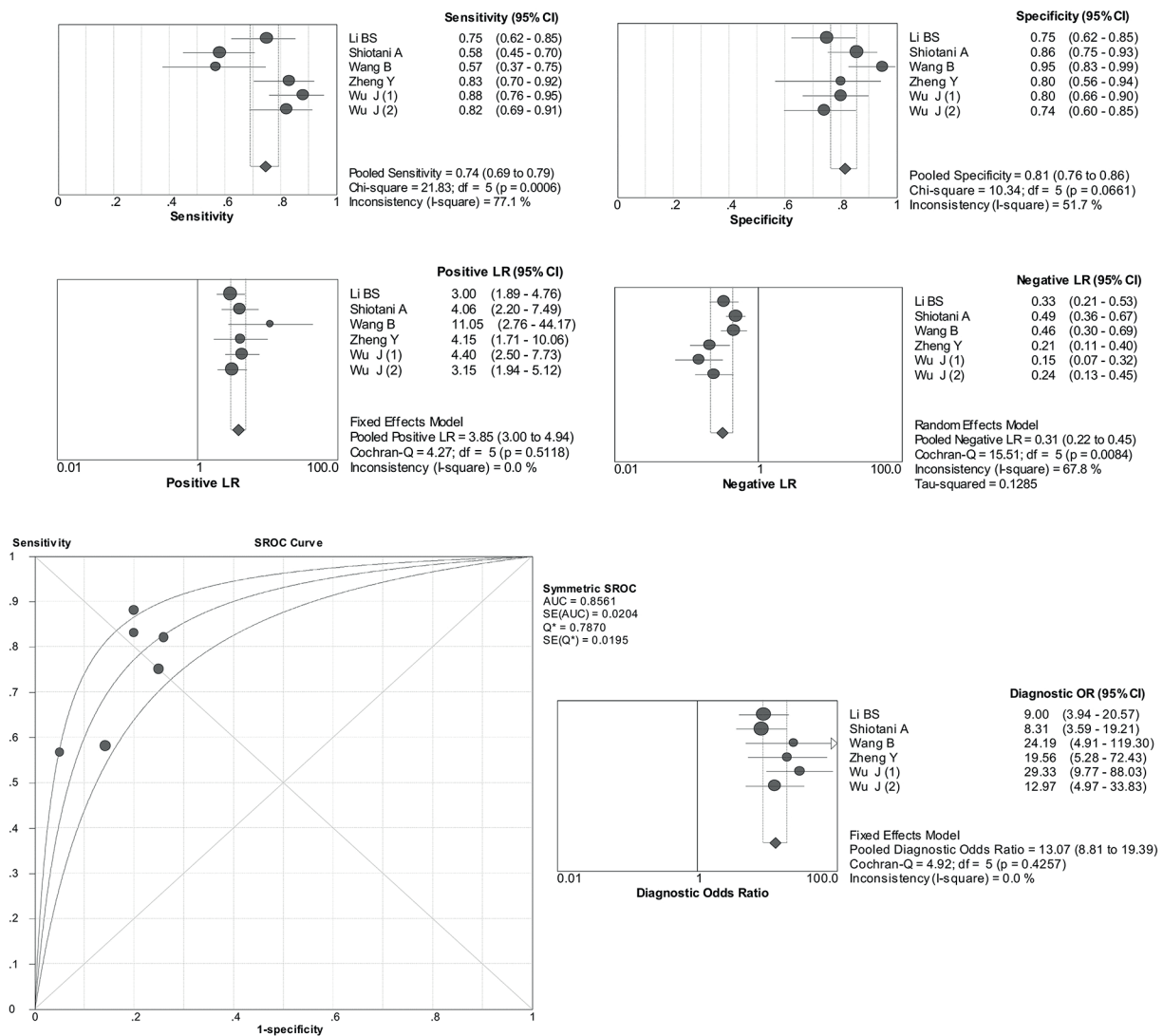


Figure 2.

Predictive Accuracy of miR-21 for Lymph Node Metastasis in Gastric Cancer

Based on the findings presented in Figure 4, we further assessed the predictive accuracy of miR-21 for lymph node metastasis in gastric cancer.

As shown in Figure 5, significant heterogeneity ($I^2 > 50\%$, $p < 0.10$) was detected among studies for sensitivity, specificity, PLR, and DOR. Thus, the random-effects model was used. Meanwhile, fixed effects model was applied when pooling the NLR data because no significant heterogeneity was observed among the included studies ($I^2 < 50\%$, $p > 0.10$). The po-

oled sensitivity (0.56, 95% CI: 0.48-0.64), specificity (0.62, 95% CI: 0.53-0.71), PLR (2.02, 95% CI: 0.90-4.54), NLR (0.58, 95% CI: 0.45-0.75), and DOR (3.50, 95% CI: 1.04-11.83) as well as AUC (0.6673 ± 0.0469) indicated that miR-21 might be a potential marker for predicting lymph node metastasis in gastric cancer.

After removing the work of Komatsu et al²¹ (in which the level of miR-21 was detected in plasma), the results showed a visual increase of specificity, PLR, DOR, and AUC as well as a decrease of NLR. Moreover, the significant heterogeneity among studies disappeared in the analyses

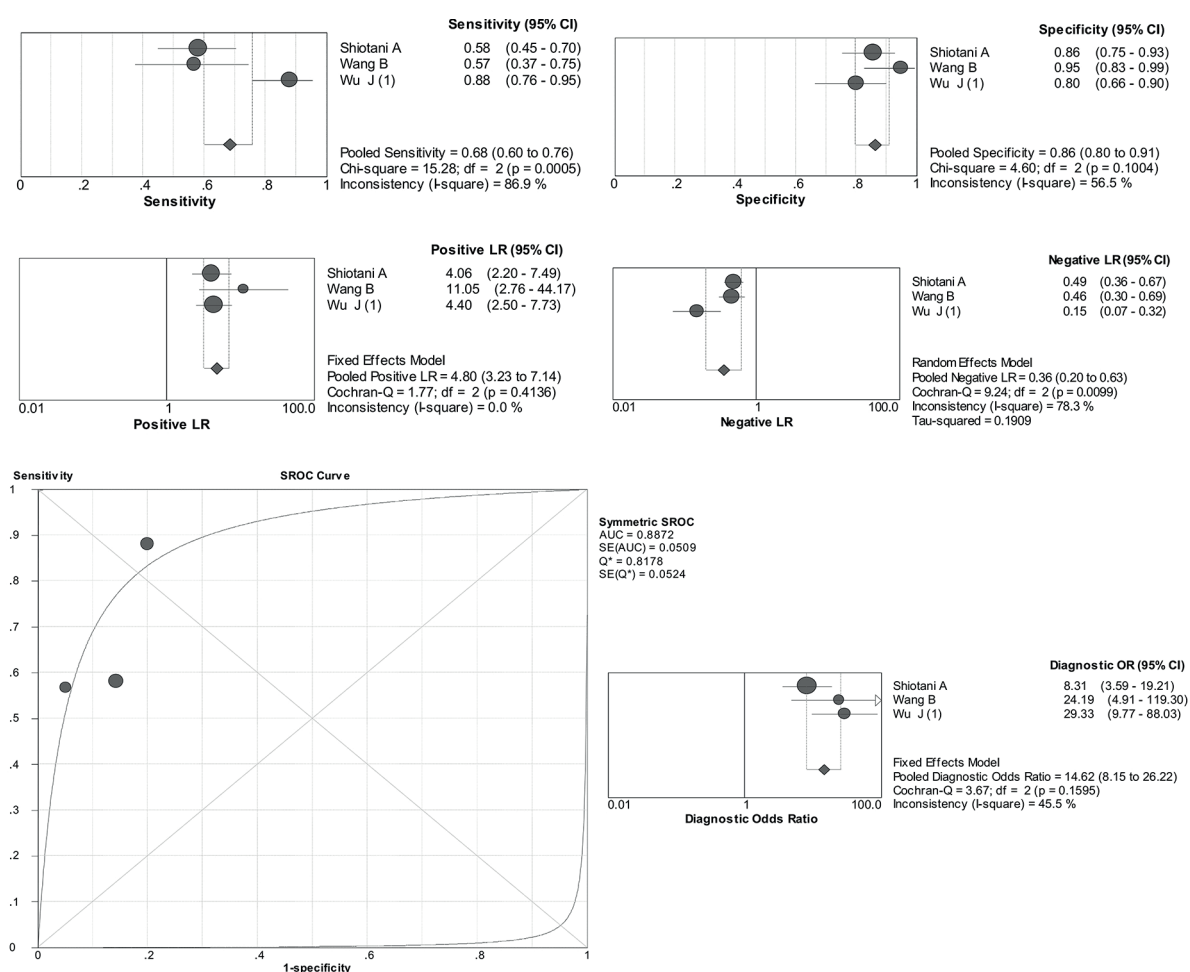


Figure 3.

when pooling the PLR and DOR data. These data indicated that the samples used to detect miR-21 might be a source of heterogeneity and could affect the predictive accuracy of miR-21 for lymph node metastasis in gastric cancer (Figure 6).

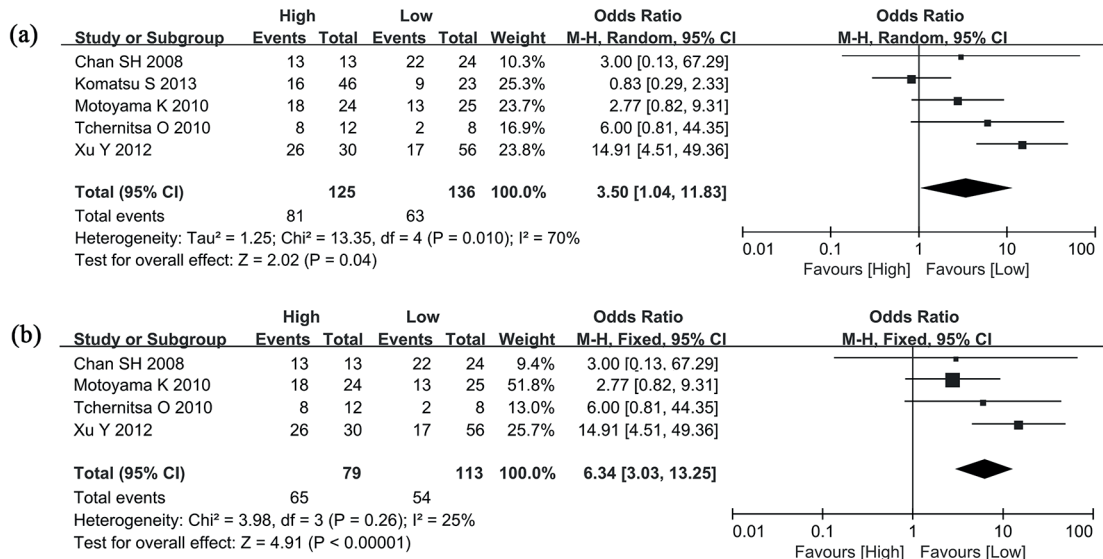
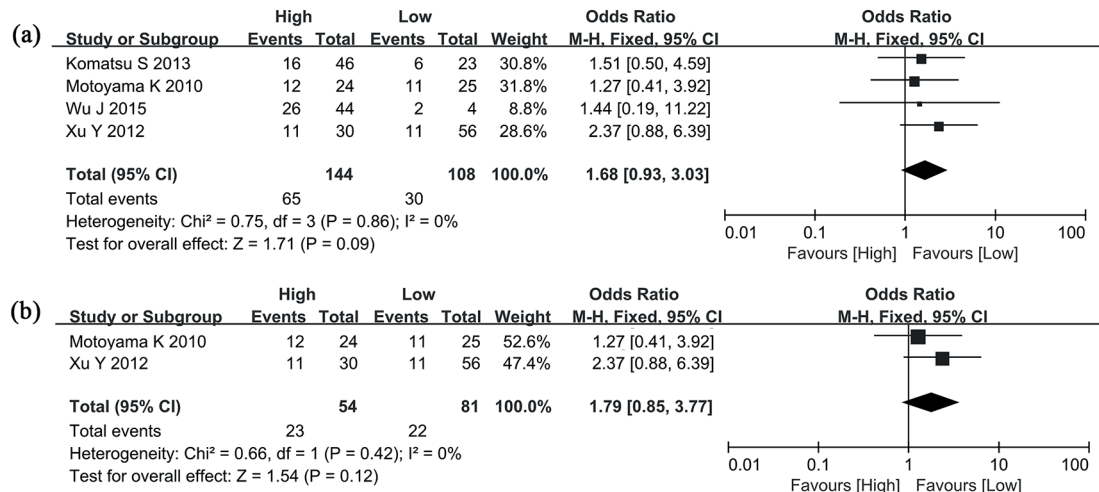
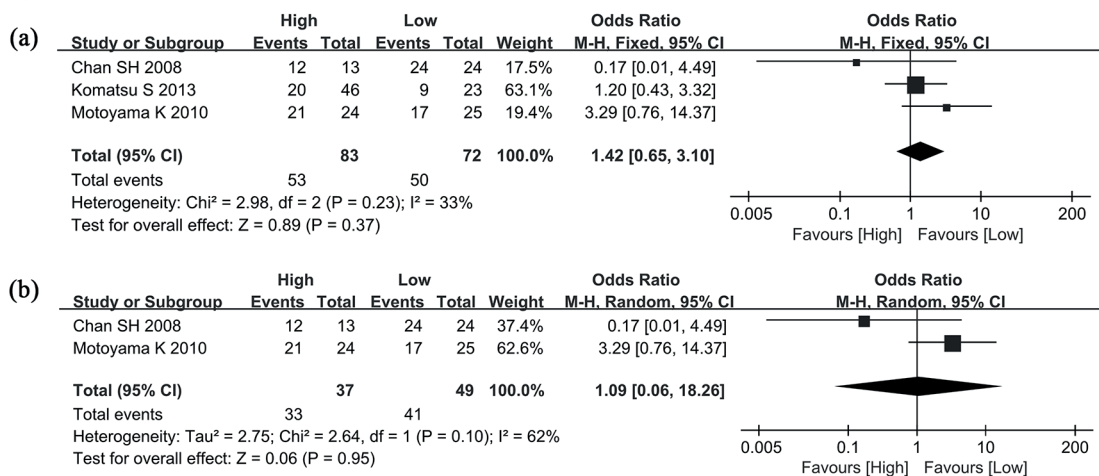
The subgroup analyses for diagnostic accuracy of serum miR-21 could not be performed due to the limited number of studies.

Discussion

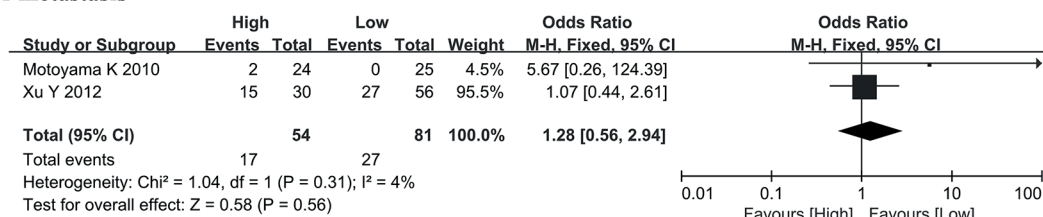
This meta-analysis confirmed the good diagnostic accuracy of miR-21 for gastric cancer, which was consistent with a previous meta-analysis¹⁵. Moreover, we also found that miR-21

was associated with lymph node metastasis in gastric cancer and can be used for predicting lymph node metastasis, which was inconsistent with a previous meta-analysis¹⁶. Also, we found that, the samples used for detecting miR-21 are one of the sources of heterogeneity and affected the diagnostic accuracy of miR-21 for gastric cancer and its predictive accuracy for lymph node metastasis in gastric cancer.

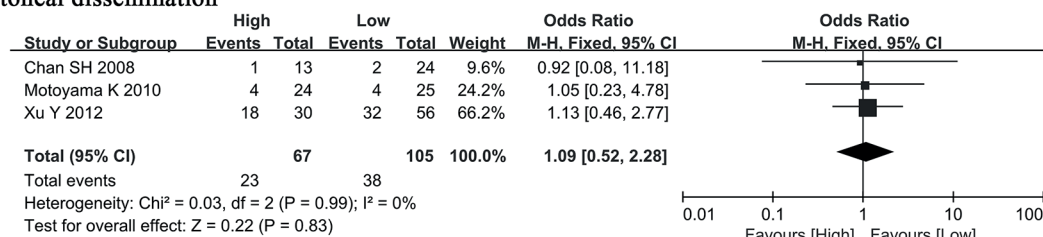
A previous report²⁷ showed that high-expression of miR-21 in gastric cancer was regulated by PTEN, which is associated with the growth and invasion of gastric cancer²⁸⁻³⁰. Thus, the role of miR-21 in the pathogenesis of gastric cancer regulated by PTEN might explain the good diagnostic accuracy of miR-21 for gastric cancer. In this meta-analysis, although the AUC

A: Lymph node metastasis**B: TNM stage (event: stage III or IV)****C: Lymphatic invasion****Figure 4.**

D: Liver metastasis



E: Peritoneal dissemination



F: Venous invasion

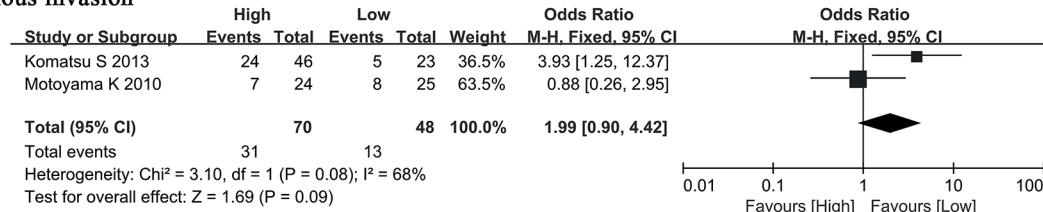


Figure 4.

(>0.80) and specificity (>0.80) of miR-21 for gastric cancer was high enough, the low-sensitivity (<0.80) indicated a high false negative rate. These results indicated that the diagnostic accuracy of miR-21 for gastric cancer should be further investigated and improved by defining the most appropriate cut-off value and the sample type for detecting miR-21.

The miR-21/PTEN pathway is also associated with the differentiation, angiogenesis, and metastasis of gastric cancer^{28,30}. Thus, the prognostic significance of miR-21 in gastric cancer has been demonstrated in many studies^{22,31}. In the present paper, we determined the potential role of miR-21 in predicting lymph node metastasis. However, despite removing the study from Komatsu et al⁹ (in which the level of miR-21 was detected in the plasma) in the subgroup analyses, the values of diagnostic parameters were still not high (sensitivity, specificity, PLR, DOR, and AUC) or low (NLR) enough, which may be explained by the different cut-off values among the included studies.

The predictive accuracy of miR-21 for lymph node metastasis in gastric cancer should be further investigated in the future.

This meta-analysis presents some limitations. Firstly, the number of included studies and the sample size were small. Thus, the results of this meta-analysis should be verified in a study with a large sample size. Secondly, although we found that the samples used for detecting miR-21 were a source of heterogeneity, the heterogeneity still existed in the subgroup analyses. The confounding factors such as cut-off values, race, and age might be the other sources of heterogeneity and affected the results of this meta-analysis. Also, the subgroup analyses by plasma and serum miR-21 were not performed due to the limited number of studies. Thus, we could not confirm the diagnostic value of plasma or tissue miR-21 or the prognostic value of plasma or serum miR-21 in gastric cancer. Further researches should be conducted to investigate these in details.

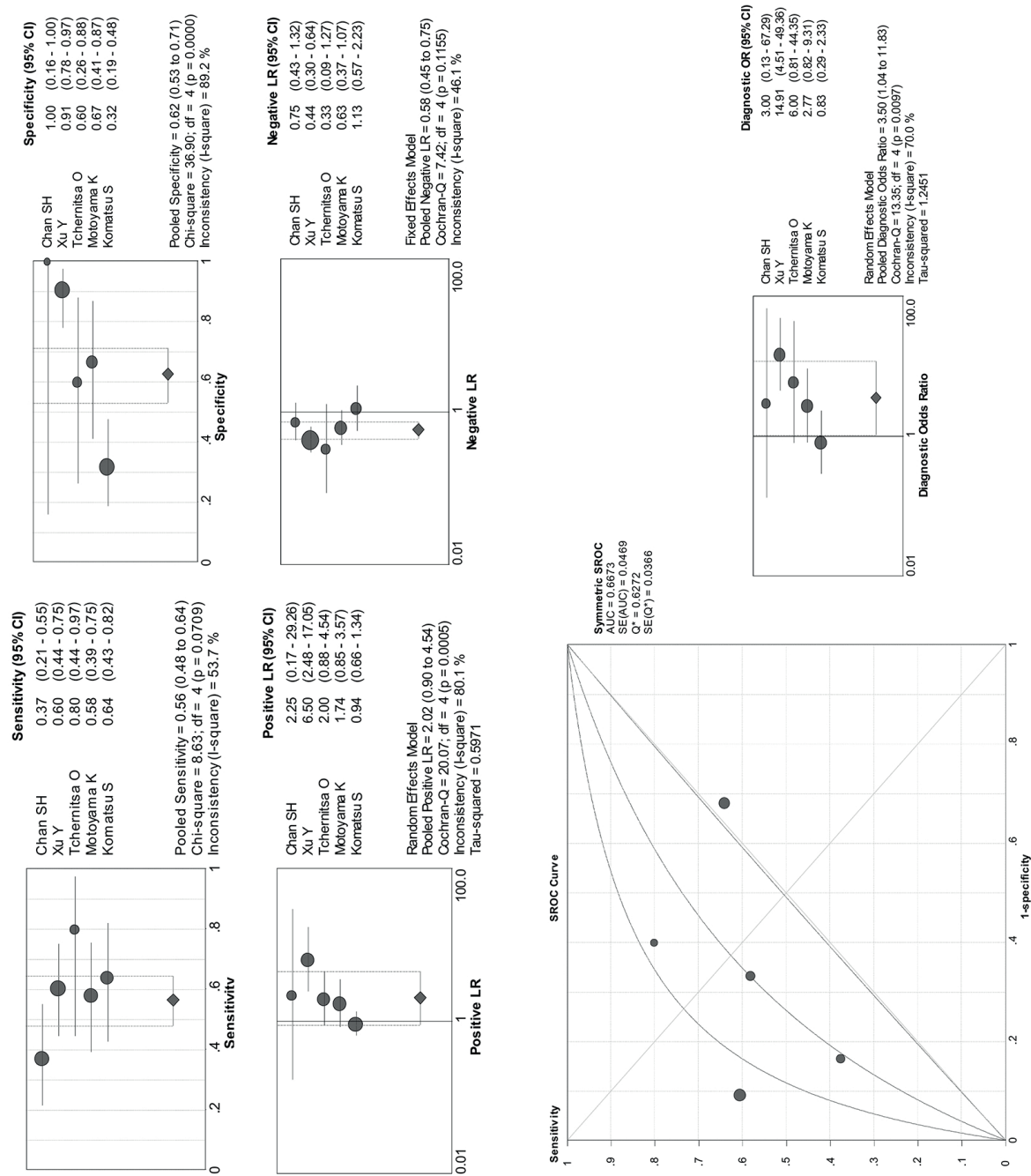


Figure 5.

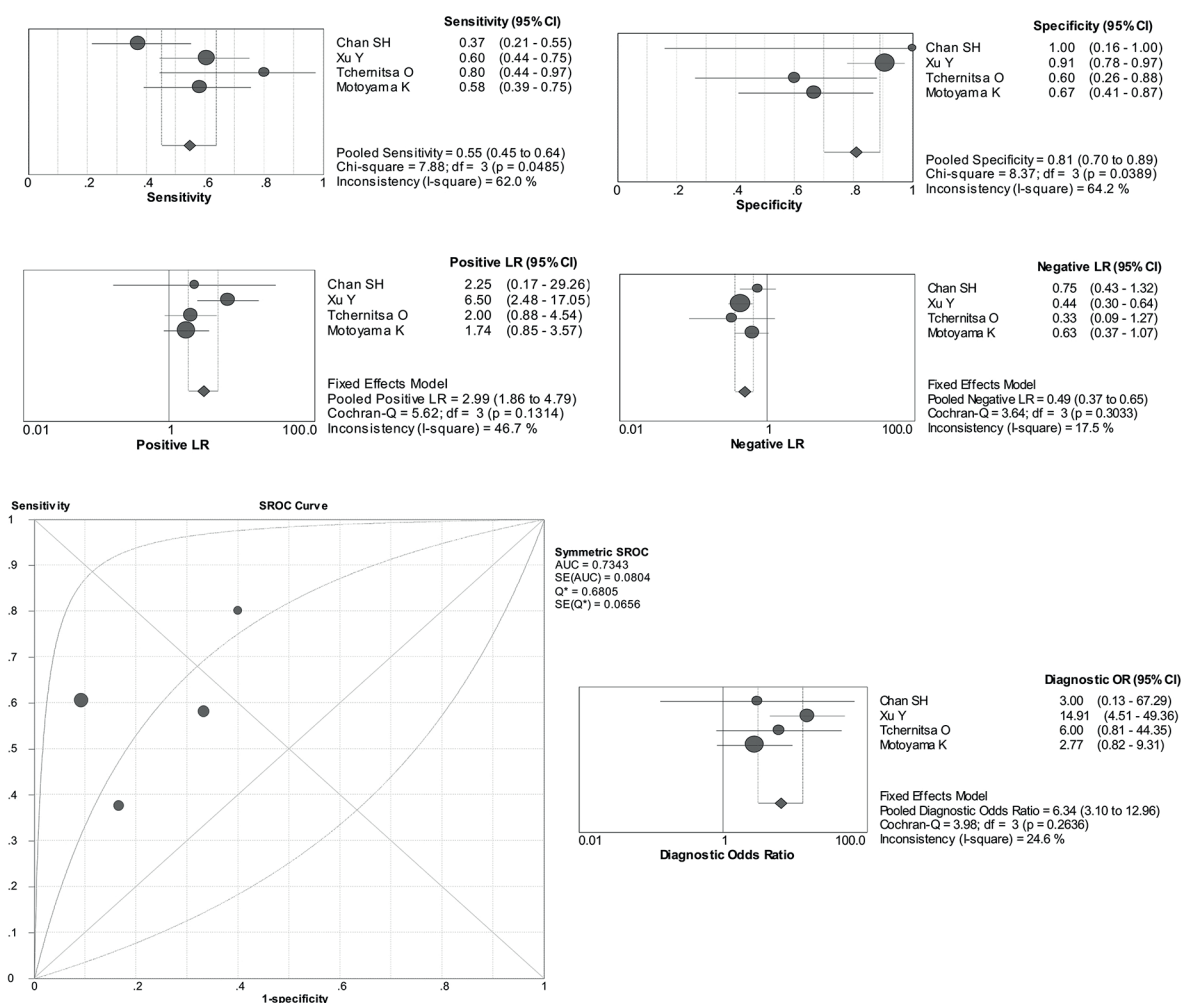


Figure 6.

Conclusions

This meta-analysis confirmed the diagnostic accuracy of serum miR-21 for gastric cancer and the prognostic value of tissue miR-21 for lymph node metastasis in gastric cancer. The diagnostic or prognostic value of serum, plasma, and tissue miR-21 should be further investigated in future studies with a large sample size.

Acknowledgments

This work was supported by Hangzhou City Science and Technology Council: Regulatory role of Yiqibushen Fang on gastric cancer stem niche and Notch signaling pathway (No. 2013073Q23) and Zhejiang Province Administration of Traditional Chinese Medicine: Anti-metastatic mechanism of Yiqibushen Fang on

gastric cancer mediated by stem cells and Notch signaling pathway (No. 2013ZB105).

Conflict of interest

The authors declare no conflicts of interest.

References

- JEMAL A, BRAY F, CENTER MM, FERLAY J, WARD E, FORMAN D. Global cancer statistics. *CA Cancer J Clin* 2011; 61: 69-90.
- CHEN W, ZHENG R, BAADE P, ZHANG S, ZENG H, BRAY F, JEMAL A, YU X, HE J. Cancer statistics in China, 2015. *CA Cancer J Clin* 2016; 66: 115-132.
- SIEGEL RL, MILLER KD, JEMAL A. Cancer statistics, 2015. *CA Cancer J Clin* 2015; 65: 5-29.
- TORRE LA, BRAY F, SIEGEL RL, FERLAY J, LORTET-TIEULENT J, JEMAL A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; 65: 87-108.

- 5) KIM JI, KIM YH, LEE KH, KIM SY, LEE YJ, PARK YS, KIM N, LEE DH, KIM HH, PARK DJ, LEE HS. Type-specific diagnosis and evaluation of longitudinal tumor extent of borrmann type IV gastric cancer: CT versus gastroscopy. *Korean J Radiol* 2013; 14: 597-606.
- 6) MUSRI FY, MUTLU H, KARAAGAC M, ERYILMAZ MK, GUNDUZ S, ARTAC M. Primary tumor resection and survival in patients with stage iv gastric cancer. *J Gastric Cancer* 2016; 16: 78-84.
- 7) SYRIOS J, SOUGIOULTZIS S, XYNOS ID, KAVANTZAS N, KOSMAS C, AGROGIANNIS G, GRINIATOS J, KARAVOKYROS I, PIKOULIS E, PATSOURIS ES, TSAVARIS N. Survival in patients with stage IV noncardia gastric cancer - the influence of DNA ploidy and *Helicobacter pylori* infection. *Bmc Cancer* 2012; 12: 264.
- 8) TOKUNAGA M, TANIZAWA Y, BANDO E, KAWAMURA T, TERASHIMA M. Poor survival rate in patients with postoperative intra-abdominal infectious complications following curative gastrectomy for gastric cancer. *Ann Surg Oncol* 2013; 20: 1575-1583.
- 9) GARZON R, CALIN G, CROCE C. MicroRNAs in cancer. *Annu Rev Med* 2009; 60: 167-179.
- 10) ZIMMERMAN AL, WU S. MicroRNAs, cancer and cancer stem cells. *Cancer Lett* 2011; 300: 10-19.
- 11) SONG MY, PAN KF, SU HJ, ZHANG L, MA JL, LI JY, YUASA Y, KANG D, KIM YS, YOU WC. Identification of serum microRNAs as novel non-invasive biomarkers for early detection of gastric cancer. *PLoS One* 2012; 7: e33608.
- 12) BRENNER B, HOSHEN MB, PURIM O, DAVID MB, ASHKENAZI K, MARSHAK G, KUNDEL Y, BRENNER R, MORGENSTERN S, HALPERN M, ROSENFELD N, CHAJUT A, NIV Y, KUSHNIR M. MicroRNAs as a potential prognostic factor in gastric cancer. *World J Gastroenterol* 2011; 17: 3976-3985.
- 13) CHAN SH, WU CW, LI AF, CHI CW, LIN WC. miR-21 microRNA expression in human gastric carcinomas and its clinical association. *Anticancer Res* 2008; 28: 907-911.
- 14) ZHANG Z, LI Z, GAO C, CHEN P, CHEN J, LIU W, XIAO S, LU H. miR-21 plays a pivotal role in gastric cancer pathogenesis and progression. *Lab Invest* 2008; 88: 1358-1366.
- 15) ZENG Z, WANG J, ZHAO L, HU P, ZHANG H, TANG X, HE D, TANG S, ZENG Z. Potential role of microRNA-21 in the diagnosis of gastric cancer: a meta-analysis. *PLoS One* 2013; 8: e73278.
- 16) WANG Z, CAI Q, JIANG Z, LIU B, ZHU Z, LI C. Prognostic role of microRNA-21 in gastric cancer: a meta-analysis. *Med Sci Monit* 2014; 20: 1668-1674.
- 17) LI BS, ZHAO YL, GUO G, LI W, ZHU ED, LUO X, MAO XH, ZOU QM, YU PW, ZUO QF, LI N, TANG B, LIU KY, XIAO B. Plasma microRNAs, miR-223, miR-21 and miR-218, as novel potential biomarkers for gastric cancer detection. *PLoS One* 2012; 7: e41629.
- 18) SHIOTANI A, MURAO T, KIMURA Y, MATSUMOTO H, KAMADA T, KUSUNOKI H, INOUE K, UEDO N, IISHI H, HARUMA K. Identification of serum miRNAs as novel non-invasive biomarkers for detection of high risk for early gastric cancer. *Br J Cancer* 2013; 109: 2323-2330.
- 19) WU J, LI G, WANG Z, YAO Y, CHEN R, PU X, WANG J. Circulating MicroRNA-21 is a potential diagnostic biomarker in gastric cancer. *Dis Markers* 2015; 2015: 435656.
- 20) WHITING PF, RUTJES AW, WESTWOOD ME, MALLETT S, DEEKS JJ, REITSMA JB, LEEFLANG MM, STERNE JA, BOS-SUYT PM. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med* 2011; 155: 529-536.
- 21) KOMATSU S, ICHIKAWA D, TSUJIIURA M, KONISHI H, TAKESHITA H, NAGATA H, KAWAGUCHI T, HIRAJIMA S, ARITA T, SHIOZAKI A, KUBOTA T, FUJIWARA H, OKAMOTO K, OTSUJI E. Prognostic impact of circulating miR-21 in the plasma of patients with gastric carcinoma. *Anticancer Res* 2013; 33: 271-276.
- 22) MOTOYAMA K, INOUE H, MIMORI K, TANAKA F, KOJIMA K, UETAKE H, SUGIHARA K, MORI M. Clinicopathological and prognostic significance of PDCD4 and microRNA-21 in human gastric cancer. *Int J Oncol* 2010; 36: 1089-1095.
- 23) TCHERNITSA O, KASAJIMA A, SCHAFER R, KUBAN RJ, UNGETHUM U, GYORFFY B, NEUMANN U, SIMON E, WEICHERT W, EBERT MP, ROCKEN C. Systematic evaluation of the miRNA-ome and its downstream effects on mRNA expression identifies gastric cancer progression. *J Pathol* 2010; 222: 310-319.
- 24) WANG B, ZHANG Q. The expression and clinical significance of circulating microRNA-21 in serum of five solid tumors. *J Cancer Res Clin Oncol* 2012; 138: 1659-1666.
- 25) XU Y, SUN J, XU J, LI Q, GUO Y, ZHANG Q. miR-21 is a promising novel biomarker for lymph node metastasis in patients with gastric cancer. *Gastroenterol Res Pract* 2012; 2012: 640168.
- 26) ZHENG Y, CUI L, SUN W, ZHOU H, YUAN X, HUO M, CHEN J, LOU Y, GUO J. MicroRNA-21 is a new marker of circulating tumor cells in gastric cancer patients. *Cancer Biomark* 2011; 10: 71-77.
- 27) ZHANG BG, LI JF, YU BQ, ZHU ZG, LIU BY, YAN M. microRNA-21 promotes tumor proliferation and invasion in gastric cancer by targeting PTEN. *Oncol Rep* 2012; 27: 1019-1026.
- 28) KIM SJ, LEE HW, BAEK JH, CHO YH, KANG HG, JEONG JS, SONG J, PARK HS, CHUN KH. Activation of nuclear PTEN by inhibition of notch signaling induces G2/M cell cycle arrest in gastric cancer. *Oncogene* 2016; 35: 251-260.
- 29) CANBAY E, KAHRAMAN OT, BUGRA D, CAYKARA B, SEYHAN MF, BULUT T, YAMANER S, OZTURK O. Increased gastric cancer risk with PTEN IVS4 polymorphism in a turkish population. *Genet Test Mol Biomarkers* 2013; 17: 249-253.
- 30) ZHENG HC, LI YL, SUN JM, YANG XF, LI XH, JIANG WG, ZHANG YC, XIN Y. Growth, invasion, metastasis, differentiation, angiogenesis and apoptosis of gastric cancer regulated by expression of PTEN encoding products. *World J Gastroenterol* 2003; 9: 1662-1666.
- 31) SONG J, BAI Z, ZHANG J, MENG H, CAI J, DENG W, BI J, MA X, ZHANG Z. Serum microRNA-21 levels are related to tumor size in gastric cancer patients but cannot predict prognosis. *Oncol Lett* 2013; 6: 1733-1737.