

58771_Auto_Edited.docx

Name of Journal: *World Journal of Clinical Cases*

Manuscript NO: 58771

Manuscript Type: ORIGINAL ARTICLE

Retrospective Study

A circulating immune parameters-based nomogram for predicting malignancy in laryngeal neoplasm

Abstract

BACKGROUND

Malignancy prediction remains important to preoperative diagnosis and postoperative follow-up in laryngeal neoplasm.

AIM

To evaluate the circulating immune population and develop a nomogram for prediction of malignancy in patients with laryngeal neoplasm.

METHODS

A primary cohort of 156 patients was divided as laryngeal benign lesion, premalignant lesion, and malignant lesion. Peripheral blood from patients was measured by blood routine test and flow cytometry. Nomogram was developed and applied to a validation cohort contained 55 consecutive patients.

RESULTS

Age, gender, and seven circulating immune parameters exhibited significantly differences between laryngeal benign lesion and premalignant lesion. The nomogram incorporated predictors, including gender, age, smoke index, proportions of monocytes, CD8+ T cells, CD4+ T cells, B cells, and CD4/CD8 + T cell ratio, showed good discrimination between laryngeal premalignant lesion and malignant lesion, with a C-

index of 0.844 for the primary cohort. Application of this nomogram in the validation cohort (C-index, 0.804) still had good discrimination and good calibration. Decision curve analysis revealed nomogram was clinically useful.

CONCLUSION

Not only laryngeal cancer but also laryngeal premalignant lesion exhibit changes in circulating immune phenotype. The novel nomogram, incorporating both clinical risk factors and circulating immune parameters, could be appropriately applied in preoperative individualized prediction of malignancy in patients with laryngeal neoplasm.

Key Words: Laryngeal premalignant lesion; Laryngeal malignant lesion; Circulating immune cell; Nomogram; Laryngeal neoplasm; Malignancy prediction

Chen M, Fang Y, Yang Y, He P, Cheng L, Wu H. A circulating immune parameters-based nomogram for predicting malignancy in laryngeal neoplasm. *World J Clin Cases* 2020; In press

Core Tip: Malignancy prediction remains important to preoperative diagnosis and postoperative follow-up in laryngeal neoplasm. There are continuing problems in differentiating premalignant lesion before surgery from malignant lesion of the larynx. Our finding suggested that not only laryngeal malignant lesion but also premalignant lesion exhibit changes in circulating immune phenotype. This circulating immune parameters-based novel nomogram could be appropriately applied in preoperative individualized prediction of malignancy in patients with laryngeal neoplasm.

INTRODUCTION

Laryngeal cancer remains one of the most common tumors of the upper respiratory tract. The American Cancer Society estimates 177,422 new cases of laryngeal cancer and

94,771 deaths from laryngeal cancer worldwide in 2018¹. When analyzed by tumor stage, cure rates for patients diagnosed with limited disease (T1,2) are excellent, ranging from 80 to 90%. Unfortunately, approximately 60% of patients are still diagnosed with locally advanced (T3,4) disease or regional nodal metastases, where survival rates are generally less than 50%². Diagnostic delay is one of the main factors influencing prognosis in laryngeal cancer. According to the data from the English National Audit of Cancer Diagnosis in Primary Care of 28 cancers, laryngeal cancer had the fifth-longest delay in primary care referral³. Anything that can improve early diagnosis is an essential step in the right direction⁴.

Laryngeal premalignant lesion is defined as an altered epithelium with an increased tendency of progression to laryngeal cancer. The altered epithelium exhibits diverse cytological and architectural changes that have traditionally been brought under the common denominator of dysplasia^{5, 6}. Laryngeal premalignant lesion differed from benign lesion is up to the 14% rate of malignant transformation⁷. There are continuing problems in differentiating premalignant lesion before surgery from malignant lesion of the larynx. Similar to other solid tumors, the exploration to define biomarkers with a discriminated impact on disease nature is a promising and diagnostic relevant research topic.

Classically, genetic markers were described in laryngeal cancer⁸. Besides, innate and adaptive immune system in the carcinogenesis of many cancers is a topic of primary interest in the past decades^{9, 10}. The immune surveillance theory nominates specific functions to different leukocyte populations: neutrophils, macrophages, and CD4+ T cells induce a cytokine environment favoring chronic inflammation, whereas natural killer cells and CD8+ T cells comprise tumor suppressor populations¹¹. Although those traditional groups have provided specific genetic or immune mediators in laryngeal cancer, reliable circulating immune markers with diagnostic value in early stage are still lacking¹². In addition, accurately predicted tools will augment the ability to diagnosis of laryngeal cancer.

A nomogram is a graphic representation of a statistical model¹ specifically designed to maximize predictive accuracy^[13]. In contrast to traditional models that allocate outcomes based on risk group, nomogram provides predictive information based on a combination of variables that take a more individualized prediction of outcome into consideration. Several studies have indicated the superiority of more multiplex predictive modeling in providing improved accuracy compared with risk group assignment techniques^[14]. Nomogram is useful to surgeons in identifying patients “at risk” who should be targeted for aggressive treatment in the field of cancer^[15].

Here, frequencies of circulating immune populations in patients with laryngeal lesions were investigated. We developed and validated a nomogram that incorporated both the clinical risk factors and circulating immune parameters for individual prediction of cancer in patients with laryngeal neoplasm.

MATERIALS AND METHODS

Study subjects

Ethical approval was obtained and informed consent was signed for this study. The primary cohort comprised patients who underwent microlaryngoscopic surgery after general anesthesia from January 2019 to September 2019 at Eye, Ear, Nose, and Throat Hospital of Fudan University (Shanghai, China). Inclusion criteria included the following: initial pathological diagnosis of laryngeal polyps or cysts, laryngeal dysplasia, and laryngeal squamous cell carcinoma; with lesions of glottis; no previous history of laryngeal cancer. Exclusion criteria were as follows: with medical history of other malignancies or autoimmune disease; treatment with radiotherapy or chemotherapy; with use of steroid during the previous 6 mo; blood transfusion with the previous 12 mo.

From September 2019 to January 2020, an independent validation cohort of 55 consecutive patients with laryngeal dysplasia or laryngeal squamous cell carcinoma was included using the same criteria as that for the primary cohort.

Clinical data

Information of participants containing gender, age, smoking status, alcohol consumption, laryngoscopic images, and pathological results was extracted. Smoking status was classified as non-smokers (with no history of smoking or having not smoked for at least 5 years) and smokers. Alcohol consumption was classified as drinkers (with consumption of >80 mL pure alcohol per day) and non-drinkers. Smoking index was calculated as number of cigarettes consumed per day multiply by years of smoking. Drinking index was calculated as volume of pure alcohol consumed per day multiply by years of drinking. Based on pathological results, laryngeal benign lesion was defined as laryngeal polyps or cysts; laryngeal premalignant was defined as laryngeal dysplasia; laryngeal malignant lesion was defined as laryngeal squamous cell carcinoma.

Measurement of circulating immune cells

Routine blood examination was performed within one week before surgery. LMR was calculated as the absolute lymphocyte counts divided by the absolute monocyte counts. NLR was calculated as the absolute neutrophil counts divided by the absolute lymphocyte counts. 50ul whole blood from patient was monitored by TBNK multitest allowing determination of absolute number of CD45+ cell, CD3+ T cell, CD4+ T cell, CD8+ T cell, NK cell, and B cell. After lysis of red blood cells, samples were analyzed using FACS Canto flow cytometer and FACS Canto software (both from BD Biosciences). Proportion of CD3+ T cell, CD4+ T cell, CD8+ T cell, NK cell, and B cell were calculated based on CD45+ cell.

Statistical analysis

Analysis was carried out using SPSS version 20.0 (IBM Corporation, Chicago, Illinois, USA) and R software (version 3.6.1; <http://www.R-project.org>) with the ggplot2, magrittr, ggpubr, glmnet, rms, and Hmisc libraries added. The P values<0.05 were considered statistically significant. Comparisons of categorical variables and quantitative variables in three groups were compared by Kruskal-Wallis test followed by Nemenyi test and one-way analysis of variance followed by least significant difference test (LSD test), respectively. The optimal cut-off value of variables was

defined by receiver operating characteristic (ROC) curve analysis of which point closest to both maximum sensitivity and specificity. And data was converted to binary variables based on the optimal cut-off points. The least absolute shrinkage and selection operator (LASSO) method was used to select the potential predictive features from the primary cohort with laryngeal premalignant lesion and laryngeal malignant lesion. Then multivariable logistic regression analysis was performed in immune features selected from LASSO method. Screened predictors were applied to develop a predictive model for risk of laryngeal cancer which was presented by a nomogram. Nomogram validation contained two activities. Firstly, Harrell's C-index was measured to quantify the discrimination performance of this nomogram. The C-index greater than 0.75 is generally recognized as relatively good discrimination. Secondly, calibration curve was depicted to evaluate the calibration of the nomogram. In addition, we evaluated the clinical utility through decision curve analysis, which quantifying net benefit at different threshold probabilities in the validation data.

RESULTS

Clinical characteristics

Clinical data and immune parameters of the primary cohorts were given in Table 1. Of 156 patients who met the criteria for inclusion, 50 (32.1%) patients were grouped as benign lesions, 54 (34.6%) patients as premalignant lesions, and 52 (33.3%) patients as malignant lesions.

Age, gender, smoke status, and index among the three groups were significantly different (Table 1). Following pairwise comparisons, patients with laryngeal benign lesions had a significantly lower age and male proportion than those with premalignant and malignant lesion (age, benign vs. premalignant, $P<0.001$, benign vs. malignant, $P<0.001$, LSD test; gender, benign vs. premalignant, $P<0.001$, benign vs. malignant, $P<0.001$, Nemenyi test). Patients with laryngeal malignant lesion had a significantly more smokers and higher smoke index than those with benign and premalignant lesion (smokers, malignant vs. benign, $P=0.013$, malignant vs.

pre-malignant, $P=0.045$, Nemenyi test; smoke index, malignant vs. benign, $P<0.001$, malignant vs. pre-malignant, $P=0.001$, LSD test;).

Violin plot analysis revealed 7 circulating immune parameters exhibited different distribution and significantly different levels between benign lesion and pre-malignant lesion (Figure 1). Although it showed a tendency of lowering in LMR, CD3+ T cell proportion, and CD4+ T cell proportion and a tendency of increasing in NK cell proportion, NK/CD3+ T cell ratio, NK/CD4+ T cell ratio, and NK/CD8+ T cell ratio among three groups, there was no significant difference in these levels between pre-malignant lesion and malignant lesion.

Feature selection

Feature selection was conducted in 54 patients with laryngeal pre-malignant lesions and 52 patients with laryngeal malignant lesions in the primary cohort. According to the optimal cut-off values defined by ROC curves (supplementary Table 1, supplementary Figure 1), data were converted to binary variables. Of demographic, clinical, and immune features, 20 features were lessened to 13 preliminary predictors on the basis of 106 patients in the primary cohort, and were features with nonzero coefficients in the LASSO logistic regression model (Figure 2A and 2B).

These features included age, gender, smoke index, NLR, LMR, proportion of monocyte, CD8+ T cells, CD4+ T cells, B cells, CD4/CD8+ T cell ratio, NK/CD3+ T cell ratio, NK/CD4+ T cell ratio, and NK/CD8+ T cell ratio.

Development of an individualized prediction model

Furthermore, the multivariable logistic regression analysis identified the proportions of monocyte, CD8+ T cells, CD4+ T cells, B cells, and CD4/CD8 + T cell ratio as independent immune predictors (Table 2). The model integrated the above circulating immune parameters and clinical factors (age, gender, smoke index) was developed and presented as the nomogram (Figure 3) yielded a C-index of 0.844 (95%CI, 0.765-0.923) for primary cohort.

Independent validation of the nomogram

Clinical data and immune parameters of 55 patients in the validation cohort were provided in supplementary Table 2. The calibration curve of the nomogram for the prediction of cancer risk exhibited good agreement in the validation cohort (Figure 4A). And the C-index for the prediction nomogram was 0.804 (95%CI, 0.688-0.920).

Clinical use

The decision curve analysis for cancer prediction nomogram is showed in Figure 4B. The decision curve displayed that if the threshold probability of >22% and <96%. Within this range, net benefit was comparable with the several overlaps, on the basis of cancer prediction nomogram.

DISCUSSION

Laryngeal dysplasia was considered as laryngeal premalignant lesion due to its possibility to become malignancy^[16]. Demographic distinctions, advanced age and male predominance, were found between laryngeal benign lesion and laryngeal premalignant lesion. Etiological studies of laryngeal dysplasia have restricted to external factors such as tobacco, alcohol, pepsin, and microorganism^[5]. Internal factors, especially abnormal immunity, were ignored in previous researches. Abundance evidence derived from studies in cancer immunosurveillance suggested that variations of immune cells influence cancer development^[17]. In our study, we focused on the analysis of circulation immune populations in different conditions of laryngeal lesions. Our data illustrated the circulating immune cells were involved in the stage of laryngeal premalignant lesion. These results corroborate the findings of the previous work that not only cancers but also premalignant lesions correlating with an alteration of immune cells [18, 19].

Host immune response to tumors is lymphocyte dependent. Decreased LMR, proportions of CD3+ T cell, and CD4+ T cell in patients with laryngeal premalignant lesions mean relative lymphocytopenia and a deficient immune response to tumor mediated by CD4+ T cells^[20]. Opposite changes in the proportion of NK cell might in that the abnormal condition in premalignant lesion triggers innate immunity. The

increases in NK cell to major lymphocyte subpopulations ratios were due to increase in NK cells and decreases in lymphocytes. The altered circulating immune phenotype of laryngeal premalignant lesion was similar to the findings of studies on HNSCC^[21] and other cancers^[22]. The field of immunotherapy, which targets immune cells to augment antitumor immune responses, has led to crucial clinical advances and supplied a new weapon against cancer^[23]. Whether adjustment of immune abnormality in the stage of premalignant lesion could prevent or reduce the formation of tumor need to be further studied.

In the present study, circulating immune cells exhibited different distribution patterns in laryngeal premalignant lesion and laryngeal malignant lesion, however these levels showed no significant difference (Figure 1). For the construction of the determinants which could be used to establish the true premalignant and malignant character of laryngeal neoplasm, 20 candidate features were lessened to 13 preliminary predictors by testing the predictor-status correlation by LASSO logistic regression model (Figure 2A, 2B). This method enables variable selection and regularization on the basis of a model fitting. Multimarker screens that incorporate individual markers into panels have been generalized to recent researches, for instance, genomic markers and protein molecular which was identified to assessment of gastric cancer and lung cancer^[24, 25]. Similarly, 10 immune parameters combined with 3 well-known risk factors were considered as potential predictors for laryngeal malignant lesion. Furthermore, 10 immune parameters were reduced to 5 final predictors following logistic regression analysis in the primary cohort (2:1 ratio; Table 2), which demonstrated more adequate discrimination between laryngeal premalignant lesion and laryngeal malignant lesion. These results support previous researches into HNSCC which links changes in immune cells to tumorigenesis^[26]. A major strength of this study is unlike most other works, laryngeal malignant lesion was compared with premalignant lesion instead of normal condition. In agreement with several studies focused on other premalignant lesions, including pancreatic intraepithelial neoplasias (PanIN), gastric dysplasia, cervical

precancerous lesion, we suggested monocytes, T cells, and B cells possible roles for immunosurveillance of cancer^[27-29].

On the basis of clinical risk factors and circulating immune parameters, we proposed and validated a diagnostic nomogram integrating eight items for preoperative individualized prediction risk of malignancy in patient with laryngeal neoplasm (Figure 3). An advantage of this nomogram is that it is a weighted model, combining multiple predictors and enabling appreciation of the magnitude of impact of each of the predictors on the risk of malignancy. Gender and CD4/CD8+ T cell ratio were the 2 major, heavily weighted, factors in this model. Laryngeal cancer has one of the highest male-to-female ratios among all malignancies^[30, 31]. Not surprisingly, when evaluating for laryngeal cancer, gender remains a more clinically relevant predictive factor. An increased CD4/CD8+ T cell ratio means a relative reduction of CD8+ T cells which were the principal weapons of immunity anti-tumor^[32]. As mentioned in the literature^[33, 34], CD4/CD8+ T cell ratio represented a marker of immune dysfunction and affected tumor progression. These factors may explain the correlation between CD4/CD8+ T cell ratio and risk of laryngeal cancer. And it is noteworthy to mention that we first emphasized its role in the risk prediction of laryngeal cancer.

This nomogram performed well in discriminating cancer, and its discrimination ability was supported by the C-index (0.844 and 0.804 for the primary and validation cohorts, respectively). The calibration plot demonstrated a good agreement of predictive probabilities from the validation cohort (Figure 4A). We also performed a decision curve analysis, as proposed by, to assess the clinical utility of the nomogram by quantifying the net benefit when different threshold probabilities are considered (Figure 4B). Thus, we found that the application of our nomogram to the present validation cohort provided an increase in the net benefit for threshold probability of >22% and <96%.

The nomogram is a predictive tool that is more multiplex than simple risk biomarkers and provides a tailored assessment of a given patient's risk of malignancy. Diagnosis of laryngeal neoplasm is established by operative tissue biopsy and follow-up needed for

high-risk patients. This, on one side, could inform clinicians on diagnostic sampling of suspicious laryngeal lesions that reduces delay in diagnosis, on the other side, could be used to monitor the onset and progression of laryngeal premalignant or malignant lesions in these individuals during postoperative follow-up period. However, the association between this nomogram and tumor node metastasis (TNM) classification, the most common way of staging laryngeal malignant lesion, was not explored. We speculated that TNM classification could be predicted after the adjustments of this nomogram for the following reasons. The component parameters of nomogram, clinical factors and immune index, were related to the clinical stage of laryngeal cancer. Chen *et al* constructed a nomogram model integrating clinical factors to evaluated lymph node metastasis of laryngeal squamous cell carcinoma (LSCC)^[35]. The significance of immune cells for the development of laryngeal cancer has been reported previously. In addition, the nomogram based on immune score and TNM classification for prognostic evaluation of patients with LSCC was proposed^[36]. For predicting TNM classification accurately, future research should be undertaken to update this nomogram by specific clinical and immune parameters screening.

Although we have established an novel discriminating model based on risk factors and immune panels, this study indicated that there are several limitations. First, this is a single center study with small sample size. Although this nomogram is internally validated, it would be consolidated by external validation with patients assessed at multiple institutions. Second, the circulating immune panels were found only as biomarker results of laryngeal premalignant and malignant lesion. Confirmatory studies investigating functional mechanism for carcinogenesis are desirable. Thirdly, tumor infiltration lymphocytes (TILs) were not considered currently. Recently, TILs has attracted increasing interest in the field of cancer research. However, it is yet to be decided whether the nomogram that applies the circulating immune cells combined with TILs to predict risk of laryngeal cancer is more accuracy. Finally, it is not clear whether the observed circulating immune panels would influence the prognostic phenomenon.

CONCLUSION

In summary, we suggest that not only laryngeal cancer but also laryngeal premalignant lesion exhibit changes in circulating immune phenotype. The novel nomogram, incorporating both clinical risk factors and circulating immune parameters, could be appropriately applied in preoperative individualized prediction of malignancy in patients with laryngeal neoplasm.

ARTICLE HIGHLIGHTS

Research background

Malignancy prediction remains important to preoperative diagnosis and postoperative follow-up in laryngeal neoplasm.

Research motivation

There are continuing problems in differentiating premalignant lesion before surgery from malignant lesion of the larynx.

Research objectives

To evaluate the circulating immune population and develop a nomogram for prediction of malignancy in patients with laryngeal neoplasm.

Research methods

Peripheral blood from patients with laryngeal neoplasm was analyzed by blood routine test and flow cytometry. Circulating immune population and clinical parameters were screened to develop a predictive model for risk of laryngeal cancer which was presented by a nomogram.

Research results

The nomogram incorporated predictors, including gender, age, smoke index, proportions of monocytes, CD8+ T cells, CD4+ T cells, B cells, and CD4/CD8 + T cell ratio, showed good discrimination between laryngeal premalignant lesion and malignant lesion.

Research conclusions

This circulating immune parameters-based novel nomogram could be appropriately applied in preoperative individualized prediction of malignancy in patients with laryngeal neoplasm.

Research perspectives

Future research should be undertaken to assess the external validation of the nomogram in multiple institutions. And confirmatory studies investigating functional mechanism of circulating immune populations for laryngeal carcinogenesis are desirable.

ACKNOWLEDGEMENTS

We thank the technical assistance of peripheral blood test kindly provided by the ¹¹Department of Clinical Laboratory at Eye, Ear, Nose, and Throat Hospital of Fudan University in Shanghai, China.

10%

SIMILARITY INDEX

PRIMARY SOURCES

- 1 ascopubs.org 68 words — 2%
Internet
- 2 Ganesh V. Raj, R. Houston Thompson, Bradley C. Leibovich, Michael L. Blute, Paul Russo, Michael W. Kattan. "Preoperative Nomogram Predicting 12-Year Probability of Metastatic Renal Cancer", The Journal of Urology, 2008 34 words — 1%
Crossref
- 3 hdl.handle.net 30 words — 1%
Internet
- 4 Huijing Wang, Le Zhang, Zhe Liu, Xiaodong Wang, Shikai Geng, Jiaoyu Li, Ting Li, Shuang Ye. "Predicting medication nonadherence risk in a Chinese inflammatory rheumatic disease population: development and assessment of a new predictive nomogram", Patient Preference and Adherence, 2018 28 words — 1%
Crossref
- 5 Enfa Zhao, Hang Xie, Yushun Zhang. "A Nomogram for the Prediction of Cessation of Migraine Among Patients With Patent Foramen Ovale After Percutaneous Closure", Frontiers in Neurology, 2020 23 words — 1%
Crossref
- 6 Liwei Wu, Wei Chang, Yanzheng Song, Lin Wang. "Predicting treatment failure risk in a Chinese Drug-Resistant Tuberculosis with surgical therapy: Development and assessment of a new predictive nomogram", International Journal of Infectious Diseases, 2020 20 words — 1%
Crossref

7 Ling Chen, Dan Luo, Xiajuan Yu, Mei Jin, Wenzhi Cai. 19 words — 1%
"Predicting stress urinary incontinence during pregnancy: combination of pelvic floor ultrasound parameters and clinical factors", *Acta Obstetrica et Gynecologica Scandinavica*, 2018

Crossref

8 Liwei Wu, Wei Chang, Yanzheng Song, Lin Wang. 19 words — 1%
"Predicting treatment failure risk in a Chinese Drug-Resistant Tuberculosis with surgery therapy: development and assessment of a new predictive nomogram", *International Journal of Infectious Diseases*, 2020

Crossref

9 Filippo Marchi, Francesco Missale, Fabiola Incandela, Marta Filauro et al. 18 words — 1%
"Prognostic Significance of Peripheral T-Cell Subsets in Laryngeal Squamous Cell Carcinoma", *Laryngoscope Investigative Otolaryngology*, 2019

Crossref

10 Wenwen Sun, Lu Gui, Xulei Zuo, Lingyun Zhang, Daibing Zhou, Xiaoling Duan, Weimin Ren, Guoxiong Xu. 17 words — < 1%
"Human epithelial-type ovarian tumour marker beta-2-microglobulin is regulated by the TGF- β signaling pathway", *Journal of Translational Medicine*, 2016

Crossref

11 www.spandidos-publications.com 15 words — < 1%
Internet

12 Borque, Ángel, Jose Rubio-Briones, Luis M. Esteban, Gerardo Sanz, Jose Domínguez-Escrig, Miguel Ramírez-Backhaus, Ana Calatrava, and Eduardo Solsona. 15 words — < 1%
"Implementing the use of nomograms by choosing threshold points in predictive models: 2012 updated Partin Tables vs a European predictive nomogram for organ-confined disease in prostate cancer : Thresholds for predicting organ-confined prostate cancer using nomograms", *BJU International*, 2014.

Crossref

13 Jin-Long Huang, Yi-Peng Fu, Chu-Yu Jing, Yong Yi, Jian Sun, Wei Gan, Zhu-Feng Lu, Jian Zhou, Jia 15 words — < 1%

Fan, Shuang-Jian Qiu. "A novel and validated prognostic nomogram based on liver fibrosis and tumor burden for patients with hepatocellular carcinoma after curative resection", Journal of Surgical Oncology, 2018

Crossref

14 www.nature.com 13 words — < 1%
Internet

15 Stefano Molica, Diana Giannarelli, Luciano Levato, Rosanna Mirabelli, Massimo Gentile, Mirella Lentini, Fortunato Morabito. "A prognostic algorithm including a modified version of MD Anderson Cancer Center (MDACC) score predicts time to first treatment of patients with clinical monoclonal lymphocytosis (cMBL)/Rai stage 0 chronic lymphocytic leukemia (CLL)", International Journal of Hematology, 2014 12 words — < 1%
Crossref

16 Min Chen, Yi Fang, Lei Cheng, Haitao Wu. "Helicobacter pylori is associated with poor prognosis of laryngeal precancerous lesion", Auris Nasus Larynx, 2019 12 words — < 1%
Crossref