MicroRNA-21 as a potential biomarker for detecting esophageal carcinoma in Asian populations: A meta-analysis

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Background: MicroRNA-21 (miR-21) is significantly expressed in a variety of cancers and could be used as a tumor biomarker. However, the results are varied, and no studies on the diagnostic usefulness of miR-21 in Asian esophageal cancer (EC) patients have been published. This meta-analysis was aimed at exploring whether miR-21 can be used as a diagnostic marker and assessing its effectiveness. Methods: The relevant literature was identified in six main databases: Ovid MEDLINE, PsycINFO, PubMed MEDLINE, Embase, Web of Science, and the Cochrane Library. Two researchers independently selected the literature based on the inclusion and exclusion criteria, extracted data, and evaluated the risk of bias. The Meta-analysis was carried out using Review Manager 5.4, Meta-Disc 1.4 and STATA 15.1 software. In the end, 987 patients from 12 different studies were included. Quality evaluation of diagnostic accuracy studies 2 (QUADAS – 2) was used to examine the risk of bias. Results: The pooled sensitivity (SEN) was 0.72 (95%CI: 0.69-0.75), the pooled specificity (SPE) was 0.78 (95% CI: 0.75-0.81), the pooled positive likelihood ratio (PLR) was 2.87 (95% CI: 2.28–3.59), the pooled negative likelihood ratio (NLR) was 0.36 (95% CI: 0.31–0.43), the pooled diagnostic odds ratio (DOR) was 10.00 (95% CI: 7.73–12.95), and the area under the curve 0.82 (95%CI: 0.79-0.85). A Deeks' funnel plot shows that there was no publication bias (P=0.99). Conclusion: Our findings suggest miR-21 might be the potential biomarker for detecting EC in Asian populations, with a good diagnostic value.

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29 **Abstract**

30 Background: MicroRNA-21 (miR-21) is significantly expressed in a variety of cancers and could be used as a

31 tumor biomarker. However, the results are varied, and no studies on the diagnostic usefulness of miR-21 in Asian

32 esophageal cancer (EC) patients have been published. This meta-analysis was aimed at exploring whether miR-

33 21 can be used as a diagnostic marker and assessing its effectiveness.

34 Methods: The relevant literature was identified in six main databases: Ovid MEDLINE, PsycINFO, PubMed

35 MEDLINE, Embase, Web of Science, and the Cochrane Library. Two researchers independently selected the

36 literature based on the inclusion and exclusion criteria, extracted data, and evaluated the risk of bias. The Meta-

37 analysis was carried out using Review Manager 5.4, Meta-Disc 1.4 and STATA 15.1 software. In the end, 987

38 patients from 12 different studies were included. Quality evaluation of diagnostic accuracy studies 2 (QUADAS

39 − 2) was used to examine the risk of bias.

40 Results: The pooled sensitivity (SEN) was 0.72 (95%CI: 0.69-0.75), the pooled specificity (SPE) was 0.78 (95%

41 CI: 0.75-0.81), the pooled positive likelihood ratio (PLR) was 2.87 (95% CI: 2.28–3.59), the pooled negative

42 likelihood ratio (NLR) was 0.36 (95% CI: 0.31–0.43), the pooled diagnostic odds ratio (DOR) was 10.00 (95%

43 CI: 7.73–12.95), and the area under the curve 0.82 (95%CI: 0.79-0.85). A Deeks' funnel plot shows that there

44 was no publication bias (P=0.99).

45 Conclusion: Our findings suggest miR-21 might be the potential biomarker for detecting EC in Asian

46 populations, with a good diagnostic value.

47 **Keywords**: MicroRNA-21; Esophageal carcinoma; Diagnosis; Meta-analysis; Asian

48

Introduction

50 Esophageal carcinoma (EC) is the seventh most frequent cancer in terms of incidence, affecting more than

51 450,000 people globally (Li et al., 2020). The majority of esophageal cancers can be subdivided into two main

52 histological subtypes: adenocarcinomas (AC) and squamous cell carcinomas (SCC). Esophageal AC incidence

53 has mysteriously increased in white people in high-income nations in recent years. This increase has largely

54 been attributed to the rising prevalence of obesity, although some controversy exists around this hypothesis

55 (Arnold et al., 2015). SCC accounts for about 88 percent of esophageal cancer cases all over the world,

56 particularly in Central Asia and South-eastern. In 2017, China accounted for nearly half of all new cases of EC

57 (234,624 Chinese cases out of 472,525 cases worldwide, or 49.7%) (Li et al., 2021). Nitrosamine compounds, a

58 class of the most probable carcinogens concerned with EC, are found more frequently and at a higher

59 concentration in Asian foods than in Western foods (Jakszyn & Gonzalez, 2006; Zhao et al., 2020; Stoner et al.,

60 2007). A recent comprehensive analysis predicts that the global burden of EC will continue to rise (Ma et al.,

61 2020).

62 EC is a devastating disease, with a 5-year survival rate of fewer than 20% and the highest mortality rate in Asia

63 (Yu et al., 2019). One positive is that if only the mucosal layer of the esophagus or the superficial submucosal

64 layer is invaded, the 5-year survival rate can be as high as 95%. Unfortunately, the majority of EC patients are

65 diagnosed at an advanced stage of the disease (Guo et al., 2016). As a result, early detection of EC can

66 dramatically improve the prognosis and decrease mortality. Biopsy and endoscopy, the current gold standard

67   procedures for detecting and diagnosing EC, are intrusive and accurate diagnosis can be attained by biopsy only

68   when patient have reached advanced stage (Fan et al., 2019; Wang et al., 2014; Liu et al., 2016; Zhao, 2012).

69   Meanwhile, endoscopic and pathological doctors require a lengthy qualification process (Zhao, 2012). A number

70   of biomarkers reflecting the molecular status or activity within the tumors have been used for the clinical

71   diagnosis of cancers (Xie et al., 2022; Qiu et al., 2021; Qiu et al., 2022). Conventional tumor markers, such as

72   carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 19-9, and squamous cell carcinoma antigen (SCC),

73   have been used in diagnostic assays for early detection and monitoring the tumor dynamics of EC. These tumor

74   markers, however, lack sufficient sensitivity and specificity to facilitate early detection of EC (Zarrilli et al.,

75   2021).

76   MicroRNA-21 (miR-21) is found in a range of extracellular fluids, including cerebrospinal fluid (CSF), saliva,

77   serum, plasma, and blood, and has been reported to be highly expressed in a variety of cancer types (Bautista-

78   Sánchez et al., 2020). MiR-21 has several isoforms, the most important, miR-21-3p and miR-21-5p. Gao et al.

79   reported that up-regulation of hsa-miR-21-3p was associated with a high risk of EC (Gao et al., 2019).

80   Meanwhile, miR-21-5p, contributing to the polarization of M2 macrophages in tumor micro-environment, was

81   also increased in patients with EC (Song et al., 2021).

82   A growing number of studies have suggested the feasibility of miRNAs to diagnose EC patients. However, these

83   studies have returned inconsistent results. (Liu et al., 2016) reported a good diagnostic characteristic with 79.9%

84   sensitivity, whereas (Xie et al., 2013) reported a much lower specificity (51.3%) for miR-451. (Wang et al.,

85   2014) conducted a meta-analysis including only eight publications whose publication years ranged from 2010

86   to 2013, and yielded moderate diagnostic accuracy, with 78.5% sensitivity and 96% specificity. To advance

87   knowledge based on the existing evidence and to address the limitations of the previous reviews, we undertook

88 this systematic analysis to further explore the clinical applicability of miR-21 as novel biomarker for the

89 diagnosis of EC in Asians.

90

91 **Methods**

92 We conducted and reported this systematic review according to prespecified criteria outlined by the PRISMA

93 guidelines; the corresponding checklists are shown in online supplemental file. The study protocol was registered

94 with the PROSPERO international prospective register of systematic reviews (number CRD42021289781).

95 **Search strategy**

96 One investigator (HZ), a health information specialist, searched 6 databases: Ovid MEDLINE, PsycINFO,

97 PubMed MEDLINE, Embase, Web of Science, and the Cochrane Library. There were no specified date, age,

98 sex, or language restrictions. The coverage dates for this study began from each database's inception

99 (MEDLINE, 1946; PsycINFO, 1806; Embase, 1947; Web of science,1900; and Cochrane Library, 1995) and

100 ended on December 1, 2021. The search strategy contained three core components, which were linked using the

101 AND operator: disease type (eg, Esophageal Neoplasms, Neoplasm Esophageal); research method (eg,

102 sensitivity, predictive value of tests); diagnostic method (eg, MicroRNA, Primary miRNA). The search was

103 developed initially for PubMed MEDLINE and then adapted for each of the other five databases by mapping the

104 search terms to additional controlled vocabulary and subject heading terminology.

105 **Inclusion criteria**

106 (1) Asian people diagnosed with esophageal cancer (AC, SCC or both);

107 (2) The included article at least reported sensitivity and specificity of miR-21 in diagnosis of EC patients;

108 (3) Studies providing the diagnostic performance of miR-21 in blood, serum, plasma, tissue for EC including

109   World Health Organization (WHO) grade I–IV gliomas;

110   **Exclusion criteria**

111   (1) Meta-analysis, reviews, letters, comments, and duplicated studies;

112   (2) Studies that did not estimate the diagnostic role of miR-21 in EC (AC, SCC or both);

113   (3) Articles that were published repeatedly or had qualitative outcomes;

114   **Data extraction**

115   The first author's name, publication year, country, specimen, sample size, specificity, sensitivity, number of

116   patients and controls were all obtained from each study's patients. Any disagreements among the researchers

117   were worked out with the help of a third researcher.

118   **Quality assessment**

119   Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) was appraising the applicability of

120   included studies using Review Manager 5.4 software. The patient selection, index test, reference standard, and

121   flow and timing domains made up the four domains of this scale. With the exception of the flow and timing

122   domain, where the applicability concern did not apply, each signaling question was rated as "yes," "no," or

123   "unclear," and each study's risk of bias and worry for application were estimated as "high," "low," or "unclear,"

124   respectively. A "yes" response suggested that the risk of bias could be regarded as low, whereas a "no" or

125   "unclear" response indicated that the risk of bias might be rated as high.

126   **Q tests and I^2 statistics**

127   Q tests and I^2 statistics were used to estimate the heterogeneity caused by a non-threshold effect among the

128   included studies. Meta-Disc1.4 software was used to perform the statistical analysis for this meta-analysis.

129   According to the Cochrane handbook, either $P<0.1$ or $I^2 >50\%$ suggested the existence of substantial

130 heterogeneity, a random-effects model was applied to quantify the pooled sensitivity, specificity, the positive

131 likelihood ratio (PLR), negative likelihood ratio (NLR), summary diagnostic odds ratio (DOR) and area under

132 curve (AUC). Otherwise, a fixed-effects model was used.

133 **Subgroup analysis**

134 Subgroup analysis was carried out to investigate the potential influential factors on the summary sensitivity

135 and specificity, which included Country (China vs Others), Specimen types (Plasma vs Serum vs Blood vs

136 Saliva), Cancer Stage (I-IV vs Others), Gender (Male vs Female) and Age (>60 vs ≤ 60). The analyses were

137 performed in StataSE15.1 (Stata Corp LP, College Station, TX, USA) and Meta-Disc1.4.

138 **Sensitivity analysis**

139 To determine whether our analysis was stable, sensitivity analysis was conducted by removing the included

140 studies one by one and analyzing the SROC curve. Sensitivity analysis was conducted with StataSE15.1.

141 **Fagan's nomogram**

142 Fagan's nomogram was performed using the "Midas" module in the STATA 15.1 to calculate pre-test

143 probability and post-test probability to assess the diagnostic power of miR-21 in clinical practice.

144 **Publication bias**

145 In addition, we adopted Deeks' funnel plots to examine the possibility of publication bias across studies, with

146 values showing $P < 0.10$ considered to have significant statistical publication bias. StataSE15.1 software was

147 used to perform the statistical analysis.

Study	Country	Year	Sample type	Cancer	Control	TNM	SEN	SPE	Median age	Male (P)	Female (P)	Male (C)	Female (C)
Guiying Sun (Sun et al., 2019)	China	2019	Plasma	125	125	I-IV	61.00%	90.00%	63(59-68)	76	49	76	49
Wen-Tao Wang (a) (Wang et al., 2019)	China	2019	Plasma	128	45	I	80.00%	71.43%	N/A	N/A	N/A	N/A	N/A
Wen-Tao Wang (b)	China	2019	Plasma	128	45	II	67.02%	79.41%	N/A	N/A	N/A	N/A	N/A
Wen-Tao Wang (c)	China	2019	Plasma	128	45	III	80.85%	70.00%	N/A	N/A	N/A	N/A	N/A
Hadiseh Samiei (Samiei et al., 2019)	Iran	2019	Plasma	34	34	I-IV	77.78%	65.38%	50(48-52)	5	29	5	29
Lu Zhang (Zhang et al., 2018)	China	2018	Blood	125	125	I-IV	74.00%	78.00%	N/A	N/A	N/A	N/A	N/A
Kai Wamg (Wang et al., 2018)	China	2018	Serum	35	32	I-IV	77.40%	82.90%	51(27-83)	26	9	19	13
Priyanka Sharma (Sharma et al., 2018)	India	2018	Blood	24	21	I-IV	83.33%	57.15%	74	18	6	N/A	N/A
Shuhei Komatsu (Komatsu et al., 2016)	Japan	2016	Plasma	37	20	I-IV	54.20%	94.30%	64	30	7	N/A	N/A
Zijun Xie (Xie et al., 2013)	China	2013	Saliva	39	19	II-IV	89.70%	47.40%	58(46-88)	31	8	15	4
Zi-Jun Xie (Xie et al., 2012)	China	2012	Saliva	32	16	I-IV	87.50%	62.50%	61(43-88)	26	6	13	3
Bing Wang (Wang & Zhang, 2012)	China	2012	Serum	31	39	I-IV	71.00%	69.20%	61(46-82)	23	8	9	30
Junji Kurashige (Kurashige et al., 2012)	Japan	2012	Serum	71	39	I-IV	46.50%	100.00%	70	66	5	N/A	N/A
S Komatsu (KOMATSU et al., 2011)	Japan	2011	Plasma	50	20	I-IV	88.00%	70.00%	66(58-74)	44	6	N/A	N/A

148   TABLE 1. Characteristics of the Studies Included in This Systematic Review.

149   Note: TNM: Cancer Stage; SEN: Sensitivity; SPE: Specificity; P: Patient; C: Control; NA: Not Available.; AUC: Area under curve.

150

152   TABLE 2. The results of subgroup analysis for the diagnosis of EC.

153   Note: SEN: Sensitivity; SPE: Specificity; PLR: Positive likelihood ratio; NLR: Negative likelihood ratio; DOR: Diagnostic odds ratio; NA: Not Available; AUC: Area under

Subgroup	n	SEN (95%CI)	SPE (95%CI)	PLR (95%CI)	NLR (95%CI)	DOR (95%CI)	AUC
Country							
China	7	0.74(0.71-0.77)	0.78(0.74-0.81)	2.94(2.30-3.77)	0.35(0.31-0.41)	9.94(7.50-13.10)	0.82
Others	5	0.66(0.59-0.72)	0.79(0.71-0.86)	2.98(1.57-5.65)	0.39(0.27-0.57)	10.77(5.22-22.22)	0.84
Specimen types							
Plasma	5	0.73(0.69-0.76)	0.80(0.75-0.84)	3.16(2.36-4.24)	0.36(0.30-0.44)	10.28(7.33-14.40)	0.83
Serum	3	0.60(0.51-0.68)	0.85(0.76-0.91)	4.59(1.38-15.23)	0.42(0.26-0.67)	13.05(3.66-46.61)	0.80
Blood	2	0.75(0.68-0.82)	0.75(0.67-0.82)	2.64(1.55-4.48)	0.33(0.25-0.45)	9.30(5.46-15.80)	N/A
Saliva	2	0.89(0.79-0.95)	0.54(0.37-0.71)	1.88(1.31-2.71)	0.21(0.10-0.43)	9.47(3.49-25.73)	N/A
Stage							
I-IV	10	0.69(0.65-0.73)	0.81(0.77-0.84)	3.13(2.28-4.30)	0.38(0.31-0.47)	10.63(7.70-14.67)	0.83
Others	2	0.77(0.73-0.81)	0.70(0.62-0.77)	2.47(1.84-3.30)	0.32(0.25-0.41)	8.97(5.83-13.80)	N/A
Gender							
Male	6	0.73(0.66-0.79)	0.79(0.71-0.85)	2.73(1.67-4.46)	0.39(0.31-0.50)	10.82(6.09-19.20)	0.83
Female	6	0.72(0.62-0.80)	0.77(0.69-0.84)	2.84(1.93-4.17)	0.39(0.29-0.52)	8.97(5.83-13.80)	0.82
Age							
＞60	7	0.66(0.61-0.71)	0.83(0.78-0.87)	3.29(1.98-5.47)	0.41(0.32-0.53)	11.47(7.36-17.88)	0.83
≤60	3	0.81(0.73-0.88)	0.68(0.57-0.78)	2.40(1.40-4.11)	0.29(0.19-0.45)	9.10(4.54-18.21)	0.84
Total	12	0.72(0.69-0.75)	0.78(0.75-0.81)	2.87(2.28-3.59)	0.36(0.31-0.43)	10.00(7.73-12.95)	0.82

154   curve.

155

156

Results

157

158 The search identified 532 full texts, of which 520 articles were omitted for the following reasons: (1) reviews,

159 letters or comments; (2) laboratory studies; (3) not directly related to specific outcome; (4) non-digestive

160 system cancer; (5) Outcomes were not protein expression. Manual search of references cited in the published

161 studies did not reveal any additional articles. A flow diagram of the search process is shown in Fig. 1.

162 The meta-analysis includes 12 studies with 14 sets of data involving 987 participants that were published

163 between 2011 and 2019. Table 1 shows the baseline characteristics of the 12 studies.

164 **Quality assessment**

165 The QUADAS-2 checklist revealed that all of the articles included were of medium to high quality (Fig. 2).

166 **Threshold effect and Non threshold effect**

167 Spearman correlation coefficient of sensitivity and 1-specificity yielded −0.12 (p = 0.86), indicating no

168 heterogeneity resulting from threshold effect.

169 **Data analysis**

170 The random-effects model was used in this study. We analyzed the combined results of some diagnostic study

171 statistic measures and found a sensitivity of 0.72 (95%CI: 0.68-0.75) (Fig. 3A), specificity of 0.78 (95%CI:

172 0.75-0.81) (Fig. 3B). The summary receiver operating characteristic curve (SROC) for the included studies

173 showed the area under curve (AUC) was 0.82 (95%CI: 0.79-0.85) (Fig. 4), indicating a good diagnostic

174 accuracy. PLR and NLR have been considered more clinically valuable compared to the specificity and

175 sensitivity. In our meta-analysis, the combined NLR was 0.36 (95% CI, 0.31–0.43) (Fig. 3C) and the combined

176 PLR was 2.87 (95%CI: 2.28–3.59) (Fig. 3D). A PLR value of 2.87 indicated that the probability of a person

177 with a positive test result having EC was about three-fold higher compared to patients without EC. The NLR

178 refers to the probability of a person who has EC testing negative divided by the probability of a person who

179 does not have EC testing negative. Here, in this meta-analysis, we found an NLR value of only 0.36. These

180 results revealed that MiRNA-21 is clearly able to distinguish EC patients from healthy people. The DOR was

181 10.00 (95% CI 7.73–12.95) (Fig. 5), suggesting the potential of a 10-fold higher level of miRNA-21 in subjects

182 with positive EC diagnosis compared with subjects with negative results, indicating a high diagnostic

183 accuracy.

184 **Subgroup analysis**

185 As for diagnostic performance, we performed a subgroup analysis on Country (China vs Others), Specimen

186 types (Plasma vs Serum vs Blood vs Saliva), Cancer Stage (I-IV vs Others), Gender (Male vs Female) and Age

187 ($>$60 vs ≤60). The results revealed that: I-IV stage had better diagnostic values than non-I-IV (DOR, 10.63 vs

188 8.97); serum had better diagnostic values than other specimen types (DOR, 13.05$>$10.28$>$9.47$>$9.30) (PLR,

189 4.59$>$3.16$>$2.64$>$1.88) (Figs. 6A-10E); male had better diagnostic values than female (DOR, 10.82 vs

190 8.97); Age$>$60 had better diagnostic values than Age≤60 (DOR, 11.47 vs 9.10). Table 2 shows the detailed

191 results of subgroup analysis.

192 **Sensitivity analysis**

193 We used sensitivity analysis to better identify how the aggregate impact size varies when an individual study is

194 removed. Practically every study had sufficient weight in this meta-analysis (Fig. 11).

195 **Fagan's nomogram**

196 When we set the pre-test probability at 20%, the post-test chance of positive tests increased to 44%, while the

197 post-test likelihood of negative tests decreased to 7%, according to the Fagan's Nomogram (Fig. 12).

198 **Publication bias**

199 Deeks' asymmetry test showed a statistically non-significant value (p = 0.99), confirming no evident

200 publication bias (Fig. 13).

201

Discussion

203 EC is one of the most lethal malignancies in the world with a high mortality rate and correspondingly low

204 survival rate (Takashi Kojima et al., 2020). About 50% of the new cases of EC were in Asia due to its high

205 incidence rate and increasing popularity of nitrosamine food in Asian countries (Li et al., 2021; Jakszyn &

206 Gonzalez, 2006; Zhao et al., 2020; Stoner et al., 2007). Accurate diagnosis can be attained by biopsy only

207 when patients have reached advanced stage. Meanwhile, the number of endoscopic and pathological doctors is

208 too small in terms of the large quantities of Asian patients (Zhao, 2012). Thus, novel methods for the early

209 detection of SCC and AC are urgently required to reduce mortality and improve treatment.

210 As a potential diagnostic biomarker for EC, miR-21 possesses several unique advantages. First, serum miR-21

211 expression levels are stable and reproducible. One explanation for this remarkable stability is that miRNAs

212 might be chemically modified (e.g., methylation), making them resistant to ribonu-clease activity (Miyoshi et

213 al., 2022). Second, when compared with histopathological examination, serum or plasma miR-21 is

214 characterized by minimal invasion and convenience and easily detected by qRT-PCR. Last but not least, its

215 concentration is specifically correlated with certain types of cancer. MiR-21 modulates the expression of

216 multiple cancer-related target genes such as PTEN, TPM1, and PDCD and significant over-expression of

217 plasma miR-21 was observed even in patients with early-stage EC.

218 Several studies have reported results of MicroRNAs as diagnostic markers in EC (Wang et al., 2014; Liu et al.,

219 2016; Xie et al., 2013), and we read these studies for reference. (Liu et al., 2016) conducted a meta-analysis to

220 assess the overall accuracy of circulating miRNAs in serum/plasma in the diagnosis of ESCC and yielded good

221 diagnostic accuracy, with 79.9% sensitivity, whereas (Xie et al., 2013) reported a much lower specificity

222 (51.3%) for miR-451 and 47.4% for miR-144. Meanwhile, age and gender have been shown in other

223 pathologies that could modify the expression of circulating miRNAs. Both of them didn't explore whether

224 these variables affect the miRNA expression in this pathology. (Wang et al., 2014) undertook a systematic

225 analysis to further explore the clinical applicability of miRNAs as novel biomarkers for the diagnosis of EC in

226 Asians. Since only eight publications ranged from 2010 to 2013 were included, it is necessary to perfect the

227 conclusions by further research.

228 To our knowledge, this is the largest and most thorough examination of miR-21's diagnostic accuracy in Asian

229 EC patients. We did not limit publication date during the search of the six main databases: Ovid MEDLINE,

230 PsycINFO, PubMed MEDLINE, Embase, Web of Science, and the Cochrane Library, making it unlikely to

231 miss important publications. This quantitative analysis includes 14 studies from 12 articles, with a total of 987

232 EC patients and 625 healthy controls.

233 Every study in this meta-analysis had sufficient weight, indicating the stability of this meta-analysis. With

234 regard to subgroup analysis, results revealed that: I-IV stage had better diagnostic values than non-I-IV (DOR,

235 10.63 vs 8.97); serum had better diagnostic values than other specimen types (DOR, 13.05＞10.28＞9.47＞

236 9.30); male had better diagnostic values than female (DOR, 10.82 vs 8.97); Age＞60 had better diagnostic

237 values than Age≤60 (DOR, 11.47 vs 9.10). Deeks' asymmetry test showed a statistically non-significant value,

238 confirming no evident publication bias.

239 Pooled sensitivity, specificity, and AUC revealed satisfactory diagnostic accuracy of this meta-analysis (Liao

240 et al., 2015). Positive and negative likelihood ratios are two extremely stable indicators that can fully reflect

241   the diagnostic value of a screening test (Fuchs et al., 2018). As shown in this meta-analysis, we found a

242   pooled PLR of 2.87 which indicated about three-fold increment in the probability of EC in an Asian with a

243   positive relative to a healthy individual. The pooled NLR of only 0.36, on the other hand, meant that the risk of

244   EC in an Asian with a negative test was 36 percent higher than in a healthy person. In addition, DOR was

245   found to have a strong diagnostic effectiveness in 12 investigations.

246   Despite our best efforts, there are several limitations to the present study: (1) study is based on published

247   articles, and unpublished studies or on-going were not included; (2) the sample size in our meta-analysis was

248   small and more high-quality clinical studies are needed to be carried out

249   for validation; (3) Ten of the studies in this meta-analysis had obvious differences in time or place. However,

250   there may have few overlapping patients between two studies (with obvious differences in specificity and

251   sensitivity) (Guiying Sun et al. Zhang et al.), and emails sent to the corresponding authors got no response.

252

253

Conclusions

255   Our meta-analysis findings imply that circulating miR-21 has potential diagnostic utility for EC in Asians, with

256   good sensitivity and specificity. However, further high-quality research on the diagnostic function of circulating

257   miR-21 should be undertaken in the future.

258

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372

Figure 1

Study flow diagram

```
┌──────────────────────────────┐   ┌──────────────────────────────┐
│ 754 potentially eligible studies │   │ 0 additional records identified │
│ identified by database search │   │ through other sources        │
└──────────────────────────────┘   └──────────────────────────────┘
```

┌──┐
│ 532 records after duplicates removed   │
└──┘

┌──────────────────────────────────────┐
│ 82 records were excluded, due   │
│ to: │
│ 45 reviews, letters, or comment   │
│ 13 laboratory studies   │
│ 24 not directly related to   │
│ specific outcome   │
└──────────────────────────────────────┘

┌──┐
│ 450 full-text articles assessed for eligibility │
└──┘

┌──────────────────────────────────────┐
│ 438 articles were excluded,   │
│ due to: │
│ 372 non-digestive system   │
│ cancer   │
│ 66 Outcomes were not protein   │
│ expression   │
└──────────────────────────────────────┘

┌──┐
│ 12 articles application of inclusion   │
└──┘

┌──┐
│ 12 studies included in qualitative synthesis   │
└──┘

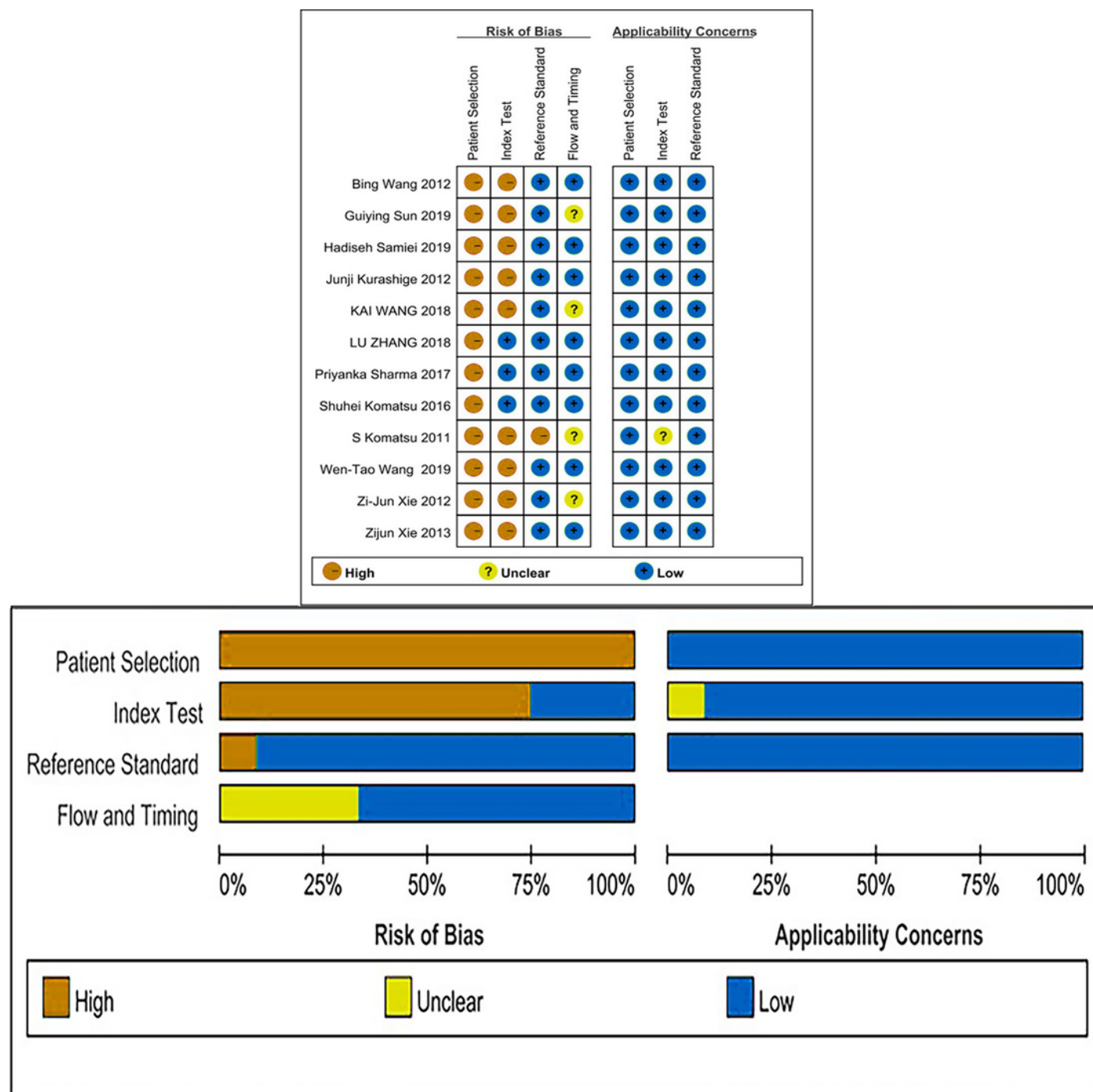
Characteristics of the 12 Studies Included in This Systematic Review

1   TABLE 1. Characteristics of the 12 Studies Included in This Systematic Review

Study	Country	Year	Sample type	Cancer	Control	TNM	SEN	SPE	Median age	Male (P)	Female (P)	Male (C)	Female (C)
Guiying Sun (Sun et al., 2019)	China	2019	Plasma	125	125	I-IV	61.00%	90.00%	63(59-68)	76	49	76	49
Wen-Tao Wang (a) (Wang et al., 2019)	China	2019	Plasma	128	45	I	80.00%	71.43%	N/A	N/A	N/A	N/A	N/A
Wen-Tao Wang (b)	China	2019	Plasma	128	45	II	67.02%	79.41%	N/A	N/A	N/A	N/A	N/A
Wen-Tao Wang (c)	China	2019	Plasma	128	45	III	80.85%	70.00%	N/A	N/A	N/A	N/A	N/A
Hadiseh Samiei (Samiei et al., 2019)	Iran	2019	Plasma	34	34	I-IV	77.78%	65.38%	50(48-52)	5	29	5	29
Lu Zhang (Zhang et al., 2018)	China	2018	Blood	125	125	I-IV	74.00%	78.00%	N/A	N/A	N/A	N/A	N/A
Kai Wamg (Wang et al., 2018)	China	2018	Serum	35	32	I-IV	77.40%	82.90%	51(27-83)	26	9	19	13
Priyanka Sharma (Sharma et al., 2018)	India	2018	Blood	24	21	I-IV	83.33%	57.15%	74	18	6	N/A	N/A
Shuhei Komatsu (Komatsu et al., 2016)	Japan	2016	Plasma	37	20	I-IV	54.20%	94.30%	64	30	7	N/A	N/A
Zijun Xie (Xie et al., 2013)	China	2013	Saliva	39	19	II-IV	89.70%	47.40%	58(46-88)	31	8	15	4
Zi-Jun Xie (Xie et al., 2012)	China	2012	Saliva	32	16	I-IV	87.50%	62.50%	61(43-88)	26	6	13	3
Bing Wang (Wang & Zhang, 2012)	China	2012	Serum	31	39	I-IV	71.00%	69.20%	61(46-82)	23	8	9	30
Junji Kurashige (Kurashige et al., 2012)	Japan	2012	Serum	71	39	I-IV	46.50%	100.00%	70	66	5	N/A	N/A
S Komatsu (KOMATSU et al., 2011)	Japan	2011	Plasma	50	20	I-IV	88.00%	70.00%	66(58-74)	44	6	N/A	N/A

3   Note: TNM: Cancer Stage; SEN: Sensitivity; SPE: Specificity; P: Patient; C: Control; NA: Not Available.; AUC: Area under curve.

Figure 2

Risk of bias and applicability concerns summary and graph.

The results of subgroup analysis for the diagnosis of EC.

1 TABLE 2. The results of subgroup analysis for the diagnosis of EC.

2 Note: SEN: Sensitivity; SPE: Specificity; PLR: Positive likelihood ratio; NLR: Negative likelihood ratio; DOR: Diagnostic odds ratio; NA: Not Available; AUC: Area under

Subgroup	n	SEN (95%CI)	SPE (95%CI)	PLR (95%CI)	NLR (95%CI)	DOR (95%CI)	AUC
Country							
China	7	0.74(0.71-0.77)	0.78(0.74-0.81)	2.94(2.30-3.77)	0.35(0.31-0.41)	9.94(7.50-13.10)	0.82
Others	5	0.66(0.59-0.72)	0.79(0.71-0.86)	2.98(1.57-5.65)	0.39(0.27-0.57)	10.77(5.22-22.22)	0.84
Specimen types							
Plasma	5	0.73(0.69-0.76)	0.80(0.75-0.84)	3.16(2.36-4.24)	0.36(0.30-0.44)	10.28(7.33-14.40)	0.83
Serum	3	0.60(0.51-0.68)	0.85(0.76-0.91)	4.59(1.38-15.23)	0.42(0.26-0.67)	13.05(3.66-46.61)	0.80
Blood	2	0.75(0.68-0.82)	0.75(0.67-0.82)	2.64(1.55-4.48)	0.33(0.25-0.45)	9.30(5.46-15.80)	N/A
Saliva	2	0.89(0.79-0.95)	0.54(0.37-0.71)	1.88(1.31-2.71)	0.21(0.10-0.43)	9.47(3.49-25.73)	N/A
Stage							
I-IV	10	0.69(0.65-0.73)	0.81(0.77-0.84)	3.13(2.28-4.30)	0.38(0.31-0.47)	10.63(7.70-14.67)	0.83
Others	2	0.77(0.73-0.81)	0.70(0.62-0.77)	2.47(1.84-3.30)	0.32(0.25-0.41)	8.97(5.83-13.80)	N/A
Gender							
Male	6	0.73(0.66-0.79)	0.79(0.71-0.85)	2.73(1.67-4.46)	0.39(0.31-0.50)	10.82(6.09-19.20)	0.83
Female	6	0.72(0.62-0.80)	0.77(0.69-0.84)	2.84(1.93-4.17)	0.39(0.29-0.52)	8.97(5.83-13.80)	0.82
Age							
＞60	7	0.66(0.61-0.71)	0.83(0.78-0.87)	3.29(1.98-5.47)	0.41(0.32-0.53)	11.47(7.36-17.88)	0.83
≤60	3	0.81(0.73-0.88)	0.68(0.57-0.78)	2.40(1.40-4.11)	0.29(0.19-0.45)	9.10(4.54-18.21)	0.84
Total	12	0.72(0.69-0.75)	0.78(0.75-0.81)	2.87(2.28-3.59)	0.36(0.31-0.43)	10.00(7.73-12.95)	0.82

3 curve.

Figure 3

Forest plots of (A) sensitivity, (B) specificity, (C) pooled specificity and (D) the pooled sensitivity , of miRNA for diagnosis of EC in Asia.

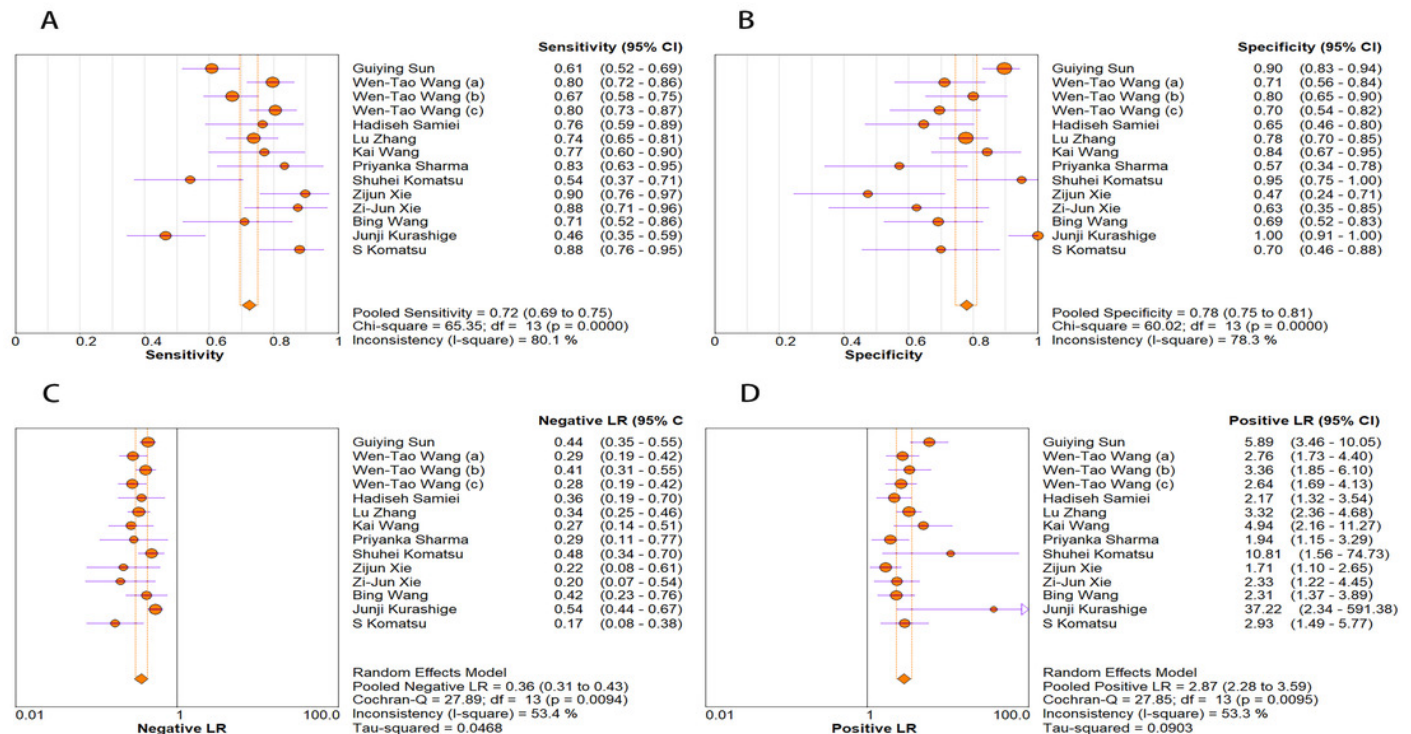



Figure 4

SROC curve for miR-21 in EC diagnosis.

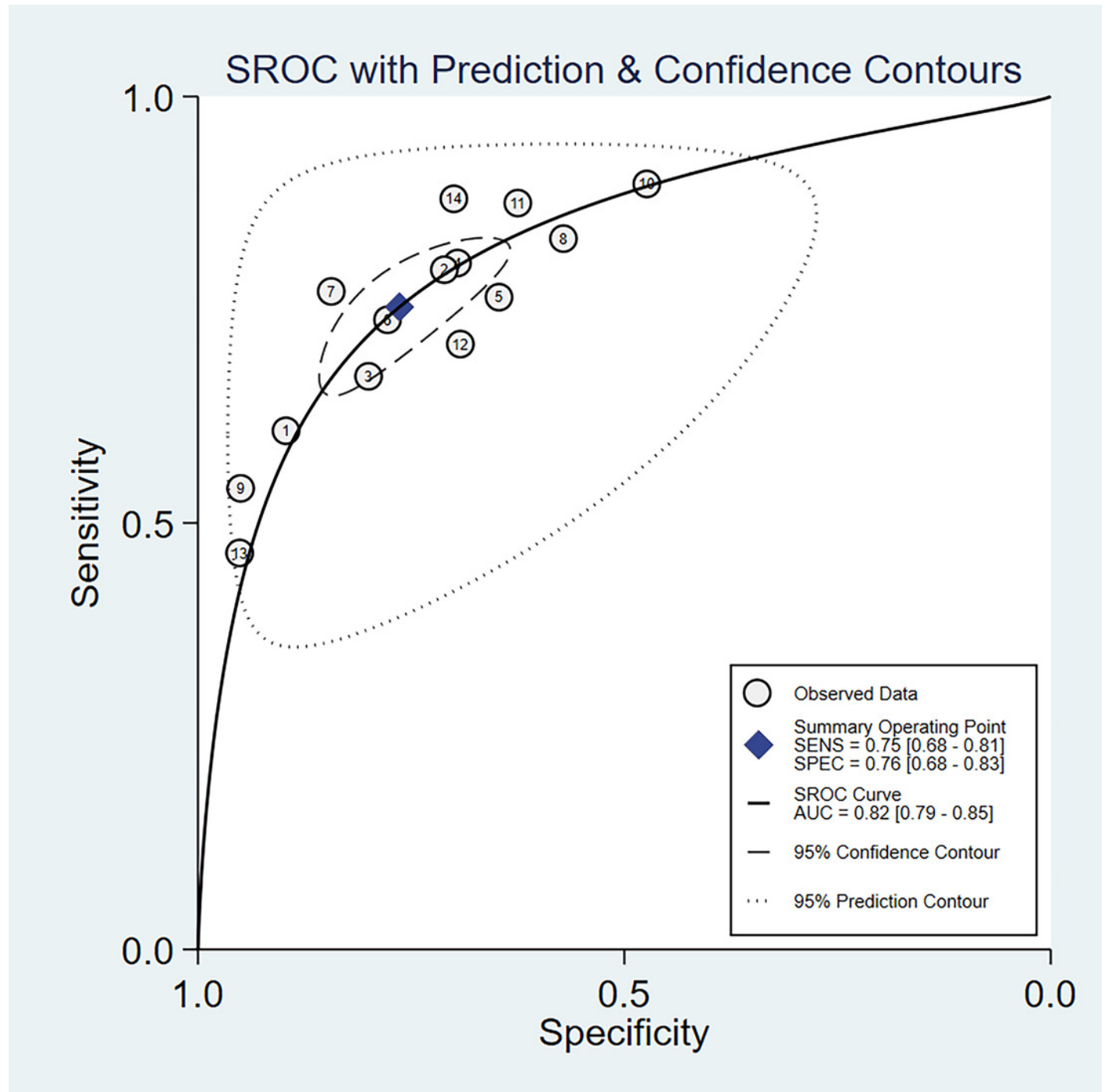



Figure 5

Forest plot of included studies assessing the DOR of miR-21 in EC.

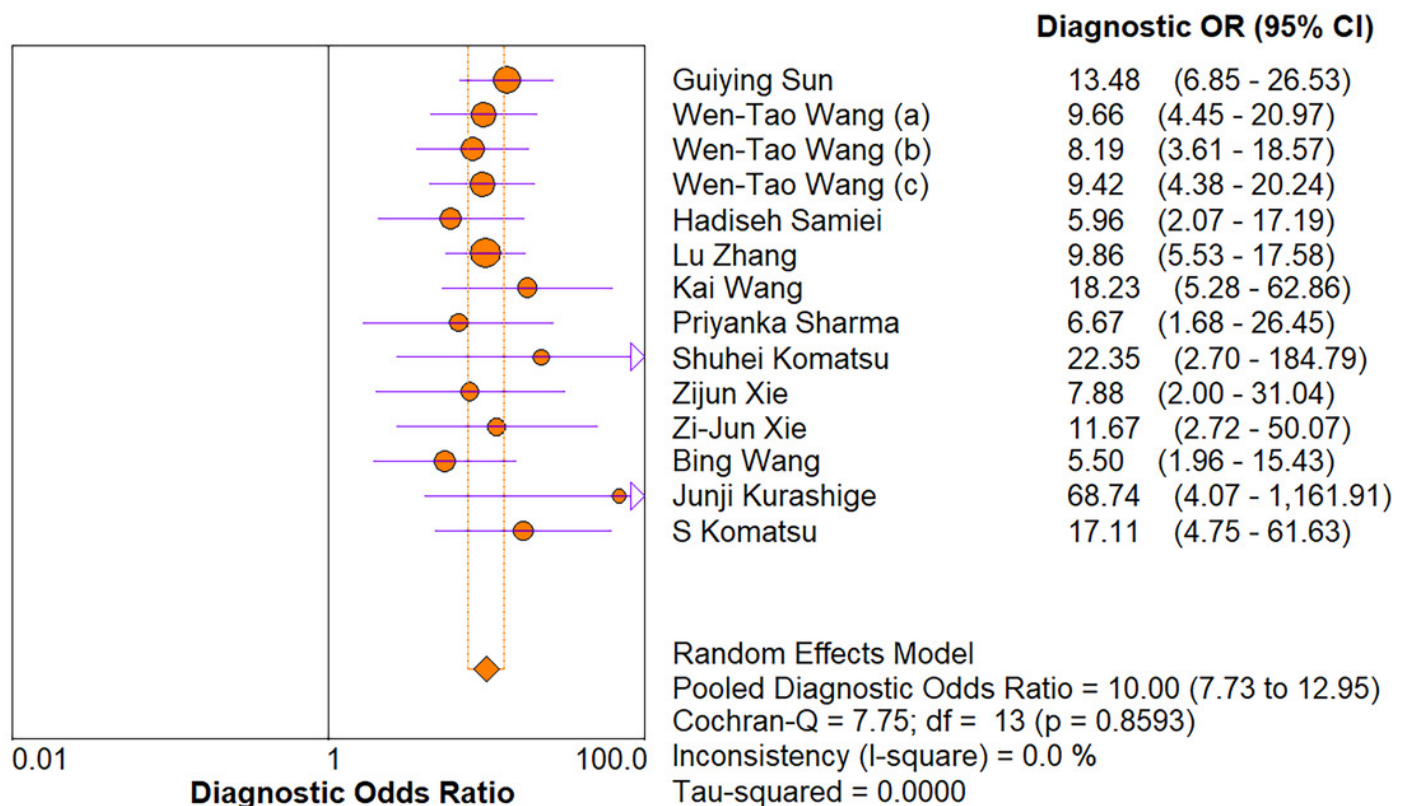



Figure 6

Forest plots of (A) sensitivity, (B) specificity, (C) pooled specificity and (D) the pooled sensitivity of plasma miRNA for diagnosis of EC in Asia.

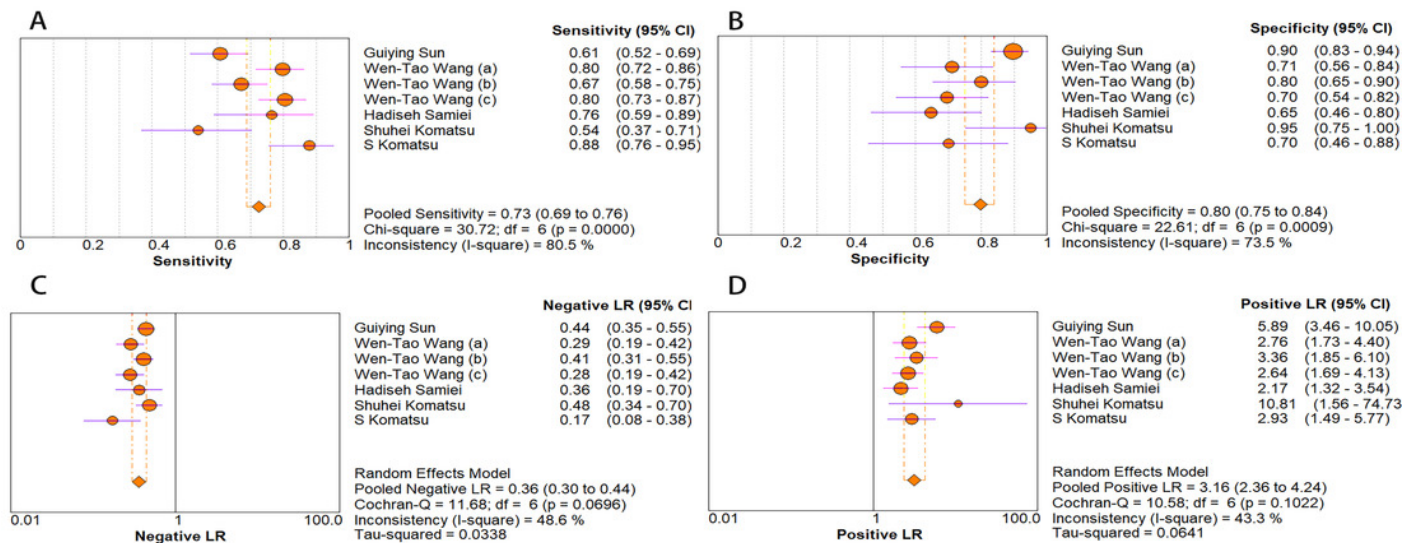



Figure 7

Forest plot of included studies assessing the DOR of plasma miR-21 in EC.

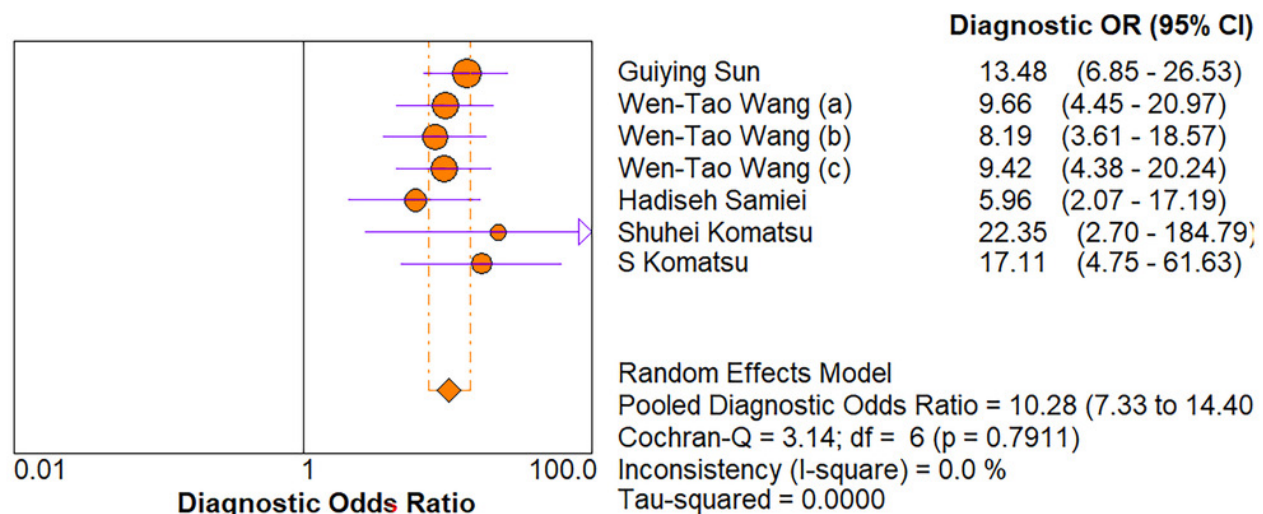



Figure 8

Forest plots of (A) sensitivity, (B) specificity, (C) pooled specificity, (D) the pooled sensitivity and (E) the pooled diagnostic odds ratio of saliva miRNA for diagnosis of EC in Asia.

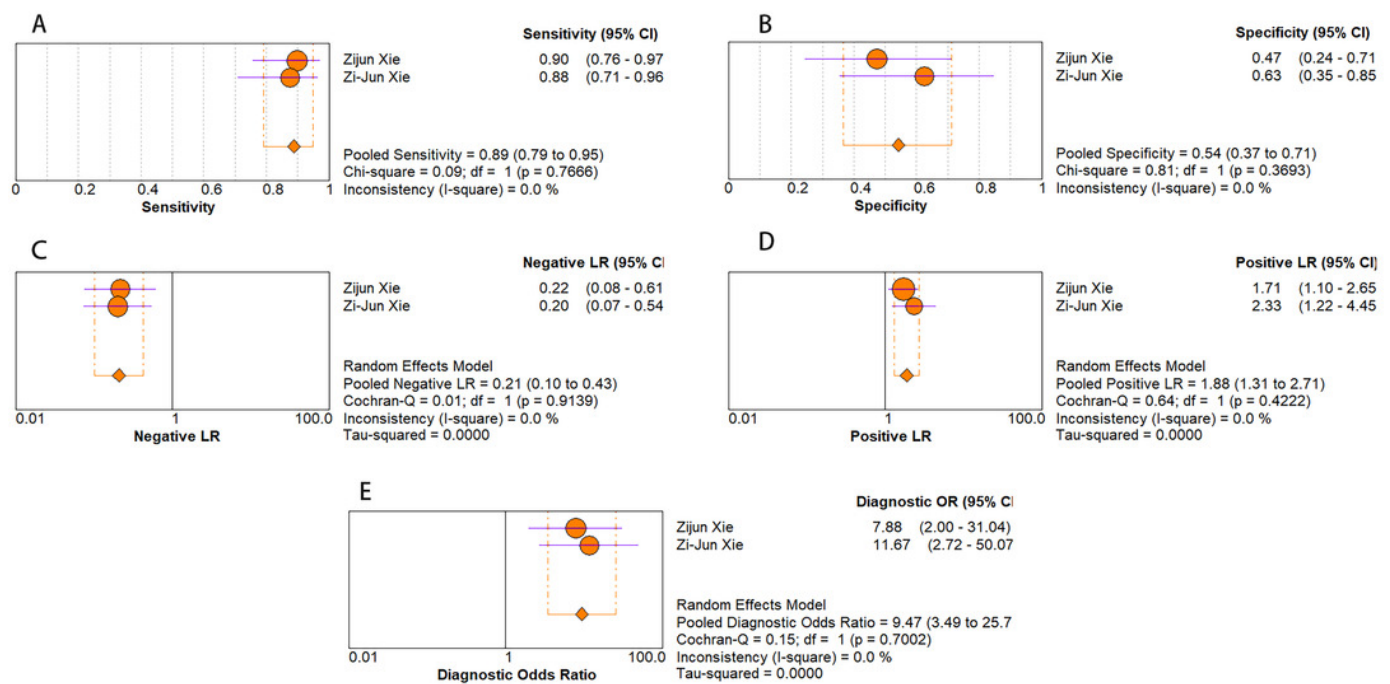



Figure 9

Forest plots of (A) sensitivity, (B) specificity, (C) pooled specificity, (D) the pooled sensitivity and (E) the pooled diagnostic odds ratio of blood miRNA for diagnosis of EC in Asia.

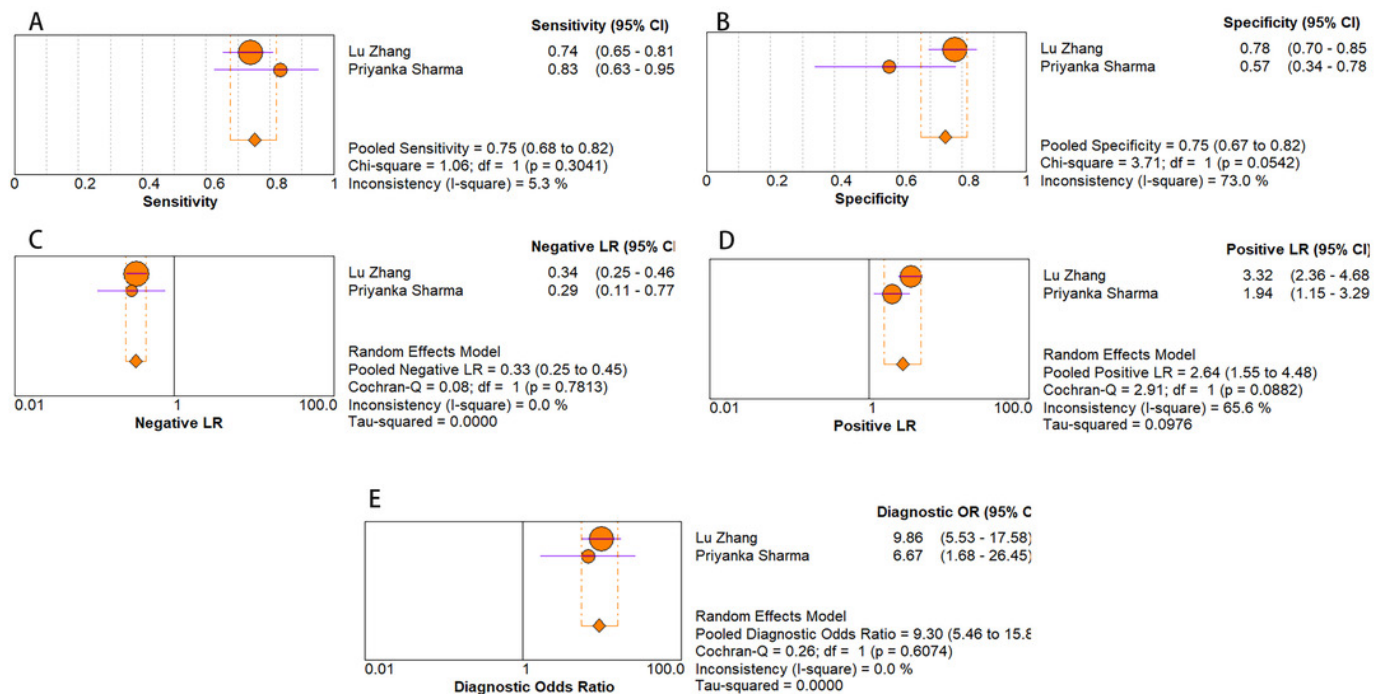



Figure 10

Forest plots of (A) sensitivity, (B) specificity, (C) pooled specificity, (D) the pooled sensitivity and (E) the pooled diagnostic odds ratio of serum miRNA for diagnosis of EC in Asia.

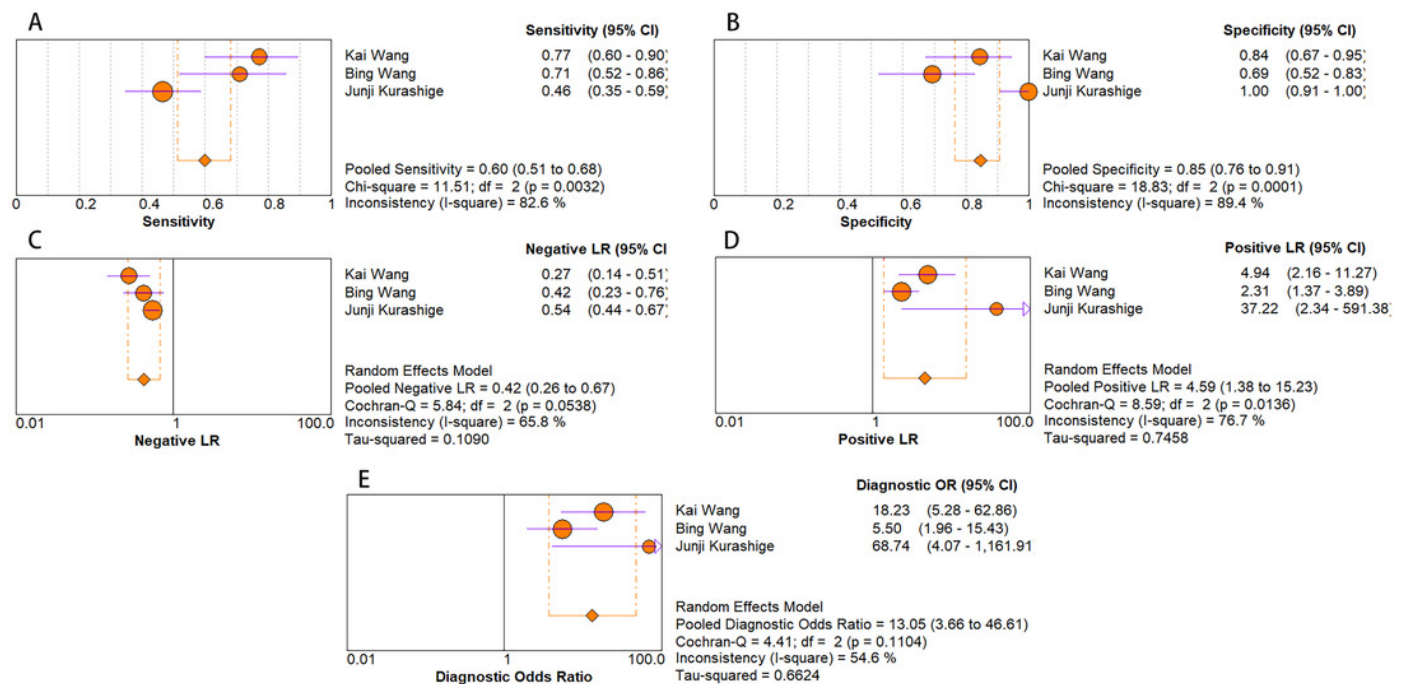



Figure 11

Sensitivity analysis of the included studies. (a) Goodness-of-fit, (b) bivariate normality, (c) influence analysis, and (d) outlier detection.

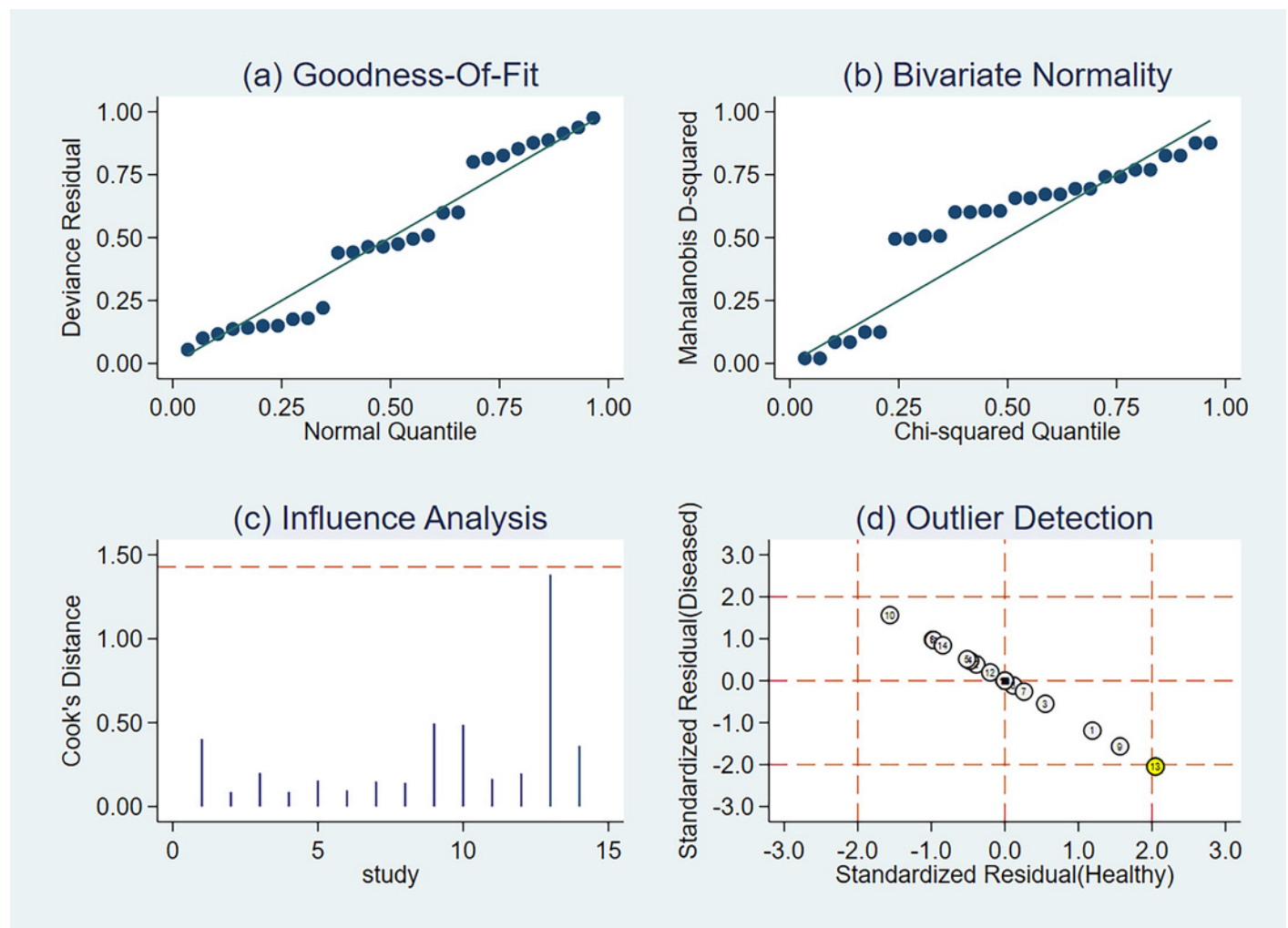



Figure 12

Fagan's nomogram of miRNA-21 for the diagnosis of EC.

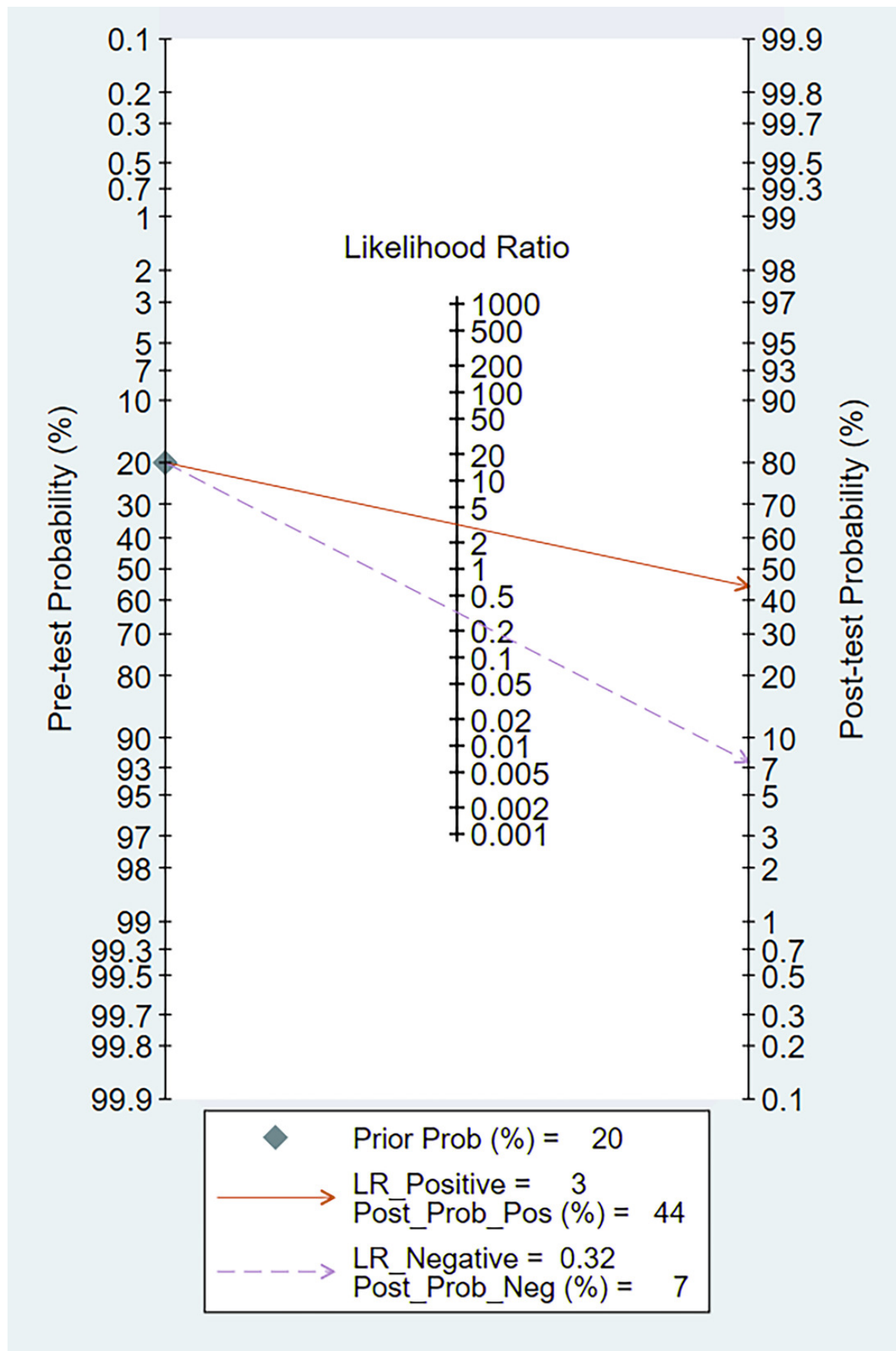



Figure 13

Deeks' funnel plot of miRNA-21 for the diagnosis of EC.