

MiR-210 has the capacity to serve as a diagnostic biomarker for laryngeal carcinoma patient

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Abstract

Background

Early diagnosis represents a great challenge for laryngeal carcinoma patients. *MiR-210* is involved in various human cancers. In this study, we aimed to investigate the diagnostic performance of serum *miR-210* in laryngeal carcinoma.

Methods

In our study, qRT-PCR was performed to determine the serum *miR-210* level in 137 laryngeal carcinoma patients and 79 healthy volunteers. The association of serum *miR-210* level with clinical characteristics of the patients was estimated by chi-square test. ROC analysis was applied to evaluate the diagnostic value of *miR-210* in laryngeal carcinoma.

Results

Serum *miR-210* level was higher in laryngeal carcinoma patients than that in healthy group ($P < 0.001$). Moreover, its elevated expression was positively associated with TNM stage ($P = 0.000$) and distant metastasis ($P = 0.001$). The AUC value of the ROC curve was 0.893, suggesting the possibility of serum *miR-210* as a diagnostic biomarker for the disease. The cut-off value was 4.685, with the sensitivity of 83.2% and the specificity of 84.8%.

Conclusion

MiR-210 serves as an oncogene in progression of laryngeal carcinoma. Serum *miR-210* may be a potential diagnostic biomarker for laryngeal carcinoma.

Background

Laryngeal carcinoma is one of the most common cancers in head and neck region, with increasing morbidity and high mortality around the world [1]. Several risk factors are confirmed for laryngeal carcinoma, such as smoking, alcohol abuse, and exposure to carcinogens, however, the etiology of the cancer still remains unclear [2]. The cancer can be curable by surgical strategies or radiotherapy at early stages, but the prognosis of patients with advanced stages are far from satisfactory [3]. Tumor stage at initial diagnosis is a pivotal factor for outcomes of patients with laryngeal carcinoma [4]. Until now, early diagnosis of laryngeal carcinoma is mainly based on conventional biopsy which is frequently performed under local or general anesthesia [5]. In addition, CT, MRI, ultrasonography, and noninvasive imaging techniques are also used for early detection of the disease, but their diagnostic accuracy can not meet the

clinical requirements [6–8]. Thus it is urgent to identify a novel and valuable biomarker which can help to achieve the early diagnosis for the laryngeal cancer patients.

MicroRNAs (miRNAs) are a class of short and non-coding RNAs with the length of 20–23 nucleotides [9]. Given their regulatory roles in gene expression at post-transcriptional level, miRNAs are involved in a variety of biological processes, including cell differentiation, proliferation, and apoptosis [10]. Dysregulation of miRNAs may contribute to occurrence and development of diseases, including cancers. MiRNAs can function as oncogenes or tumor suppressors in tumorigenesis. The expression patterns of miRNAs show significant association with tumor initiation, development and progression, suggesting their potential as predictive biomarkers for human malignancies [11]. MiRNA-210 (*MiR-210*), a common member of miRNAs family, was reported to be involved in various human cancers, such as breast cancer, lung cancer, pancreatic carcinoma, etc [12–14]. However, the expression profile of serum *miR-210* and its clinical significance in laryngeal carcinoma were still poorly known.

In this study, we aimed to explore the expression profile of serum *miR-210* in laryngeal carcinoma, as well as its association with clinicopathologic characteristics. The potential diagnostic value of *miR-210* in laryngeal carcinoma was also estimated in the current study.

Methods

Collection of patients and specimens

A total of 137 newly diagnosed laryngeal carcinoma patients were finally recruited in this study. Our study was approved by the ethics committee of Chinese PLA General Hospital. All the patients or their family signed informed consents in advance. In addition, 79 gender and age matched healthy volunteers were collected as the control group. In the control group, no one had been diagnosed with any malignancies. The blood specimens were taken from all the participants on the morning in a collection tube with EDTA. Then the samples were centrifugated at 3000 rpm for 10 min to isolate the serum sample. The supernate was stored at -80°C until RNA extraction. The basic data of those patients with laryngeal cancer were recorded in Table 1, including age, sex, tumor size, subsite, histologic type, TNM stage, and distant metastasis.

Table 1
Association of *miR-210* expression with clinicopathological characteristics of laryngeal carcinoma patients

| Characteristics | Number N = 137 | <i>miR-210</i> expression | | <i>P</i> values |
|-----------------------|-------------------|---------------------------|---------------|-----------------|
| | | Low (n = 59) | High (n = 78) | |
| Age (years) | | | | 0.800 |
| < 60 | 61 | 27 | 34 | |
| ≥ 60 | 76 | 32 | 44 | |
| Gender | | | | 0.257 |
| Male | 69 | 33 | 36 | |
| Female | 68 | 26 | 42 | |
| Tumor size | | | | 0.374 |
| < 3 cm | 66 | 31 | 35 | |
| ≥ 3 cm | 71 | 28 | 43 | |
| Subsite | | | | 0.406 |
| Supraglottis | 46 | 20 | 26 | |
| Glottis | 51 | 25 | 26 | |
| Subglottis | 40 | 14 | 26 | |
| Histologic type | | | | 0.301 |
| preinvasive carcinoma | 65 | 25 | 40 | |
| LSCC | 72 | 34 | 38 | |
| TNM stage | | | | 0.000 |
| I-II | 71 | 43 | 28 | |
| III-IV | 66 | 16 | 50 | |
| Distant metastasis | | | | 0.001 |
| yes | 69 | 20 | 49 | |
| no | 68 | 39 | 29 | |

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted with the miRNeasy Mini Kit (Qiagen, Hilden Germany) following the manufacturer's instructions. The first strand of cDNA was synthesized with the One Step PrimeScript

miRNA cDNA Synthesis Kit (Takara Bio, Shiga, Japan). The relative expression level of *miR-210* was measured using qRT-PCR method which was performed with a SYBR Premix Ex Taq™ kit (Takara, Dalian, China) on the Applied Biosystems 7900 Fast Real-Time PCR system (Applied Biosystems, Foster City, California, USA). *U6* snRNA was used as an internal control, and the primers of *miR-210* were forward: 5'-ACACTCCAGCTGGGCTGTGCGTGACAGCGG-3', reverse: 5'-CTCAACTGGTGTGCGTGGA-3', and primers of *U6* were forward: 5'-CTCGCTTCGGCAGCACA-3', reverse: 5'-AACGCTTCACGAATTTGCGT-3'. The relative expression level of *miR-210* was normalized to that of *U6* and calculated using $2^{-\Delta\Delta Ct}$ method. Each test was performed in triple.

Statistical analysis

In this study, all statistical analyses were performed with software of SPSS 19.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5 (GraphPad, San Diego, CA, USA). The serum *miR-210* expression level was expressed as mean \pm SD, and student's t-tests was used to compare its differences between case and control groups. The relationship between serum *miR-210* expression and various clinicopathological characteristics were assessed using Chi-square tests. To determine the diagnostic performance of serum *miR-210* expression in laryngeal carcinoma, the receiver operating characteristic (ROC) curve was plotted based on the serum levels of *miR-210* in healthy individuals and laryngeal cancer patients. *P* values < 0.05 were considered statistically significant.

Results

The overexpression of *miR-210* in laryngeal cancer

In order to investigate the expression profile of *miR-210* in 137 laryngeal cancer patients and 79 healthy volunteers, the qRT-PCR was performed. As shown in Fig. 1, the results suggested that *miR-210* expression was significantly higher in laryngeal cancer tissues than that in healthy volunteers ($P < 0.001$).

Association between *miR-210* and clinicopathological parameters of laryngeal cancer patients

The patients were divided into high expression ($n = 78$) and low expression ($n = 59$) groups according to their mean expression value of *miR-210*. Chi-square tests was used to evaluate the effects of *miR-210* expression on clinical characteristics of laryngeal carcinoma patients. Analysis results demonstrated that *miR-210* expression was significantly associated with TNM stage ($P = 0.000$) and distant metastasis ($P = 0.001$). However there were no obvious relationship between the expression of *miR-210* and the age, gender, tumor size, subsite or histologic type of patients with laryngeal carcinoma (all $P > 0.05$, Table 1).

Diagnostic value of *miR-210* expression in laryngeal cancer patients

In our study, ROC curve was used to determine the accuracy of *miR-210* in distinguishing laryngeal cancer patients and healthy group. The AUC value of the curve was 0.893, suggesting that the laryngeal carcinoma patients could be distinguished from the healthy group based on their serum levels of *miR-*

210. The cut-off value of serum *miR-210* for laryngeal carcinoma diagnosis was 4.685, with the sensitivity of 83.2% and the specificity of 84.8% (Fig. 2).

Discussion

Laryngeal carcinoma represents a frequently diagnosed head and neck cancer. Despite of the various available treatments, the clinical outcomes of the patients has not been significantly improved during the past three decades [15]. Low early diagnosis rate may be responsible for the high mortality [4]. Surgery and radiotherapy are effective treatments for patients diagnosed at early stages, but the therapeutic effects are limited for those diagnosed with advanced stages, due to the high recurrence rate [16]. Therefore, identification of novel biomarkers for early diagnosis may be a promising approach to improve the outcomes of the patients.

As gene expression regulators, miRNAs play important roles in various biological processes, such as development, cell proliferation, apoptosis, differentiation, as well as carcinogenesis [17, 18]. Growing evidences have demonstrated that miRNAs as oncogenes or suppressors are involved in various human malignancies. Given their functional roles in tumor progression, miRNAs are considered as promising candidate biomarkers for cancer diagnosis, prognosis, and treatments. In laryngeal cancer, a variety of miRNAs biomarkers were identified. For examples, Wu et al. reported that laryngeal cancer tissues exhibited increased expression of *miR-148a* and *miR-375* which might serve as diagnostic biomarkers for the cancer [19]. Zhang et al. reported that up-regulation of *miR-23a* in laryngeal cancer showed positive correlation with aggressive clinical parameters of the patients, moreover, its elevated expression predicted poor prognosis[20]. Based on the related studies, we speculated that the expression profile of miRNAs showed significant association with tumor development and progression, and they held the potential to serve as predictive biomarkers for human malignancies.

MiR-210, a common member of miRNA family, has been determined to play an important role in tumorigenesis. The increased expression of *miR-210* was observed in various cancer, such as lung adenocarcinoma, renal cell carcinoma, colorectal cancer, suggesting its carcinogenic function in these cancers [21–23]. In this study, we found that the expression level of *miR-210* was higher in laryngeal carcinoma patients than that in healthy group. Moreover, the increased expression of *miR-210* was significantly correlated with advanced TNM stage and positive distant metastasis. All the data revealed that *miR-210* as a tumor oncogene played a promoting role in malignant development and progression of laryngeal carcinoma. This conclusion was consistent with the previous results obtained in other types of cancer.

Given its oncogenic roles in tumorigenesis, *miR-210* was identified as a biomarker for several cancers. In clear cell renal cell carcinoma, up-regulation of *miR-210* showed significant association with tumor recurrence and poor prognosis of the patients, suggesting its capacity as a prognostic indicator for the disease [24]. Wang et al. reported that serum level of *miR-210* was significantly higher in colorectal cancer patients than that in the healthy individuals, moreover, its elevated expression was positively

correlated with malignant tumor progression and poor prognosis. Circulating *miR-210* might be a potential biomarker for early detection and prognosis evaluation of the disease [25]. In this study, we estimated the diagnostic performance of serum *miR-210* in laryngeal cancer. The results suggested that *miR-210* could distinguish the laryngeal cancer patients from the healthy individuals with high sensitivity and specificity. *MiR-210* might be a potential diagnostic biomarker for laryngeal carcinoma patients. However, the sample size was relatively small, and the application value of serum *miR-210* for laryngeal carcinoma required further identification. In addition, the carcinogenic mechanisms of *miR-210* in laryngeal cancer were poorly known. Further analysis were still needed to address the related issues.

Conclusion

In conclusion, *miR-210* is elevated in laryngeal carcinoma, and positively correlated with malignant tumor progression, Serum *miR-210* may be a candidate biomarker for early detection of laryngeal carcinoma.

Abbreviations

MicroRNAs (miRNAs)

MiRNA-210 (MiR-210)

quantitative real-time polymerase chain reaction (qRT-PCR)

Declarations

Disclosure

The authors report no conflicts of interest in this work.

Author Informatio

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Chinese PLA General Hospital and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

Consent for publication

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Data availability

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

L.W. design of the work; H.S. the acquisition, analysis, H.S., S.Y. interpretation of data; L.W., H.S. the creation of new software used in the work; L.W., H.S., S.Y. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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Figures

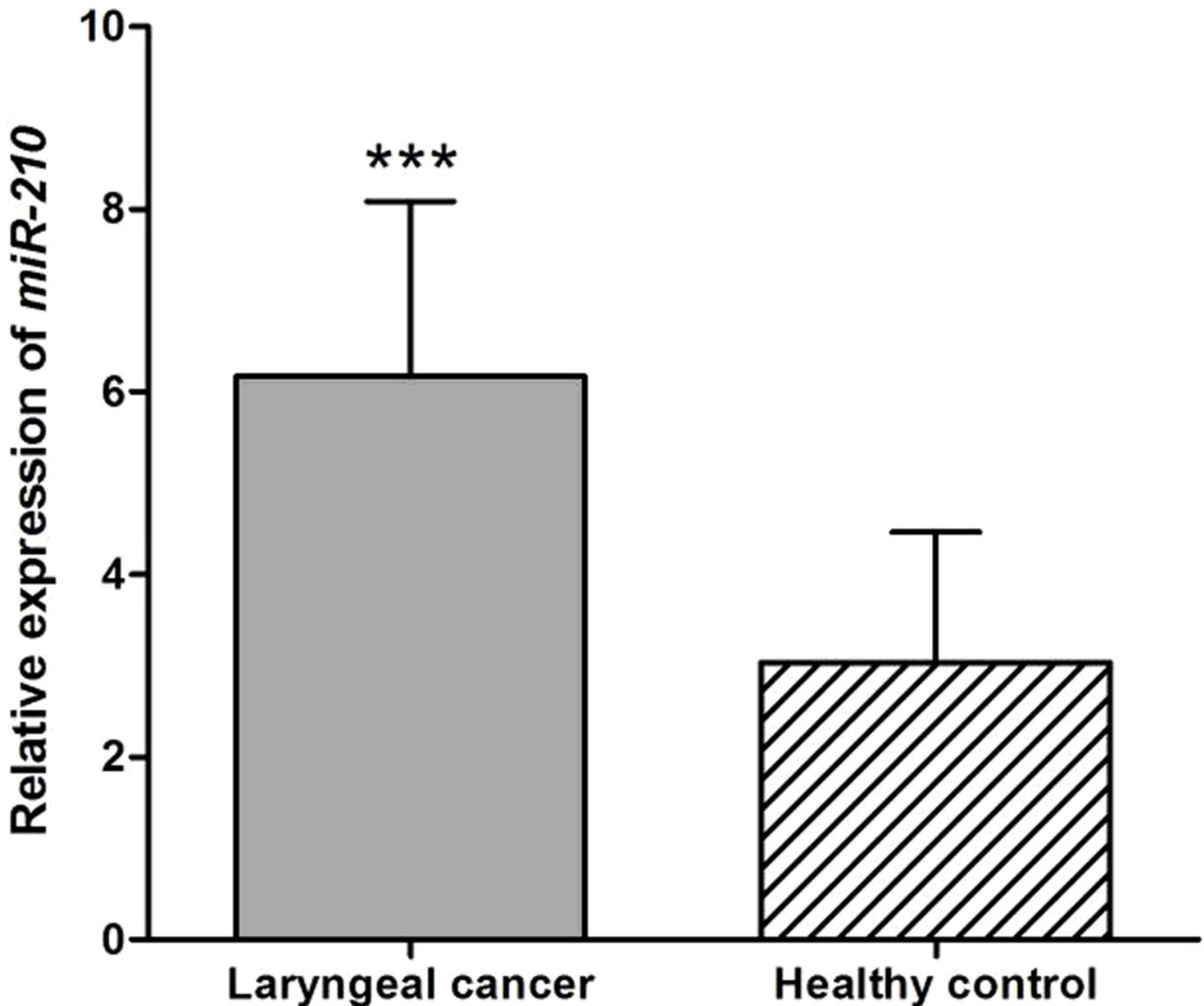


Figure 1

The relative expression of serum miR-210 in laryngeal cancer cases and healthy individuals. The expression of serum miR-210 was significantly increased in laryngeal cancer patients, compared to the

healthy controls. ***: suggested $P < 0.001$.

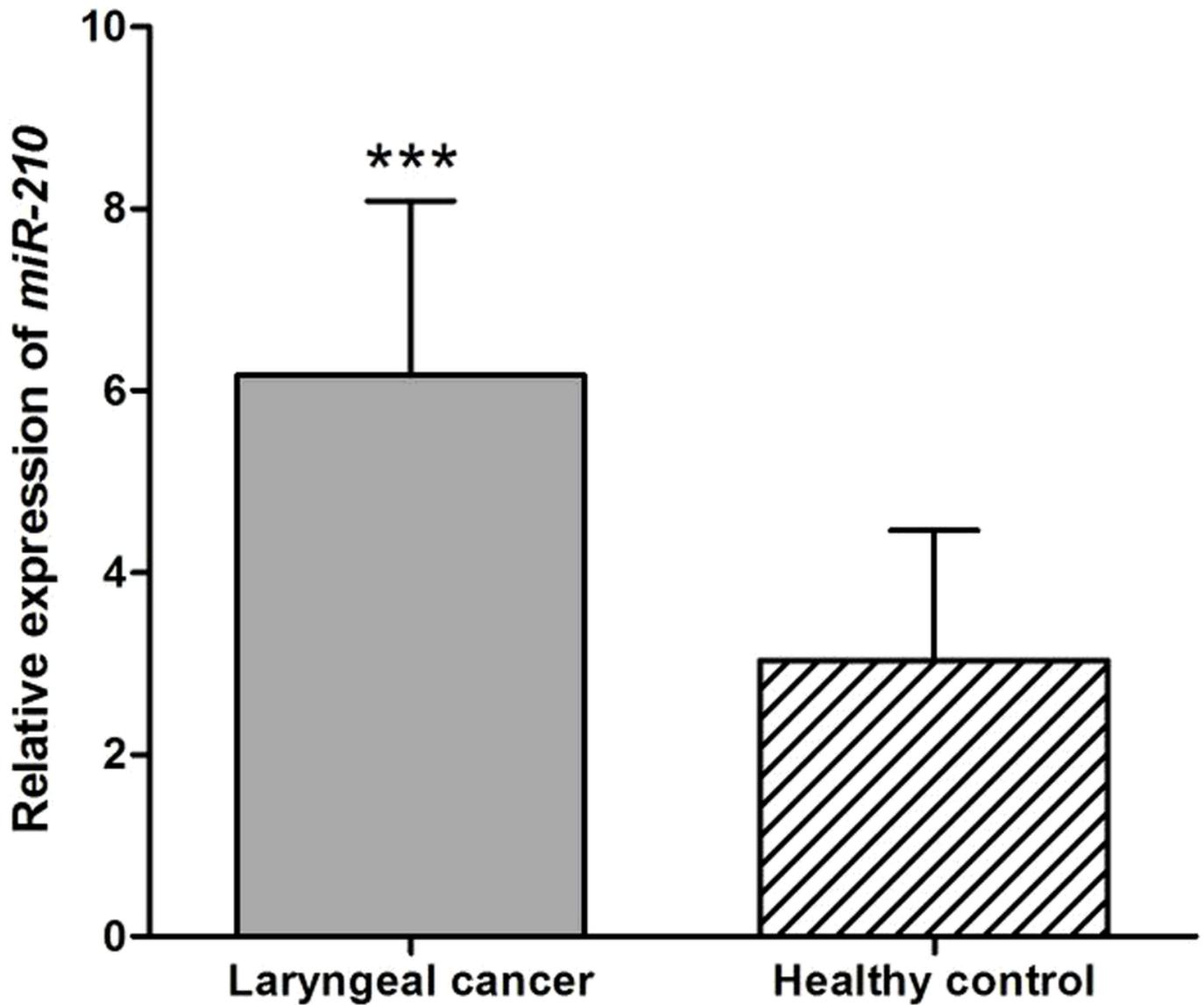


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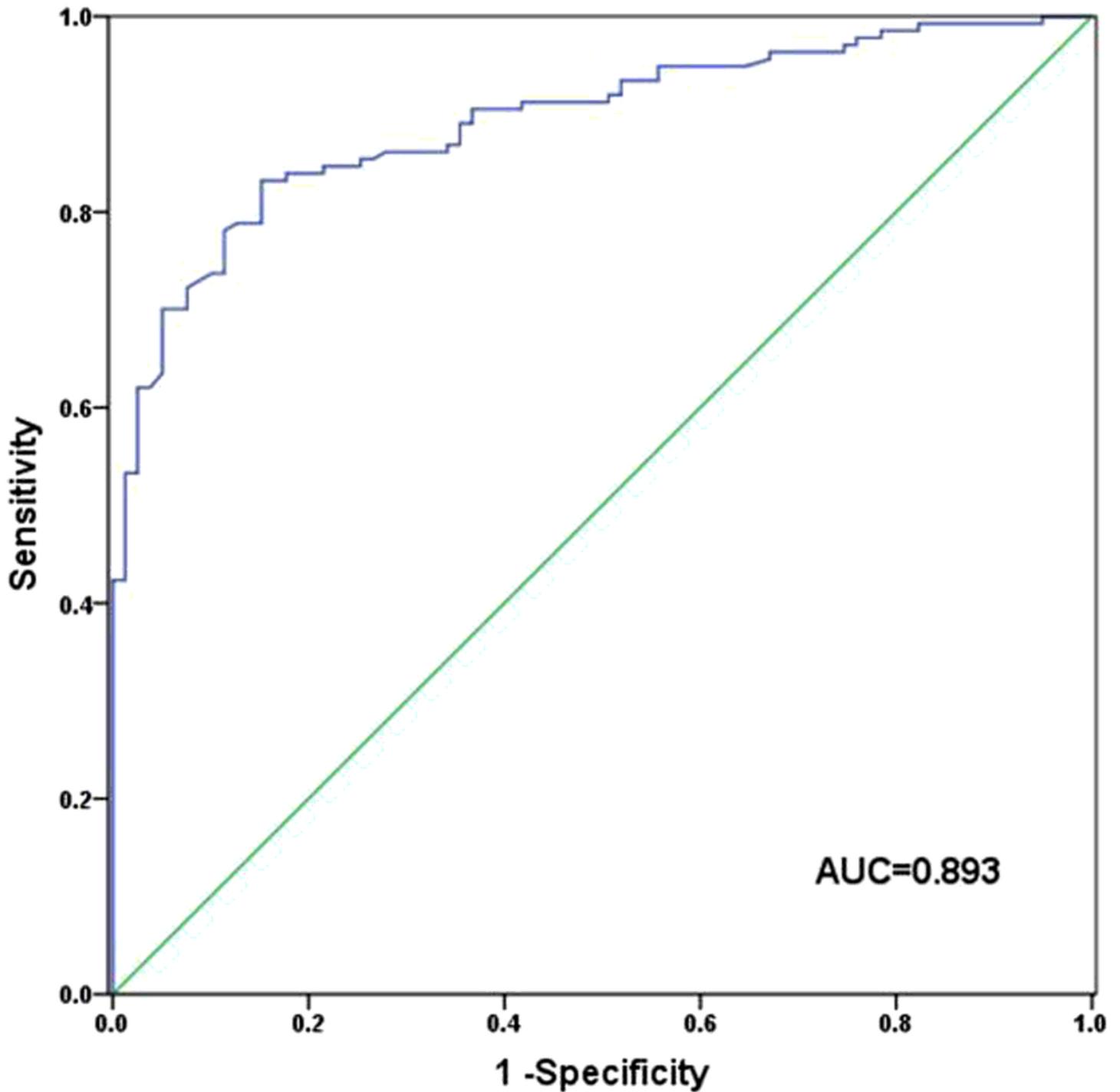


Figure 2

ROC analysis for evaluation of the diagnostic accuracy of serum miR-210 in laryngeal cancer. The curve demonstrated that serum miR-210 could discriminate between laryngeal cancer patients and healthy individuals at the cut-off value of 4.638, with the AUC value of 0.893, combining with the sensitivity of 83.2% and the specificity of 84.8%.

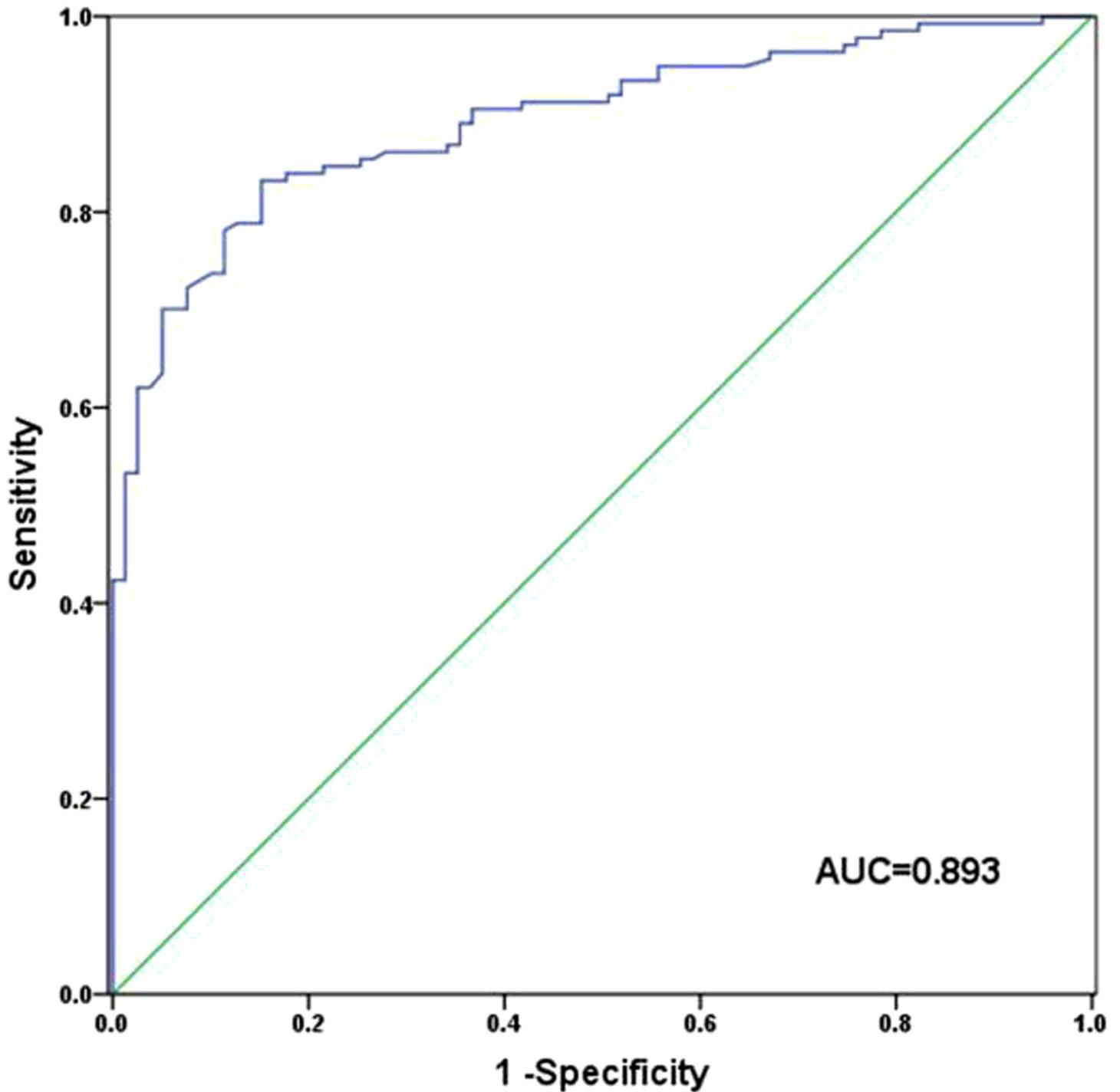


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