Increased microRNA-30a levels in bronchoalveolar lavage fluid as a diagnostic biomarker for lung cancer

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Background: MicroRNA-30a (miRNA-30a) levels have been shown to increase in the plasma of lung cancer patients. Herein, we evaluated the miRNA-30a levels in the bronchoalveolar lavage fluid (BALF) of lung cancer patients as a potential biomarker for lung cancer diagnosis.

Methods: BALF miRNA-30a expression of 174 subjects was quantified using quantitative real-time reverse transcription-polymerase chain reaction and compared between lung cancer patients and control patients with benign lung diseases. Moreover, its diagnostic value was evaluated by performing receiver operating characteristic (ROC) curve analysis.

Results: The relative BALF miRNA-30a expression was significantly higher in the lung cancer patients than in the controls (0.74 ± 0.55 versus 0.07 ± 0.48, respectively, $p<0.001$) as well as in lung cancer patients with stage I–IIA disease than in those with stage IIB–IV disease (0.98 ± 0.64 versus 0.66 ± 0.54, respectively, $p<0.05$). Additionally, miRNA-30a distinguished benign lung diseases from lung cancers, with an area under the ROC curve (AUC) of 0.822. ROC analysis also revealed an AUC of 0.875 for the Youden index-based optimal cut-off points for stage I–IIA adenocarcinoma. Thus, increased miRNA-30a levels in BALF may be a useful biomarker for non-small-cell lung cancer diagnosis.

1 RESEARCH ARTICLE

2 **Increased microRNA-30a levels in bronchoalveolar lavage**

3 **fluid as a diagnostic biomarker for lung cancer**

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22 **Abstract**

23   **Background:** MicroRNA-30a (miRNA-30a) levels have been shown to increase in the plasma of

24   lung cancer patients. Herein, we evaluated the miRNA-30a levels in the bronchoalveolar lavage

25   fluid (BALF) of lung cancer patients as a potential biomarker for lung cancer diagnosis.

26   **Methods:** BALF miRNA-30a expression of 174 subjects was quantified using quantitative real-

27   time reverse transcription-polymerase chain reaction and compared between lung cancer patients

28   and control patients with benign lung diseases. Moreover, its diagnostic value was evaluated by

29   performing receiver operating characteristic (ROC) curve analysis.

30   **Results:** The relative BALF miRNA-30a expression was significantly higher in the lung cancer

31   patients than in the controls (0.74 ± 0.55 versus 0.07 ± 0.48, respectively, $p<0.001$) as well as in

32   lung cancer patients with stage I–IIA disease than in those with stage IIB–IV disease ($0.98 \pm$

33   0.64 versus 0.66 ± 0.54, respectively, $p<0.05$). Additionally, miRNA-30a distinguished benign

34   lung diseases from lung cancers, with an area under the ROC curve (AUC) of 0.822. ROC

35   analysis also revealed an AUC of 0.875 for the Youden index-based optimal cut-off points for

36   stage I–IIA adenocarcinoma. Thus, increased miRNA-30a levels in BALF may be a useful

37   biomarker for non-small-cell lung cancer diagnosis.

39   **Keywords:** miRNA-30a, lung cancer, bronchoalveolar lavage fluid (BALF), tumor marker, early

40   diagnosis, biomarker

Introduction

43 Lung cancer is the leading cause of cancer-related mortality worldwide (Torre et al. 2016). The

44 World Health Organization estimates that the trends of lung cancer deaths will continue to rise,

45 largely due to an increase in global tobacco use, especially in Asia (Dela Cruz et al. 2011). In

46 addition, lung cancer is usually diagnosed at an advanced stage because of the lack of clinical

47 symptoms (Tissot et al. 2015), leading a poor prognosis (Alberg et al. 2013). Given its low rate

48 of early diagnosis, lung cancer not only seriously affects the survival and prognosis of patients

49 but also imposes a heavy social and economic burden. Therefore, it is essential to explore new

50 biomarkers for early detection of lung cancer.

51

52 Biopsy under flexible bronchoscopy (FB) is now established as an essential diagnostic tool for

53 patients with abnormal imaging or sputum findings, according to the routine lung cancer

54 screening process (Du Rand et al. 2013). The diagnostic yield from FB ranges from 48% to 93%,

55 depending on whether the tumor is visible within the bronchial tree (Rivera & Mehta 2007).

56 However, biopsy with FB is limited when the lesions are with obviously exposed blood vessels

57 and when patients with clinical risk factors for bleeding (such as anticoagulation therapy, renal

58 dysfunction) (Du Rand et al. 2013). Bronchial washings with FB is an alternation when biopsy is

59 limited, which increases the diagnostic yield of FB in patients with lung cancer (McLean et al.

60 1998). Therefore, alternative diagnostic process should be taken into account for individuals

61 undergoing FB but with invisible tumor in the bronchial tree or risk factors of biopsy.

62

63 MicroRNAs (miRNAs) are a class of 22-nucleotide long, non-coding, single-stranded RNAs (Yi

64 et al. 2003). There is increasing evidence that miRNA expression is associated with various

65   human cancers and may function as tumor suppressors or oncogenes (Bridge et al. 2012).

66   MiRNAs play an important role in the cancer cells' development (Bussing et al. 2008; Johnson et

67   al. 2007), invasion, metastasis(Yao et al. 2010), proliferation, differentiation, apoptosis, and

68   neoplastic transformation(Bartel 2004) (Boehm & Slack 2005), indicating that miRNAs may

69   serve as a new source of biomarkers for the diagnosis of tumors (Chen et al. 2008; Esquela-

70   Kerscher & Slack 2006; Fredsøe et al. 2018; Kim et al. 2015; Mitchell et al. 2008).

71

72   MiRNA-30a is a member of the miRNA-30 family, which contains five distinct premature

73   miRNAs sequences (miR-30a, -30b, -30c, -30d and -30e)(Bridge et al. 2012; Mao et al. 2018).

74   Increasing evidence suggested that circulating miRNA-30a has been correlated with several

75   types of human cancers(Fredsøe et al. 2018; Zeng et al. 2013; Zhou et al. 2015). miRNA-30a has

76   been found to be related to the transformation, prognosis, and diagnosis of lung

77   cancer(Kumarswamy et al. 2012; Tang et al. 2015; Wen et al. 2015a), which indicates miRNA-

78   30a may act as a biomarker for lung cancer detection. Our previous study demonstrated that

79   plasma miRNA-30a levels increased in non-small-cell lung cancer (NSCLC) with a relatively

80   high value in the diagnosis of NSCLC(Sun et al. 2016). However, the expression level of

81   miRNA-30a levels in bronchoalveolar lavage fluid (BALF) and diagnostic value of BALF

82   miRNA-30a for those individuals suspected for lung cancer with indications of flexible

83   bronchoscopy but with relatively high risk to perform biopsy still remains unknown.

84

85   In this study, we investigated the relative expression of miRNA-30a in BALF and explored its

86   diagnostic value for lung cancer, especially at an early stage.

87

Material & Methods

Study setting and subjects

The protocol for this prospective study was approved by the Medical Ethics Committee of the West China Hospital, Sichuan University (approval number 2018-83). Samples were collected and processed in a double-blind manner. All patients were grouped based on the pathological diagnosis after miRNA-30a levels were measured, and all other data were collected. By the end of this study, 99 patients with histologically proven primary lung cancer, 55 patients with benign lung diseases 20 healthy controls were recruited between December 2015 and February 2017 at our institution. All the patients have not undergone any types of therapy before sample collection.

The clinical data were acquired for each subject from electronic medical records and included information regarding a complete history and physical examination, serological examination, diagnostic imaging scans, and pathology reports. Epidemiological data were also collected using a self-reported questionnaire, which included information such as demographics, performance status, occupational history, and previous medical history.

Sample collection

Written informed consent was obtained from all patients prior to bronchoscopy. Plasma and BALF collection, miRNA extraction and analysis were performed based on the same SOP (Standard Operating Procedure) for each patient. Briefly, fasting peripheral venous blood (5 ml) and BALF (8ml) were collected within an hour before and during bronchoscopy process respectively. For patient with lung cancer, BALF specimens were collected from both the sides of the chest lung, including where the tumor was located and the contralateral side (Kim et al.

111   2015). The blood sample and BALF specimens were centrifuged immediately after collection, at

112   4°C 3,000rpm 10min and 4°C 2,000 rpm 10min respectively. The supernatants were stored at

113   4°C temporally and were transferred to −80°C within one hour. RNA sample extraction, Reverse

114   transcription (RT) Quantitative real-time polymerase chain reaction (qPCR) and ELISA

115   (enzyme-linked immunosorbent assay) procedure were performed within one week after BALF

116   and plasma sample collection.

117

118   RNA quality was measured by RNA concentration, purity and integrity. RNA concentration and

119   purity was detected by measuring the absorbance at 260 nm/280 nm(A260/A280) and 260

120   nm/230 nm(A260/A230) in a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific Inc.,

121   DE, USA). RNA integrity was measured by 1%AGE (Agarose Gel Electrophoresis). With the

122   quality of high purification(A260/A280 was in 1.8-2.1, A260/A230＞1.8) and integrate bands,

123   the RNA extracted is suitable for molecular biology operation.

124

125   ***Measurement of miRNA-30a levels***

126   Total RNA was isolated from BALF specimens using the TRIzol-based method (Life

127   Technologies, Carlsbad, CA, USA), and the RNA pellet was dissolved in 30 μL of RNAase-free

128   water as previously reported (Sun et al. 2016). The purity and concentration of RNA were

129   evaluated using a ScanDrop 100 spectrophotometer (Analytik Jena AG, Jena, Germany). Reverse

130   transcription (RT) was performed using a RevertAid RT kit (Thermo Fisher Scientific, Waltham,

131   MA, USA) according to the manufacturer's instructions.

132   Quantitative real-time polymerase chain reaction (RT-qPCR) assays were performed as

133   previously reported (Sun et al. 2016) in a 20 μL volume using a CFX96 real-time PCR system

134   (Bio-Rad, Hercules, CA, USA). The reactions were incubated at 95°C for 3 min, followed by 40

135   cycles of 10 s at 95°C, 15 s at 59°C, and 10 s at 72°C. The relative expression levels of miRNA-

136   30a were calculated as expression value of miRNA-30a to the expression value of U6 via the

137   comparative $2^{-\Delta\Delta CT}$ method (Fredsøe et al. 2018) . Finally, the results were processed and

138   analyzed using the Bio-Rad CFX Manager.

139

140   ***Statistical analyses***

141   All statistical analyses were performed using SPSS version 21 (SPSS Inc., Chicago, IL, USA).

142   The association of categorical variables was assessed using the chi-squared test. A *t*-test or

143   analysis of variance was used to explore the distribution of continuous variables among the study

144   groups, as appropriate. Receiver operating characteristic (ROC) curve analysis was applied to

145   investigate the potential of miRNA-30a and other tumour markers to distinguish between the

146   benign and malignant groups. The optimal Youden index was used as the cut-off point for lung

147   cancer diagnosis (Berezikov et al. 2006) based on the sensitivity and specificity. Values of

148   p<0.05 were considered to be statistically significant.

149

150   **Results**

151   ***Clinical characteristics of study subjects***

152   BALF samples were collected from 99 patients with histologically proven lung cancer, 55

153   patients with benign lung diseases and 20 healthy control patients. There were 62 males and 37

154   females in the lung cancer group, and the mean age was 57.97 ± 10.33 years. The pathological

155   types included 60 adenocarcinomas, 22 squamous cell carcinomas, 15 small-cell lung cancers,

156   and 2 large-cell lung cancers. According to the 8th Lung Cancer TNM Classification and Staging

157 System, 19, 8, 31, and 26 patients with stages I, II, III, and IV lung cancer, respectively, were

158 identified based on the postoperative pathology. Among patients with benign lung diseases, there

159 were 39 males and 16 females with a mean age of 54.53 ± 10.62 years, including 47 pathology-

160 verified cases of pneumonia, 2 cases of inflammatory pseudotumor, and 6 cases with benign lung

161 nodules. There were 12 males and 8 females in the healthy control group, and the mean age was

162 53.05±13.20. Patients with benign lung diseases as well as patients in the healthy control group

163 had not developed any types of cancer during the whole follow-up period. There were no

164 statistically significant differences in the clinical characteristics among three groups (Table 1).

165

166 ***BALF miRNA-30a levels in three groups***

167 MiRNA-30a was significantly upregulated in BALF specimens collected from the lesion side of

168 lung cancer patients compared with patients with lung benign lesions and healthy controls (all,

169 p<0.001) (Fig. 1).

170

171 ***MiRNA-30a levels in BALF specimens obtained from the sides with and without lung cancer***

172 ***lesions***

173 The origin of miRNA-30a in BALF is unknown. It may be secreted by tumour cells, cells

174 adjacent to tumour tissues, immune cells accumulated around the tumour, or even cells

175 circulating in the bloodstream. It is reasonable to hypothesize that the level of miRNA-30a in

176 BALF specimens obtained from the side with the lesion would be higher than in that obtained

177 from the side without the lesion. Therefore, BALF specimens were collected from lung cancer

178 patients via flexible bronchoscopy from both the sides, with and without the lesions,

179 simultaneously. No statistically significant difference was found in the BALF miRNA-30a levels

180 between the two sides (0.23 ± 0.65, p=0.06)(Fig.2). Therefore, we subsequently analyzed the

181 level of miRNA-30a in the BALF specimens obtained from the side with the lung cancer lesion.

182

183 ***Correlation between BALF miRNA-30a levels and clinicopathological features of lung cancer***

184 To explore the possible relationships between BALF miRNA-30a levels and the

185 clinicopathological features of lung cancer, we analyzed the data based on the following factors:

186 age, sex, smoking status, histological type, and TNM stage. No statistically significant

187 differences were found between miRNA-30a levels and these clinicopathological features, apart

188 from the clinical TNM stage (Table 2 and Fig. 3).

189

190 ***Diagnostic value of miRNA-30a levels in lung cancer patients***

191 Since the level of miRNA-30a in BALF specimens was significantly higher among lung cancer

192 patients compared with that of the controls, miRNA-30a might serve as a biomarker for lung

193 cancer diagnosis. ROC curve analysis was performed to evaluate the diagnostic value of

194 miRNA-30a levels. The area under the curve (AUC) for miRNA-30a was 0.822 (95% confidence

195 interval [CI]: 0.752–0.892, p<0.001) (Fig. 4).

196 As shown in Table 3, the best diagnostic cut-off value of relative miRNA-30a levels in BALF

197 was 2.0316 (with a diagnostic sensitivity and specificity of 80.8% and 69.1%, respectively).

198

199 ***Diagnostic value of BALF miRNA-30a level for stages I–IIA lung cancer and benign lung***

200 ***disease***

201 Compared with levels among patients with benign lung diseases, miRNA-30a was significantly

202 upregulated in the BALF of patients with stages I–IIA lung cancer (0.98 ± 0.64 versus 0.07 ±

203   0.48, respectively, p<0.001) (Fig. 5), with an AUC of 0.882 (95% CI: 0.806–0.958, p<0.001)

204   (Fig. 6). The best diagnostic cut-off value of relative miRNA-30a levels in BALF was 2.6508

205   (with a diagnostic sensitivity and specificity of 90.9% and 74.5%, respectively) (Table 4). This

206   indicated that the upregulated BALF miRNA-30a expression level could be used as an early

207   diagnostic marker for lung cancer.

208

209   ***Diagnostic value of miRNA-30a expression in BALF among different pathologic types and***

210   ***TNM stages of lung cancer and benign lung disease***

211   There were statistically significant differences in BALF miRNA-30a levels among different

212   pathologic types and TNM stages of lung cancer and benign lung disease (p<0.001). ROC

213   analysis revealed an AUC of 0.875 for the Youden index-based optimal cut-off points for stages

214   I–IIA adenocarcinoma (Fig. 7). Thus, increased levels of miRNA-30a in BALF may serve as a

215   diagnostic biomarker for stage I–IIA adenocarcinoma (Tables 5 and 6).

216

217   ***Combined diagnostic value of BALF miRNA-30a and plasma tumour markers***

218   In addition, we compared the diagnostic value of BALF miRNA-30a with that of traditional

219   plasma tumour markers (carcinoembryonic antigen [CEA] and CYFRA21-1) based on the ability

220   to differentiate lung cancer patients from patients with benign lung disease. We determined the

221   BALF miRNA-30a, plasma CEA, and CYFRA21-1 levels in stages I–IIA NSCLC and control

222   groups and found that the AUC of miRNA-30a was larger than that of CEA and CYFRA21-1. A

223   combination of BALF miRNA-30a levels with plasma CEA and CYFRA21-1 levels could

224   improve the diagnostic value for advanced NSCLC. When plasma CYFRA21-1, CEA, and

225   BALF miRNA-30a levels were combined, the AUC reached the maximum value of 90.3% for

226   stages I–IIA NSCLC and 92.3% for stages IIB–IV NSCLC, which makes this combination

227   preferable for the diagnosis of NSCLC (Table 7 and Fig. 8).

228

229   **Discussion**

230   Our previous data showed miRNA-30a was down-regulated and up-regulated in lung cancer

231   tissue(Sun et al. 2016) and plasma respectively (Liang et al. 2019), which arouse our interest in

232   analyzing miRNA-30a expression in BALF of lung cancer. In this hospital-based case-control

233   study, we found the expression level of miRNA-30a in BALF was significantly up-regulated in

234   lung cancer patients when both compared to those with benign lung disease and healthy control

235   patients (both p<0.001). The expression level of miRNA-30a in BALF was significantly higher

236   in individuals with early stage (stage I-IIA) of lung cancer when compared to those with stage

237   IIB-IV (p<0.05). The ROC curve analysis revealed that the BALF miRNA-30a level could well

238   distinguish lung benign disease and lung cancer as well as early stage of lung cancer with the

239   relative high AUC of 0.822 and 0.882 respectively. The diagnostic value was even higher when

240   combined BALF miRNA-30a, CEA and CYFRA21-1. All those findings indicated that BALF

241   miRNA-30a relative expression level could be used as a biomarker in early detection of lung

242   cancer for individuals undergoing FB but with invisible tumor in the bronchial tree or risk factors

243   of biopsy.

244

245   Abundant circulation miRNAs have been demonstrated to play a vital role in early cancer

246   detection (Kim et al. 2015; Mitchell et al. 2008; Tsujiura et al. 2010). The circulating cell-free

247   miRNAs have shown great promise as a new class of cancer biomarkers, owing to their

248   surprisingly high stability in body fluids, association with disease states, and ease of

249   measurement (Heinzelmann et al. 2011; Zheng et al. 2011). MiRNA-30a, as a member of the

250   miRNA-30 family, has been correlated with several types of human cancers(Saleh et al. 2019;

251   Sun et al. 2019; Wen et al. 2015b). The expression level of miRNA-30a was down-regulated in

252   breast cancer(Zeng et al. 2013) but up-regulated in prostate cancer and ovarian serous

253   adenocarcinoma (Fredsøe et al. 2018; Zhou et al. 2015). Our present study revealed that miRNA-

254   30a in BALF was correlated with different stages of lung cancer. The opposite activity of miR-

255   30a in various cancer type and different stages in one cancer type indicated that the biological

256   function of miR-30a might be complicated and associated with a specific cancer type and tumor

257   microenvironment, which needs to be further explored.

258

259   There are several potential mechanism of miRNA-30a in the development, invasion and

260   metastasis of lung cancer. First, miRNA-30a inhibits the epithelial-to-mesenchymal transition by

261   targeting SNAI1, and thus, its downregulation in lung cancer results in cancer invasion and

262   metastasis (Kumarswamy et al. 2012). Second, Guan, Y., et al. have found that miR-30a

263   suppresses lung cancer progression by targeting SIRT1 (Guan et al. 2017). In addition,

264   expression of miR-30a inhibitor the growth of lung cancer cells by targeting MEF2D (Luan et al.

265   2018). Further studies are necessary to identify the target genes of miR-30a and elucidate the

266   underlying mechanism that regulates the biogenesis of miR-30a.

267

268   The expression level of miRNA-30a in body fluids, including BALF and the plasma(Liang et

269   al. 2019) of lung cancer patients, were significantly upregulated, while the opposite trend was

270   observed in lung cancer tissue(Boeri et al. 2011; Kumarswamy et al. 2012; Tang et al. 2015).

271   There are several possible mechanisms. microRNAs are transported into the extracellular fluid

272   environment from cells by exosomes and microvesicles (Etheridge et al. 2011). Pritchard et al.

273   have presented evidence showing that blood cells are a major contributor to circulating miRNAs

274   (Pritchard et al. 2012). The extracellular circulation of miRNAs in lung cancer patients may be

275   due to a response of blood cells to the tumour state (Patnaik et al. 2012) and shifting to various

276   body fluids. However, the exact mechanism still needs to be investigated. However, the exact

277   biological basis of this discrepancy between miRNA-30a expression changes in cancer tissues

278   and in circulating body fluids in lung cancer still remains unclear and needs to be investigated in

279   the future research.

280

281   The present study does have some limitations. First, as a hospital-based case-control study, the

282   relatively small sample size of each group was unavoidable. Future studies with larger cohorts

283   are warranted to validate these findings. Second, there was no significance in BALF miRNA-30a

284   expression levels among the adenocarcinoma, squamous cell carcinoma and small cell lung

285   cancer, and it may be related to the relative small sample size of small cell lung cancer. Third the

286   underlying molecular mechanism that accounts for the contradiction between miRNA-30a

287   expression in cancer tissues and in circulating body fluids of lung cancer needs further

288   investigation.

289

290   **Conclusions**

291   In conclusion, BALF miRNA-30a was significantly up-regulated in lung cancer patients and

292   correlated with early stage lung cancer. These present findings indicated that BALF miRNA-30a

293   relative expression level could be used as a biomarker in early detection of lung cancer for

294   individuals undergoing FB but with invisible tumor in the bronchial tree or risk factors of biopsy.

295

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412

Figure legends

414 **Fig. 1** miRNA-30a levels in BALF samples obtained from lung cancer patients, patients with

415 benign lung diseases and healthy control, as determined by quantitative real-time polymerase

416 chain reaction (RT-qPCR) (n=55 in the benign control group, n=99 in the lung cancer group and

417 n=20 in the healthy control). *p<0.001. Data was log transformed to approximate a normal

418 distribution.

419

420 **Fig. 2** miRNA-30a levels in BALF specimens obtained from the sides with and without lung

421 cancer lesions (n=20, p>0.05)

422

423 **Fig. 3** Bronchoalveolar lavage fluid (BALF) miRNA-30a expression was measured by

424 quantitative real-time polymerase chain reaction (RT-qPCR) (n=22 for stages I–IIA and n=62 for

425 stages IIB–IV). *p<0.05. Data was log transformed to approximate a normal distribution.

426

427 **Fig. 4** Receiver operating characteristic (ROC) curve analysis of miRNA-30a level to distinguish

428 between lung cancer and benign lung diseases. The area under the curve for miRNA-30a was

429 0.822, and the Youden index was 0.499.

430

431   **Fig. 5** miRNA-30a levels in stages I–IIA lung cancer patients (n=22) and benign controls (n=55),

432   as determined by quantitative real-time polymerase chain reaction (RT-qPCR). *p<0.001. Data

433   was log transformed to approximate a normal distribution.

434

435   **Fig. 6** Receiver operating characteristic (ROC) curve analysis of miRNA-30a level to distinguish

436   between early lung cancer and benign lung diseases. The area under the curve for miRNA-30a

437   was 0.882 (95% confidence interval: 0.806–0.958, p<0.001), and the Youden index was 0.654.

438

439   **Fig. 7** Receiver operating characteristic (ROC) curve analysis of miRNA-30a level to distinguish

440   between stages I–IIA adenocarcinoma and benign lung diseases. The area under the curve for

441   miRNA-30a was 0.875 (95% confidence interval: 0.790–0.960, p<0.001).

442

443   **Fig. 8** Receiver operating characteristic (ROC) curve analysis of miRNA-30a level, CEA level,

444   and a combination of miRNA-30a and carcinoembryonic antigen (CEA) levels to distinguish

445   between stages I–IIA adenocarcinoma and benign lung diseases. The area under the curve for

446   miRNA-30a was 0.910 (95% confidence interval: 0.808–1.000, p<0.001).

447

Table 1. Demographic characteristics of study subjects

2 **Table 1**. Demographic characteristics of study subjects

	Lung cancer cases (n=99)	Benign control cases (n=55)	Healthy control (n=20)	p-value
Sex male: female	62:37	39:16	12:8	0.52
Median age(y)	57.97 ± 10.33	54.53 ± 10.62	53.05±13.20	0.061
BMI, kg·m^{-2}	22.77 ± 2.80	23.11 ± 3.97	22.55±1.94	0.728
Smoking status				
Smoker	56 (57%)	26 (47%)	9(45%)	0.426
Never smoked	43 (43%)	29 (53%)	11(55%)	

3 Data are presented as n, n (%), or the mean ± standard deviation.

4 BMI: body mass index.

Table 2. Relationship between miRNA-30a levels and clinicopathological features among patients with lung cancer

1 **Table 2**. Relationship between miRNA-30a levels and clinicopathological features among

2 patients with lung cancer

Clinicopathological features	Subgroup	Subjects	miRNA-30a relative expression	
			Mean ± SD	p-value
Tissue type	SCLC	15	0.72 ± 0.58	
	NSCLC	84	0.74 ± 0.55	0.911
Tissue type subgroup	SCC	22	0.71 ± 0.44	
	ADC	60	0.76 ± 0.59	
	SCLC	15	0.72 ± 0.58	0.924
Age, years	≤60	51	0.69 ± 0.55	
	>60	48	0.79 ± 0.55	0.387
Sex	Male	62	0.75 ± 0.58	
	Female	37	0.71 ± 0.50	0.710
Smoking	Yes	56	0.77 ± 0.59	
	No	43	0.69 ± 0.49	0.473
Clinical TNM stage	I–IIA	22	0.98 ± 0.64	
	IIB–IV	62	0.66 ± 0.54	0.025*

3 SD, standard deviation; SCLC, small-cell lung cancer; NSCLC, non-small-cell lung cancer; SCC,

4 squamous cell carcinoma; ADC, adenocarcinoma.

5 *p<0.05

Table 3. Diagnostic value of bronchoalveolar lavage fluid miRNA-30a based on the optimal cut-off point

1   **Table 3**. Diagnostic value of bronchoalveolar lavage fluid miRNA-30a based on the optimal cut-

2   off point

	Value	95% CI
Cut-off point	2.0316	
TPR	80.8%	0.7140–0.8776
TNR	69.1%	0.5503–0.8047
LR+	2.61	1.7409–3.9260
LR−	0.27	0.1824–0.4228
PPV	82.4%	0.7313–0.8917
NPV	66.6%	0.5283–0.7825

3   CI, confidence interval; TPR, true positive rate (sensitivity); TNR, true negative rate

4   (specificity); LR+, positive likelihood ratio; LR−, negative likelihood ratio; PPV, positive

5   predictive value; NPV, negative predictive value.

Table 4. Diagnostic value of bronchoalveolar lavage fluid miRNA-30a in early (stages I–IIA) lung cancer

1 **Table 4**. Diagnostic value of bronchoalveolar lavage fluid miRNA-30a in early (stages I–IIA)

2 lung cancer

	Value	95% CI
Cut-off point	2.6508	
TPR	90.9%	0.69–0.98
TNR	74.5%	0.60–0.845
LR+	3.57	2.23–5.72
LR−	0.12	0.03–0.46
PPV	58.8%	0.41–0.75
NPV	95.3%	0.83–0.99

3 CI, confidence interval; TPR, true positive rate (sensitivity); TNR, true negative rate

4 (specificity); LR+, positive likelihood ratio; LR−, negative likelihood ratio; PPV, positive

5 predictive value; NPV, negative predictive value.

Table 5. Diagnostic value of bronchoalveolar lavage fluid miRNA-30a for different
pathologic types of lung cancer

1 **Table 5**. Diagnostic value of bronchoalveolar lavage fluid miRNA-30a for different pathologic

2 types of lung cancer

Category	Subjects	AUC	p-value	95% CI
ADC versus control	60:55	0.821	<0.001	0.75–0.90
SCC versus control	22:55	0.832	<0.001	0.74–0.92
SCLC versus control	15:55	0.817	<0.001	0.71–0.93
NSCLC versus control	84:55	0.823	<0.001	0.75–0.89

3 AUC, area under the receiver operating characteristic curve; CI, confidence interval; ADC,

4 adenocarcinoma; SCC, squamous cell carcinoma; SCLC, small-cell lung cancer; NSCLC, non-

5 small-cell lung cancer.

6 Control group (benign disease, n=55).

Table 6. Diagnostic value of bronchoalveolar lavage fluid miRNA-30a in different pathologic types and TNM stages of lung cancer

1 **Table 6**. Diagnostic value of bronchoalveolar lavage fluid miRNA-30a in different pathologic

2 types and TNM stages of lung cancer

Tissue type	Subjects	AUC	p-value	95% CI
ADC, stages I–IIA	18	0.875	<0.001	0.79–0.96
NSCLC, stages I–IIA	21	0.876	<0.001	0.80–0.96
SCC, stages IIB–IV	14	0.822	<0.001	0.72–0.93
SCLC, stages IIB–IV	10	0.773	<0.05	0.63–0.91
ADC, stages IIB–IV	37	0.783	<0.001	0.69–0.87
NSCLC, stages IIB–IV	52	0.796	<0.001	0.71–0.88

3 AUC, area under the receiver operating characteristic curve; CI, confidence interval; ADC,

4 adenocarcinoma; NSCLC, non-small-cell lung cancer; SCC, squamous cell carcinoma; SCLC,

5 small-cell lung cancer.

Table 7. Diagnostic value of bronchoalveolar lavage fluid miRNA-30a in combination with plasma tumor markers

1   **Table 7**. Diagnostic value of bronchoalveolar lavage fluid miRNA-30a in combination with

2   plasma tumor markers

Diagnostic value	Subjects	Subgroup	AUC	p-value	95% CI
ADC, stages I–IIA	10	miR-30a	0.910	<0.001	0.81–1.00
		CEA	0.572	>0.05	0.36–0.78
		CEA + miR-30a	0.903	<0.001	0.80–1.00
ADC, stages IIB–IV	29	miR-30a	0.790	<0.001	0.68–0.91
		CEA	0.840	<0.001	0.74–0.94
		CEA + miR-30a	0.880	<0.001	0.80–0.97
NSCLC, stages I–IIA	12	miR-30a	0.882	<0.001	0.78–0.99
		Cyfra21-1	0.438	>0.05	0.22–0.65
		Cyfra 21-1 + miR-30a	0.885	<0.001	0.78–0.99
NSCLC, stages IIB–IV	34	miR-30a	0.796	<0.001	0.69–0.91
		Cyfra21-1	0.813	<0.001	0.71–0.92
		Cyfra21-1+miR-30a	0.868	<0.001	0.78–0.96

3   AUC, area under the receiver operating characteristic curve; CI, confidence interval; CEA,

4   carcinoembryonic antigen; ADC, adenocarcinoma; NSCLC. non-small-cell lung cancer.

Figure 1

miRNA-30a levels in BALF samples obtained from lung cancer patients, patients with benign lung diseases and healthy control

Fig. 1 miRNA-30a levels in BALF samples obtained from lung cancer patients, patients with benign lung diseases and healthy control, as determined by quantitative real-time polymerase chain reaction (RT-qPCR) (n=55 in the benign control group, n=99 in the lung cancer group and n=20 in the healthy control). *p<0.001. Data was log transformed to approximate a normal distribution.
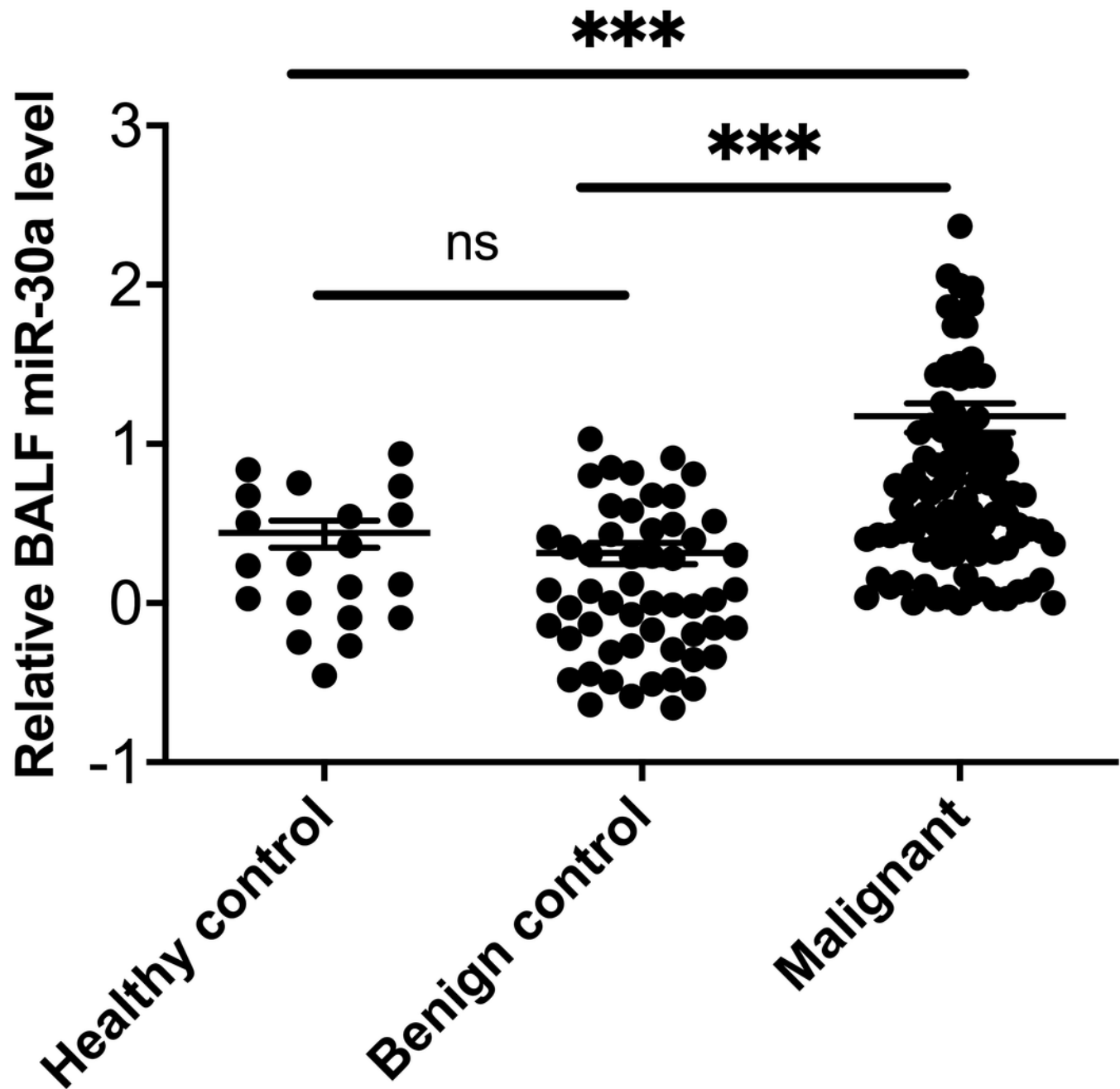
Figure 2

miRNA-30a levels in BALF specimens obtained from the sides with and without lung cancer lesions.

Fig. 2 miRNA-30a levels in BALF specimens obtained from the sides with and without lung cancer lesions (n=20, p>0.05)
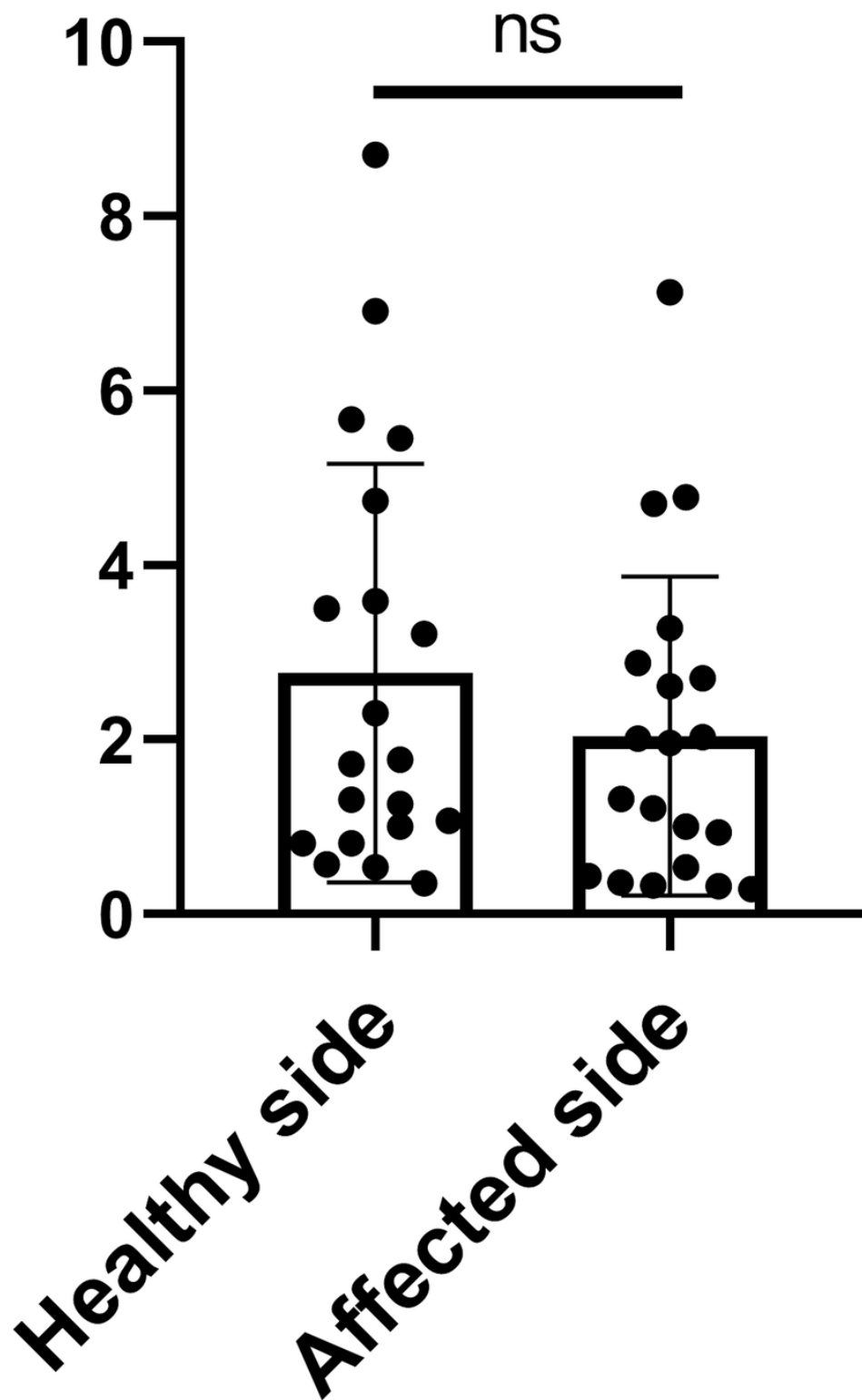
Figure 3

Correlation between BALF miRNA-30a levels and TNM stage

Fig. 3 Bronchoalveolar lavage fluid (BALF) miRNA-30a expression was measured by quantitative real-time polymerase chain reaction (RT-qPCR) (n=22 for stages I–IIA and n=62 for stages IIB–IV). *p<0.05. Data was log transformed to approximate a normal distribution.
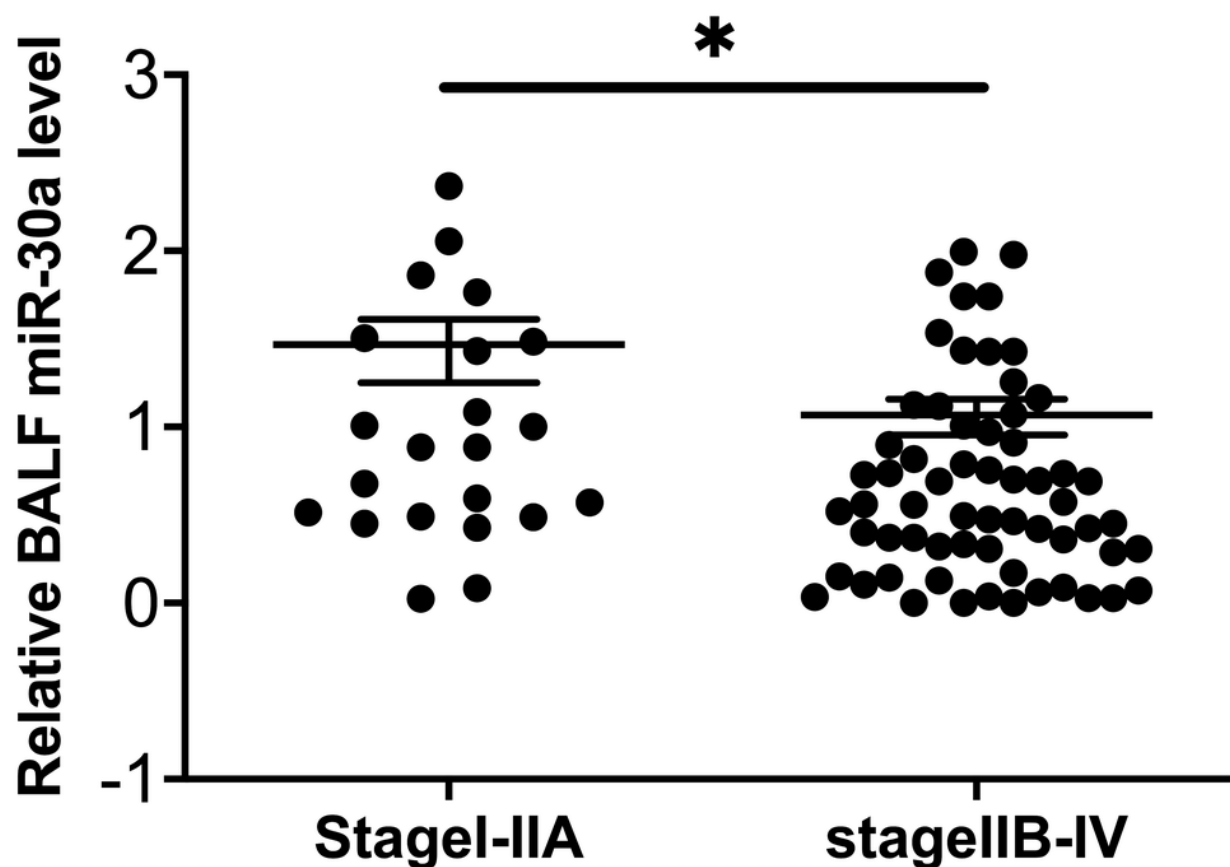
Figure 4

Receiver operating characteristic (ROC) curve analysis of miRNA-30a level to distinguish between lung cancer and benign lung diseases.

Fig. 4 Receiver operating characteristic (ROC) curve analysis of miRNA-30a level to distinguish between lung cancer and benign lung diseases. The area under the curve for miRNA-30a was 0.822, and the Youden index was 0.499.
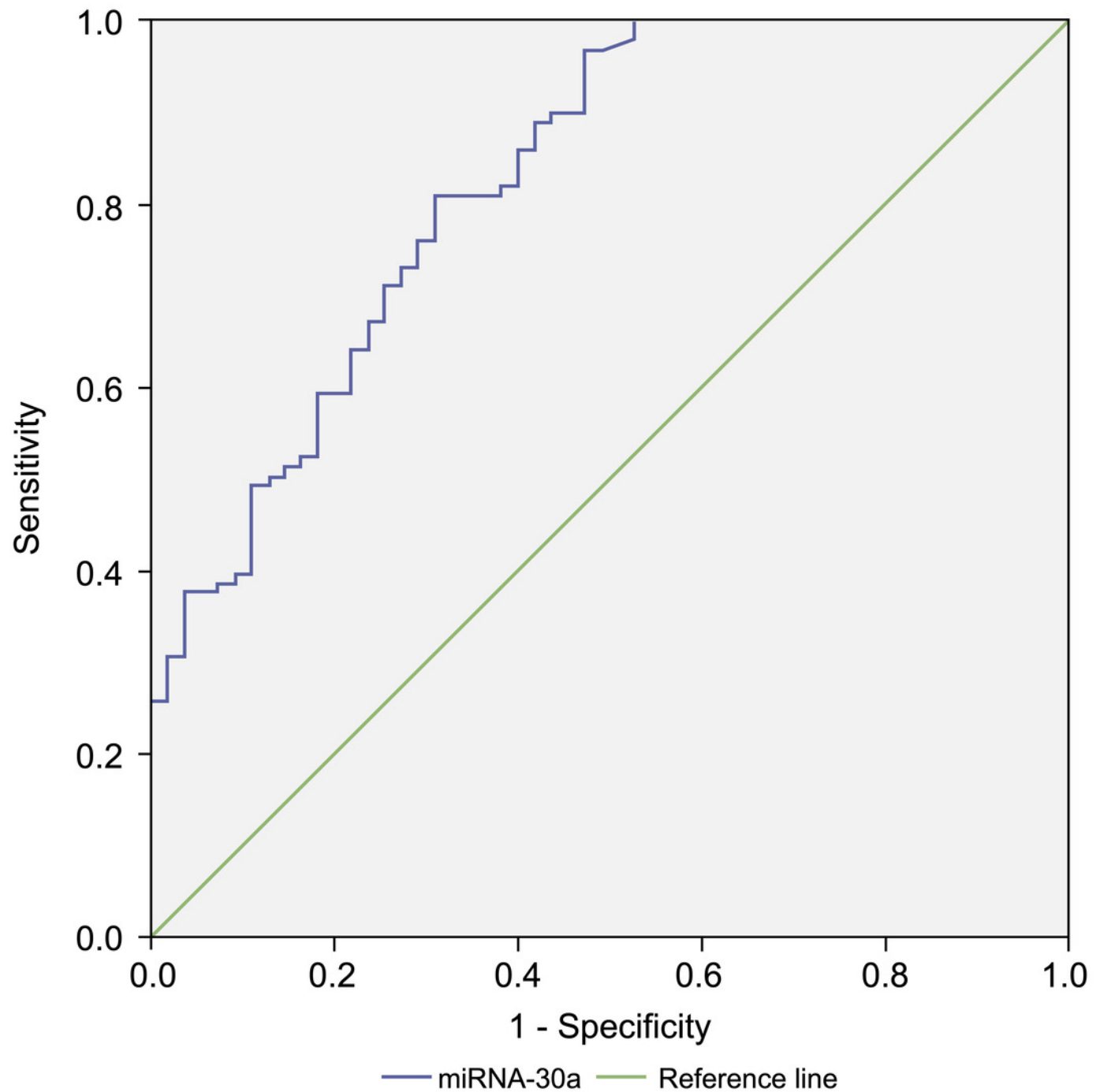
Figure 5

miRNA-30a levels in stages I–IIA lung cancer patients and benign controls.

Fig. 5 miRNA-30a levels in stages I–IIA lung cancer patients (n=22) and benign controls (n=55), as determined by quantitative real-time polymerase chain reaction (RT-qPCR). *p<0.001. Data was log transformed to approximate a normal distribution.
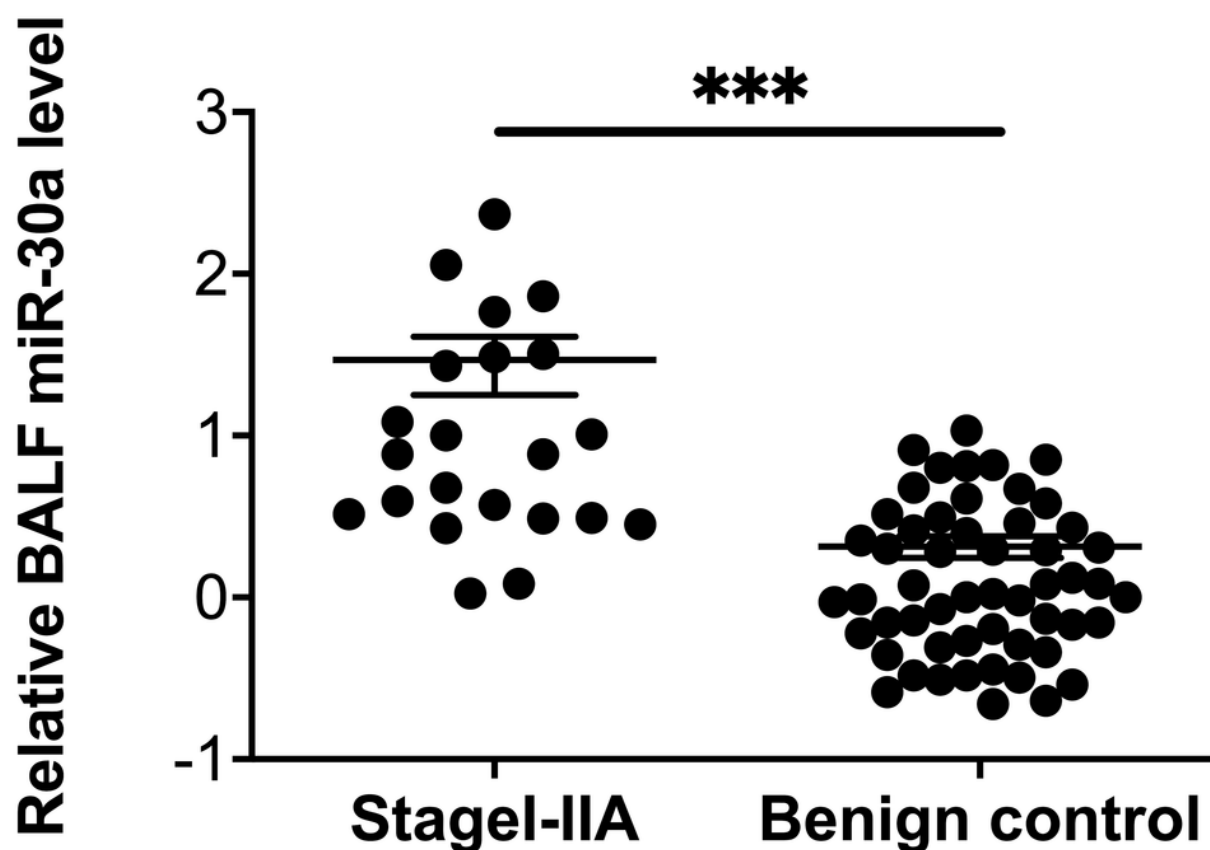
Figure 6

Receiver operating characteristic (ROC) curve analysis of miRNA-30a level to distinguish between early lung cancer and benign lung diseases.

Fig. 6 Receiver operating characteristic (ROC) curve analysis of miRNA-30a level to distinguish between early lung cancer and benign lung diseases. The area under the curve for miRNA-30a was 0.882 (95% confidence interval: 0.806–0.958, p<0.001), and the Youden index was 0.654.
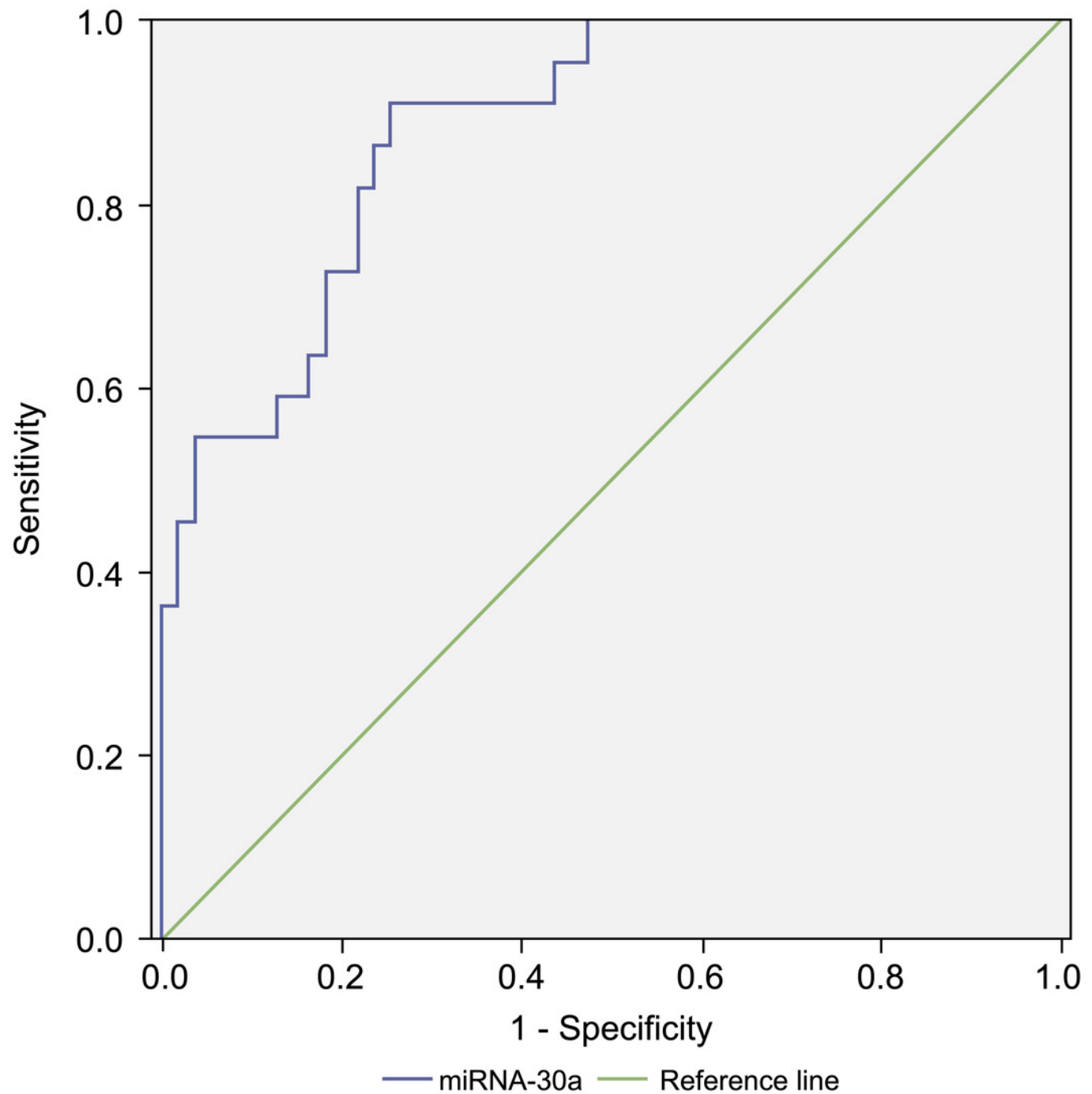
Figure 7

Receiver operating characteristic (ROC) curve analysis of miRNA-30a level to distinguish between stages I–IIA adenocarcinoma and benign lung diseases.

Fig. 7 Receiver operating characteristic (ROC) curve analysis of miRNA-30a level to distinguish between stages I–IIA adenocarcinoma and benign lung diseases. The area under the curve for miRNA-30a was 0.875 (95% confidence interval: 0.790–0.960, p<0.001).
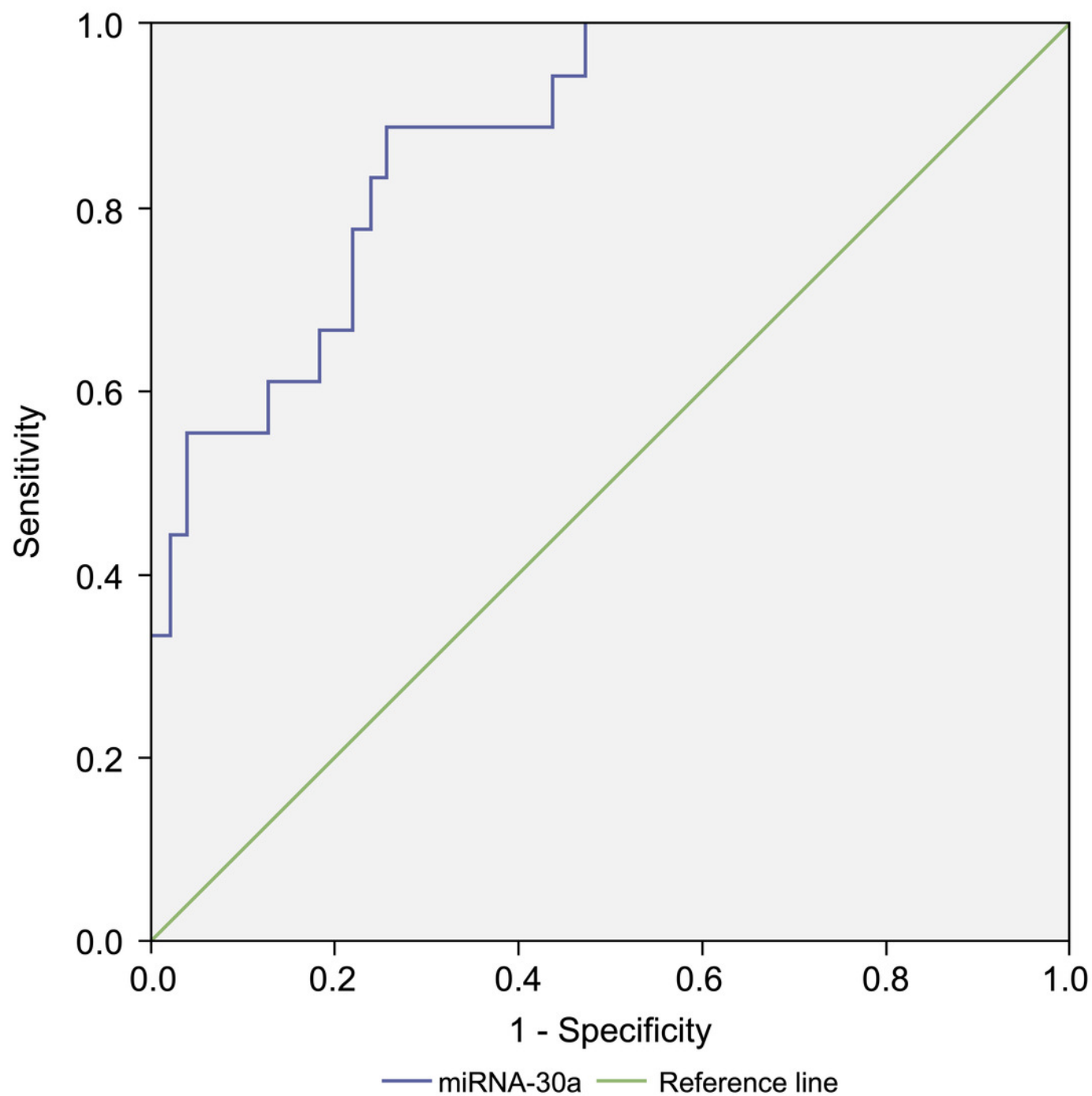
Figure 8

Receiver operating characteristic (ROC) curve analysis

Fig. 8 Receiver operating characteristic (ROC) curve analysis of miRNA-30a level, CEA level, and a combination of miRNA-30a and carcinoembryonic antigen (CEA) levels to distinguish between stages I–IIA adenocarcinoma and benign lung diseases. The area under the curve for miRNA-30a was 0.910 (95% confidence interval: 0.808–1.000, p<0.001).