Application of Circulating Genetically Abnormal Cells in the Early-stage Diagnosis of Lung Cancer

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Abstract

Background Lung cancer is the primary cause of cancer related death worldwide, early detection of the disease contributes to early diagnosis and reduce the incidence of death from lung cancer. Therefore, an effective and non-invasive method for early diagnosis of lung cancer is urgently needed.

Methods To evaluate the diagnostic performance of circulating genetically abnormal cells(CAC) in early lung cancer, a total of 63 participants who completed CAC detection by ZHUHAI SANMED BIOTECH INC. and obtained pathological results from January to December 2020 were included in our study, which were divided into 50 cases of lung cancer ,13 cases of benign lung disease. The levels of lung cancer-related markers in peripheral blood and chest computed tomography(CT) imaging characteristics of these patients were collected before pathological acquisition.

Results The positive rate of CAC was 90.0% in lung cancer group and 23.1% in benign lung disease group, the difference was statistically significant (P□0.01).The area under the receiver operating characteristic (ROC) curve of CAC was 0.837, the sensitivity was 90%, and the specificity was 76.9%.The area under the ROC curve and sensitivity were both higher than the combined or single serum tumor marker test.

Conclusions This study preliminarily concludes that CAC, as a non-invasive test, has high sensitivity and specificity for early diagnosis of lung cancer, which is expected to help early detection of disease in lung cancer patients and has certain help and guiding significance for clinical work.

Introduction

Lung cancer has always been one of the malignant tumors that seriously threaten human safety, according to the *Latest Global Cancer Data*, lung cancer accounted for 11.1% (2.2 million) of new cases of cancer worldwide in 2020,ranking second, and ranked first in mortality(18% , 1.79 million) . In 2020 , 3 million people in China will have died from cancer, including 710,000 people who died of lung cancer , 23.8% of all cancer deaths[1].These figures show that lung cancer has seriously affected people's health. The prognosis of lung cancer patients with different clinical stages was significantly different[2]. It has been reported that due to the influence of different stages and regions, the 5-year survival rate of lung cancer patients is 4% to 17%, and the primary reason for poor prognosis of lung cancer is that most patients are in advanced stage at the time of diagnosis, and many patients lack treatment response[3]. Therefore, the use of effective detection methods for early diagnosis of lung cancer is expected to provide more options for subsequent treatment, is also the key to reduce the mortality rate.

Currently, imaging-based screening, tumor markers, and histopathological examination are the primary approaches for the diagnosis of lung cancer[4]. In 2006, the results of International Early Lung Cancer Action Program published by The New England Journal of Medicine, including the United States, Europe, China and other countries, also highly affirmed the role of low-dose computed tomography(LDCT) in lung cancer screening. And in addition, the Dutch-Belgian randomised lung cancer screening trial (NELSON), has reached similar conclusions[5].The National Lung Screening Trial compared annual LDCT

examination with routine chest x-rays and found that after three rounds of screening, LDCT examination can reduce the mortality of lung cancer by 20%[6].However, it is undeniable that with the wide application of LDCT in lung cancer screening programmes, the number of nodules detected in patients has increased significantly, while the incidence of lung cancer has not increased correspondingly[7].This means that the increase in imaging and testing has produced more false positive results, failed to identify more cases of lung cancer, and may face unnecessary treatment, including overdiagnosis, which not only raises the economic burden of patients, but also causes anxiety, tension and other psychological problems[8, 9].Currently, liquid biopsies have emerged as a crucial tool in cancer management, and are diagnostic biomarkers used to detect patients with early-stage lung cancer or those at high risk of developing lung cancer. They can often be detected in body fluids, such as saliva, urine and blood[10, 11].As a new non-invasive diagnostic technique, liquid biopsy has the advantages of easy and repeatable specimen acquisition compared with other screening methods for lung cancer, and can be used for precision treatment, prognosis evaluation and efficacy monitoring of lung cancer[12].And a number of studies have reported the potential of liquid biopsy technology as a biomarker for the diagnosis of lung cancer, including cell-free DNA[13]□circulating tumour DNA[14]and exosomes[15].

One of the particularly important advances in the development of liquid biopsy was in 1869, Australian scholar Thomas Ashworth first observed tumor cells in the blood of patients with metastatic tumors, first proposed the concept of circulating tumor cell(CTC)[16].CTC is tumor cell that shed from primary tumor and released into the blood circulation, the migration of CTC is an early event of cancer progression and has important application value in the early diagnosis of tumors[17].However, when CTC technology is used in the early diagnosis of lung cancer, we need to break through its dependence on epithelial markers (EpCAM, etc.), and distinguish CTC from white blood cells in the blood at the same time[18].2010 American Anderson cancer center pathology professor Katz and others[19]in pulmonary squamous cell carcinoma and pulmonary adenocarcinoma of peripheral blood mononuclear cell (PBMC) were found in several common deletion or amplification genes, developed a sensitive and quantitative antigen-independent four-color fluorescence in situ hybridization (FISH) technique to identify the chromosome 3 (3p22.1 and CEP3), chromosome 10 (10q22.3 and CEP10) method of genetics study changes, and named this type of PBMC with the same chromosomal locus mutation as circulating genetically abnormal cell (CAC).The results of this study also showed that CAC and LDCT can be used to differentiate benign and malignant pulmonary nodules, so as to improve the diagnostic accuracy and reduce unnecessary invasive tissue examination. However, there are still few studies on the application of CAC in the clinical diagnosis of lung cancer. Therefore, 63 patients with early lung cancer who have completed the CAC test were selected in this study, which are reported as follows.

Materials And Methods

1. Study design and patients

A retrospective analysis was performed on 63 patients who were treated in Shandong,China between January and December 2020 and completed CAC test in ZHUHAI SANMED BIOTECH INC., and they were

divided into two groups according to postoperative histopathological diagnosis.In the lung cancer group, there were 27 males and 23 females, aged from 31 to 74, including 46 cases of lung adenocarcinoma, 2 of lung squamous cell carcinomas, 2 cases of small cell carcinoma(SCLC). Clinical stages were TIS (5 cases),IA (37 cases),IB (7 cases), and IIB(1 case), respectively. There were 6 males, 7 females in the benign lung disease group, aged from 34 to 78 . And there was no statistically significant differences in gender and age between the different groups.

The objects selected in this study are all meet the following points:1.all patients are not less than 18 years old in Shandong Province；2.all patients completed CAC test and obtained histopathological results after CAC results；3.all patients were in the early stage of lung cancer(TIS ~ IIB stage)；4.all patients had no history of malignancy and had not received any antitumor treatment before enrollment;5.all blood samples and computed tomography(CT) scan were obtained just prior to surgery. In addition, the measurement of tumor markers were not compulsory for enrollment in this subject. And one more thing to note, due to the retrospective character of this study, informed conssent was waived, the study was approved by the committee of Shandong First Medical University.

The diagnosis of benign disease was determined by imaging and histopathology. The staging criteria used were those from the TNM stage of American Joint Committee on Cancer[20].Tumor pathology was classified according to the World Health Organization (WHO) classification standard of lung tumors (2015 edition)[21].

2. Methods

2.1 CAC detection

CAC tests of all patients in our study were performed by the MDA TEST technology of ZHUHAI SANMED BIOTECH INC., which was originated from MD Anderson Cancer Center in the United States. CAC testing process：1.Blood collection and fixation:8-10ml of fresh venous blood was collected with EDTA vacuum anticoagulant tube, and matching cell preservation solution was added within 2 hours. The sample was gently turned upside down for 10 times (and it can be stored and transported up to 4 days at room temperature);2.Target cells enrichment and purification: peripheral blood mononuclear cell (PBMC) were enriched by automatic enrichment or Ficoll density gradient centrifugation;3.Glass slides preparation and hybridization: Cell slides were prepared and fixed solution was added and after protease digestion, multicolor FISH was detected by using mononuclear cell chromosome abnormity detection kit；4.Image scanner and interpretation： the BioView automated abnormal cell scanning analyzer can automatically complete scanning， imaging and analyzing in 30 minutes to observe the chromosome changes of the target locus . CAC interpretation criteria： the number of CAC≥3: prompts a positive test; CAC count＜3 indicates a negative test. Positive results indicate a higher malignant risk of pulmonary nodules, which is recommended to be evaluated in combination with image examination, clinical characteristics of patients and other tests. Patients may be treated with nonsurgical biopsy, aggressive surgery, or close follow-up (3 months).For negative results, it is recommended to comprehensively evaluate the clinical information of

imaging examination and other tests, and follow up patients according to the existing diagnostic and treatment standards.

2.2 Computed Tomography and Tumor biomarker

Computed tomography scanning and serum tumor markers determination were all completed in normal hospitals of Shandong Province. By analyzing the CT data, the maximum diameter of lung nodules(specifically referred to the pathological subjects in this paper) in the lung cancer group was 4-30mm, with the median and inter-quartile range of 14mm and 9-18mm, respectively. In the benign pulmonary disease group, the maximum diameter was 7-30mm, and the median inter-quartile range were 10mm and 10-18mm respectively, differences matter($P < 0.001$)means that the diameter of the nodules in the lung cancer group was generally higher than that in the benign lung disease group. According to the results of serum tumor marker determination, 65.7pg/ml, 10ng/ml, 6.0ng/ml, 1.5ng/ml, 16.3ng/ml and 35U/ ml were considered as the normal upper limits of ProGRP, CEA, CYFRA211, SCC, NSE and CA125, respectively, and there are 3, 1, 2, 0, 9 and 1 people over the upper limit respectively.

3. Statistical analysis

All statistical analyses were performed using SPSS (version 26, SPSS Inc., Chicago, IL). Receiver operating characteristics curve was used to evaluate the diagnostic efficiency of CAC and other methods in carcinoma of the lungs . And for the continuous variables, we use the Mann-Whitney U test. And for categorical variables, we used$\chi 2$ test to analyze. All P values were 2-sided, and we considered that $P < 0.05$ is statistically significant .

Results

1. Basic characteristics of Patients

There were 63 patients in this study, including 50(79.4%) patients with lung cancer , 13 (20.6%) patients with benign lung lesions, aged 31-78 years, including 33 males (52.4%) and 30 females (47.6%). Kolmogorov-Smirnov test confirmed that age, nodule size , course of disease and tumor markers in the two groups were skewed distribution data, so the median and interquartile range were selected to represent. The result showed that the time from the discovery of nodules to the operation in lung cancer group was longer than that in the benign lung lesions group, the difference was statistically significant,$P<0.001$(the data of 2 patients were missing n=61).It was considered that it might be related to the time spent in dynamic follow-up CT of patients with lung cancer before operation. Nodules with diameter $\leq$10mm were mainly found in both groups, accounting for 38% and 53.8%, respectively. The majority of lung cancer patients were mixed ground-glass nodule, accounting for 44%, while in the benign lung disease patients, solid nodules were the most common, accounting for 61.5%.In this study,49 patients completed CEA,CYFRA211 and NSE, 48 patients completed ProGRP, 47 patients completed SCC and 44 patients completed CA125.And none of the patients had a history of occupational dust exposure.

The positive rate of CAC in lung cancer patients was significantly higher than that in the control group (P < 0.001).The basic characteristics of the two groups are shown in Table 1.

2. Relationship between CAC positivity and clinical characteristics

In order to clarify whether there was difference in CAC positive expression among the basic characteristics of 63 subjects, the data were summarized as Table 2.The results showed that there were no significant statistical differences between CAC positivity and the participants demographics in age (P = 0.977), gender (P = 0.612), family history(P=1.000), smoking history (P = 1.000), and tumor nodule size (P = 0.770).Among the different pathological types, lung adenocarcinoma was the most common, with 46 cases (92%) and the positive rate of CAC was 89.1%.It should be noted that the CAC positive rate was 100% in both squamous cell carcinoma and SCLC, possibly due to small number of cases included (both 2 patients).The CAC positive rate of TIS (5 patients) and IIb (1 patient) patients was 100%, and there was no statistically significant difference from 89.2% of IA patients (37 patients).We found that the CAC positive rate of solid nodules (95%) was significantly higher than that of pure ground-glass nodules (52.9%),with a statistically significant difference(P=0.005),however, there was no significant difference between the CAC positive rate of mixed ground-glass nodules (76.9%) and the others. At the same time, for continuous variables such as the course of disease and blood tumor biomarkers, we performed the Man Whitney U test respectively and found that the course of disease in patients with CAC positive test was significantly longer than that of CAC negative patients(P=0.049), including CYFRA21-1(P=0.007), SCC(P=0.001), NSE(P<0.001) were statistically significant.

3. The diagnostic efficacy of CAC test

The previous results indicated that the CAC positive rate of solid nodules was significantly higher than that of ground-glass nodules, and the course of disease in CAC positive participants were significantly longer than that in patients with CAC negative. So we used the Spearman test to show that the correlation coefficient between CAC positive rate and course of disease was 0.045, and the nodule density was -0.430, indicating no significant correlation. Through ROC curve analysis(Figure 1-8), we obtained that the area under the curve(AUC) of CAC counts was 0.837(95% CI, 0.810–0.864, P < 0.001). In this result, using ≥2.5 CAC as the positive standard, we can obtain a sensitivity of 90.8% and a specificity of 83.9%.This result is also close to the CAC positive limit. The ROC curve of CAC was higher than the combined or single serum tumor marker test(Figure 1-8,Table 3).The data in Table 1 also showed that the positive rate of CAC in all participants was 76.2%, and the positive rate in the lung cancer group (90%) was significantly higher than that in the benign lung lesions group (23.1%), P<0.01.Therefore, we can conclude that CAC test may have a good application value in the diagnosis of early lung cancer.

At the same time, we also compared the diagnostic efficacy of CAC test with the blood tumor marker and specific signs of nodules in chest CT (Table 3), including the sensitivity, specificity, accuracy and AUC(the value of AUC has not yet been evaluated because the specific signs of nodules are both classified variables).The results showed that the sensitivity (90%) and accuracy (87.3%) of CAC test for the diagnosis of lung cancer were higher than other detection methods, including the combination of tumor

biomarkers (37.1% sensitivity and 46.5% accuracy).In our study, the sensitivity, specificity, accuracy and AUC of NSE were 23.7%, 100%, 40.8% and 0.585(95%CI, 0.552 – 0.618), which were all higher than those of other single blood tumor markers. It should be noted that the P values of CEA SCC CA125 analyzed in ROC were all above 0.05, the results are not considered to be meaningful. It may be related to the small number of overall data, such as only 1 CEA and 1 CA125 positive case,0 SCC positive case , with certain statistical errors. Among all the nodule signs reported on chest CT, vessel convergence sign has high sensitivity(56%) and accuracy(54%), while vacuolar sign has the highest specificity(92.3%) for the diagnosis of lung cancer.

Discussion

It is well known that lung cancer is a multi-factorial and highly aggressive cancer, and is the common cause of cancer-related death worldwide[22].The etiology of lung cancer is still unknown, but cancer and chronic respiratory disease are both linked to tobacco use[23].Smoking is recognized as the leading risk factor for lung cancer, but there are other risk factors such as air pollution, biomass burning, and occupational exposure (asbestos) also play an important role in the development of lung cancer[24].Lung cancer is generally divided into non-small cell lung cancer(includes lung adenocarcinoma, lung squamous cell carcinoma and large cell lung cancer) and small cell lung cancer according to pathological types, accounting for 85% and 15% of all lung cancers respectively. Significant survival differences between patients with different T and M stages of lung cancer have been reported, including differences in survival among patients with single-site or multi-site metastases involving the brain or other sites[25, 26].Therefore, in order to effectively prevent the occurrence of death from lung cancer, in addition to avoiding or reducing the exposure of risk factors as much as possible, appropriate early diagnosis is particularly important.

Low-dose computed tomography (LDCT) screening significantly reduces lung cancer mortality in high-risk populations by detecting early-stage disease, according to the results of a large randomized controlled trial published in the August 2011 issue of the New England Journal of Medicine[27]. Based on this, the American Cancer Society (ACS), American College of Chest Physicians (ACCP) and other lung cancer screening guidelines have the same inclusion criteria for the target population: annual LDCT screening for people aged 55 to 74 years, who had a smoking index of ≥30 pack years, are actively smoking or have quit smoking within the past 15 years and have no other life-limiting comorbidity[28-30].However, with the improvement of people's awareness of health examination and the popularization of LDCT application, due to the high sensitivity and lack of specificity of CT, the detection rate of clinical pulmonary nodules is getting higher, which may lead to the increase of invasive treatment, and has potential harm risk[31].Currently, pulmonary nodules are defined as focal, quasi-round, dense solid or subsolid pulmonary opacity ≤3 cm in diameter, which can be solitary or multiple pulmonary nodule, and can be benign or malignant, among which malignant nodules are lung cancer[32].The diagnosis of pulmonary nodules is mainly evaluated by clinical informational, imaging techniques, surgical and non-surgical biopsy. For low-risk patients, LDCT scan should be repeated in long-term follow-up to compare the external structure (nodule size, shape, edge, etc.) and internal characteristics (nodule density,

structure, etc.) to help distinguish benign and malignant pulmonary nodules[32].Correct differentiation of benign and malignant pulmonary nodules is helpful for early surgical treatment of malignant nodules and improvement of patient prognosis.

Dr. Ruth L. Katz of the M.D. Anderson Cancer Center, as the technical inventor of the MDA Test, developed a four-color FISH technique to identify cytogenetic changes, and proposed the concept of circulating genetically abnormal cell (CAC) as a distinct cell from circulating tumor cell (CTC)[19].This advance suggests that there are genetic abnormalities in patients with lung non-small cell carcinoma that are similar to those in the primary tumor and are strongly associated with the presence and early development the cancer, and because it is antigen-independent expression, it may show more CAC if it is not restricted by antigen detection of epithelial cell differentiation. In recent years, there has also been research data to demonstrate that, including in 2020, Katz et al. [33]used a novel antigen-independent method of 4-color FISH to detect circulating tumor cells with abnormal copy number mononuclear cells in peripheral blood of patients with lung cancer (n = 107) and non-lung cancer (n = 100), and obtained results with an accuracy of 94.2%, sensitivity of 89%, and specificity of 100%.And Wei-ran Liu et al. [34]analyzed 261 lung cancer patients, 78 healthy participants in 2020 , concluded that the number of CAC in early-stage NSCLC patients was significantly higher than the latter, and the sensitivity of CAC detection in the identification of NSCLC was 67.2%(higher than tumor markers), and the specificity was 80.8%.These studies all prompt that CAC may be an effective, specific biomarker for the diagnosis of tumor , with high potential in accurate.

In this topic, we counted the number of peripheral blood CAC of 63 patients in two groups. After excluding statistical differences in gender, age and smoking history of the two groups of patients, the results showed that the lung cancer group of CAC positive rate (90%) is significantly higher than the lung benign disease group (23.1%), the difference was statistically significant, which corresponds to the previous research suggesting that CAC may be a potential biomarker for early diagnosis of lung cancer. Since it has been previously reported that different nodular features are helpful to the diagnosis of lung cancer[35, 36], we added such indicators as density, size and special signs of nodules in this study. Through further analysis ,we found that the CAC positive rate of solid nodules (95%) was significantly higher than pure ground glass nodules (52.9%), and there was a statistically significant difference in the course of disease between CAC positive and CAC negative. Therefore we conducted correlation analysis, found that the correlation coefficient between CAC positive rate and course of disease was 0.045, and the density of nodules was -0.430, both of which were not significantly correlated. After that ,we also made a comparison in diagnostic efficacy of CAC and tumor markers and special appearance of nodules on CT. The AUC of CAC count was 0.837($P < 0.001$), higher than that of combined or single tumor marker test. The sensitivity (90%) and accuracy (87.3%) of CAC in the diagnosis of lung cancer were higher than other detection methods, including tumor biomarker combination detection (37.1% sensitivity, 46.5% accuracy) and nodular special signs, such as vessel convergence sign had the highest sensitivity (56%)and accuracy (54%) of the diagnosis of lung cancer, and signs of vacuolar in the diagnosis of lung cancer has the highest specificity (92.3%).It indicates that CAC detection has a high sensitivity and specificity in the

early-stage diagnosis of lung cancer, which is basically consistent with the results of the research by the experts mentioned previously.

As a result, we can conclude that non-invasive CAC detection may have a higher detection rate than tumor markers and chest CT in the diagnosis of early-stage lung cancer. Although the tumor markers have certain reference significance for the diagnosis of lung cancer, traditional serum tumor markers are mostly related to the pathological types of lung cancer, such as CYFRA21-1[37], often used as a serum tumor marker of non-small cell lung cancer;NSE[38] is often used as a serum tumor marker for small cell lung cancer. However, when we analyzed the relationship between CAC positive expression and pathological types of lung cancer, it was found that the positive expression of CAC in lung cancer was not affected by pathological types of lung cancer patients. Therefore, it has more advantages when applied in the diagnosis of lung cancer. Of course, if it is used for the early diagnosis of lung cancer, it has to be considered that its price is almost 10 times that of LDCT, which increases the economic burden of patients. And this study shows that CAC has some false positive or false negative results, but it is undeniable the clinical application value. However, due to the limited number of samples included in this study, such as only 1 case was positive for CEA and CA125,0 cases were positive for SCC, there may be some statistical errors, and further verification of the accuracy of the results of this study is needed in a large sample population. At present, led by Professor Chunxue Bai, the Chinese Alliance for Lung Cancer Prevention and Treatment and SANMED BIOTECH jointly launched Bai-DX & MDA TEST malignancy auxiliary diagnosis of pulmonary nodules of national multicenter clinical study has fully developed. A total of 13 regional key tertiary class A hospitals participated in the study and planned to include more than 1,000 patients with pulmonary nodules. The MDA TEST technology was combined with artificial intelligence imaging analysis to conduct a prospective validation study on the benign and malignant pulmonary nodules. We believe that there will be a large sample data of MDA TEST in China to share with you soon, in order to provide a basis for improving the early-stage detection rate of lung cancer and the prognosis of patients.

Declaration

There is no financial support or conflict of interest involved in this study. The data and materials are true and valid. And the study was approved by the committee of Shandong First Medical University.

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Tables

Table 1

Basic characteristics of the two groups

Characteristics	N(proportion)	Lung cancer group(n = 50)	Benign lung diseases group(n = 13)	P
Gender,n(%)				0.614
Male	33(52.4%)	27(54%)	6(46.2%)	
Female	30(47.6%)	23(46%)	7(53.8%)	
Age(year)				0.052
Median,(interquartile range)	57(50,62)	58(50,62)	57(49,61)	
Smoking history, n (%)				1.000
Yes[a]	20(31.7%)	16(32%)	4(30.8%)	
No	43(68.3%)	34(68%)	9(69.2%)	
Family history of cancer, n(%)				0.298
Yes[b]	14(22.2%)	13(26%)	1(7.7%)	
No	49(77.8%)	37(74%)	12(92.3%)	
Course of the disease[c](month)				
Median,(interquartile range)	2(1,10)	2(1,13)	1(1,8)	< 0.001
Tumor biomarkers Median,(interquartile range)				
ProGRP(pg/ml)	32.45(23.88,39.84)	31.41 (23.94,40.63)	27.35(22.35,40.56)	0.049
CEA(ng/ml)	1.64(1.14,2.63)	1.64(1.08,2.59)	2.06(1.12,3.66)	0.143

[a] Include current smokers or patients with a history of smoking.

[b] Parent or sibling with a malignant tumor.

[C] Time from the first discovery of the nodule to the completion of the operation.

[d] Maximum diameter of the nodule.

Characteristics	N(proportion)	Lung cancer group(n = 50)	Benign lung diseases group(n = 13)	P
CYFRA21−1(ng/ml)	2.24(1.45,3.20)	2.22(1.53,3.05)	2.20(1.45,3.33)	< 0.001
SCC(ng/ml)	0.60(0.50,0.90)	0.70(0.50,0.90)	0.60(0.50,1.10)	0.170
NSE(ng/ml)	14.82(12.88,15.67)	14.87(12.83,15.98)	13.55(12.51,15.35)	< 0.001
CA125(U/ml)	7.80(6.00,9.83)	7.44(6.00,9.96)	8.70(5.68,9.58)	0.062
Nodule size[d](n/%)				0.546
≤ 10mm	26(41.3%)	19(38%)	7(53.8%)	
10−20mm	22(34.9%)	18(36%)	4(30.8%)	
> 20mm	15(23.8%)	13(26%)	2(15.4%)	
Nodule type(n/%)				0.006
Solid nodule	17(27.0%)	9(18%)	8(61.5%)	
Mixed ground-glass nodule	26(41.3%)	22(44%)	4(30.8%)	
Pure ground-glass nodule	20(31.7%)	19(38%)	1(7.7%)	
Special signs of nodules(n/%)				
Lobulation	20(31.7%)	17(34%)	3(23.1%)	0.675
spiculation	34(54.0%)	27(54%)	7(53.8%)	0.992
pleural indentation	16(25.4%)	13(26%)	3(23.1)	1.000
vessel convergence sign	35(55.6%)	28(56%)	7(53.8)	0.889
vacuolar sign	7(11.1%)	6(12%)	1(7.7%)	1.000
CAC ≥ 3 (n/%)	48(76.2%)	45(90%)	3(23.1%)	< 0.001

[a] Include current smokers or patients with a history of smoking.

[b] Parent or sibling with a malignant tumor.

[C] Time from the first discovery of the nodule to the completion of the operation.

[d] Maximum diameter of the nodule.

Table 2

Relationship between CAC positivity and clinical characteristics

Characteristics	CAC ≥ 3 (n/%)	CAC < 3(n/%)	P
Gender			0.612
Male	26(78.8%)	7(21.2%)	
Female	22(73.3%)	8(26.7%)	
Age			0.977
≥ 60 years	19(76%)	6(24%)	
< 60 years	29(76.3%)	9(23.7%)	
Smoking history			1.000
Yes[a]	15(75%)	5(25%)	
No	33(76.7%)	10(23.3%)	
Family history of cancer			1.000
Yes[b]	10(76.9%)	3(23.1%)	
No	38(76%)	12(24%)	
Pathological types			1.000
Adenocarcinoma[c]	41(89.1%)	5(10.9%)	
Squamous cell carcinoma	2(100%)	0(0%)	
SCLC[d]	2(100%)	0(0%)	
TNM stage			1.000
Tis	5(100%)	0(0%)	
IA	33(89.2%)	4(10.8%)	

[a] Include current smokers or patients with a history of smoking.

[b] Parent or sibling with a malignant tumor.

[C] Include 5 cases of adenocarcinoma in situ, 8 cases of minimally invasive adenocarcinoma and 33 cases of invasive adenocarcinoma.

[d] SCLC,small cell lung cancer.

[e] Maximum diameter of the nodule.

Characteristics	CAC ≥ 3 (n/%)	CAC < 3(n/%)	P
IB	6(85.7%)	1(14.3%)	
IIB	1(100%)	0(0%)	
Nodule size[e]			0.770
≤ 10mm	21(80.8%)	5(19.2%)	
10–20mm	16(72.7%)	6(27.3%)	
> 20mm	11(73.3%)	4(26.7%)	
Nodule type			0.008
Solid nodule	9(52.9%)	8(47.1%)	
Mixed ground-glass nodule	20(76.9%)	6(23.1%)	
Pure ground-glass nodule	19(95%)	1(5%)	
Special signs of nodules			
Lobulation	16(80%)	4(20%)	0.868
spiculation	28(82.4%)	6(17.6%)	0.214
pleural indentation	10(62.3%)	6(37.6%)	0.137
vessel convergence sign	24(68.6%)	11(31.4%)	0.197
vacuolar sign	4(57.1%)	3(42.9%)	0.433

[a] Include current smokers or patients with a history of smoking.

[b] Parent or sibling with a malignant tumor.

[C] Include 5 cases of adenocarcinoma in situ, 8 cases of minimally invasive adenocarcinoma and 33 cases of invasive adenocarcinoma.

[d] SCLC,small cell lung cancer.

[e] Maximum diameter of the nodule.

Table 3
Comparison in different diagnostic methods

Diagnostic methods	N[c]	Sensitivity	Specificity	Accuracy	AUC[a] (95% CI)
CAC test	63	90.0%	76.9%	87.3%	0.837(0.810–0.864)
ProGRP	48	8.1%	100%	29.2%	0.462(0.426–0.498)
CEA	49	0	90.9%	20.4%	0.472(0.430–0.513)
CYFRA21–1	49	5.3%	100%	26.5%	0.569(0.527–0.611)
SCC	47	0	100%	23.4%	0.474(0.431–0.516)
NSE	49	23.7%	100%	40.8%	0.585(0.552–0.618)
CA125	44	2.8%	100%	20.5%	0.541(0.495–0.586)
All tumor biomarkers[b]	43	37.1%	87.5%	46.5%	0.299(0.268–0.329)
Lobulation	63	34%	76.9%	42.9%	.
spiculation	63	54%	46.2%	52.4%	.
pleural indentation	63	28%	76.9%	38.1%	.
vessel convergence sign	63	56%	46.2%	54%	.
vacuolar sign	63	12%	92.3%	28.6%	.
Sensitivity, specificity and accuracy all depend on the positive criteria of various detection methods to obtain data, AUC obtains results based on the continuous values of each detection method.					
[a] AUC, area under curve;					
[b] All tumor biomarkers,include ProGRP + CEA + CYFRA21−1 + SCC + NSE + CA125;Positive means that any one of them is positive.					
[C] Refer to the number of people who have completed each test.					

Figures

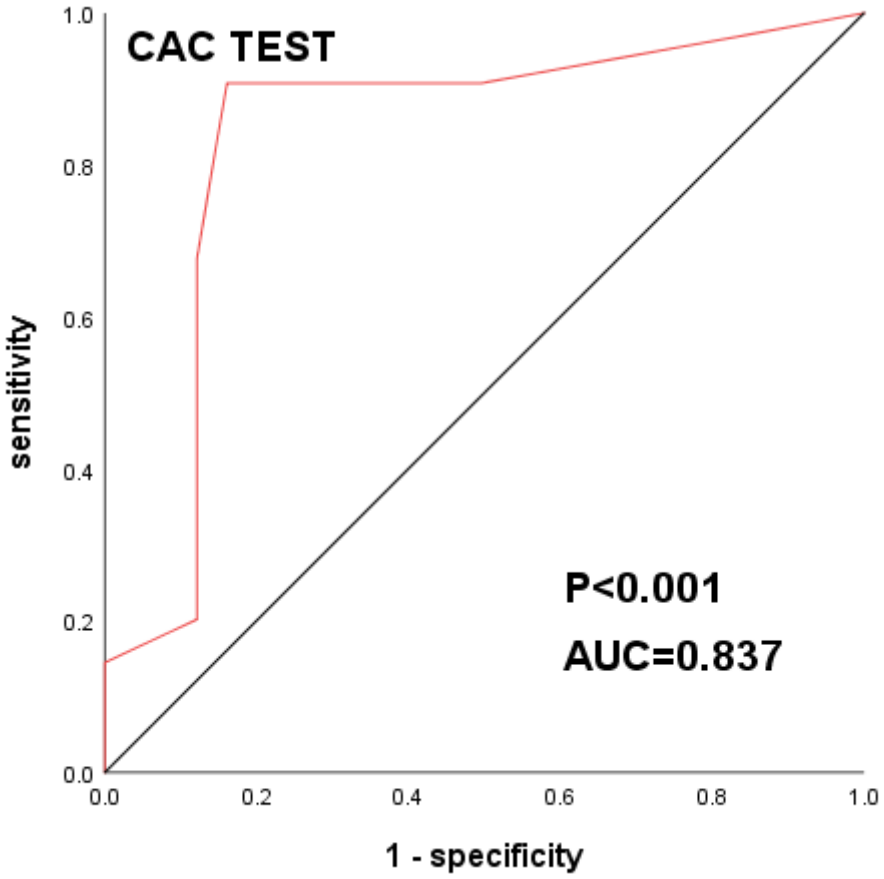

Figure 1

CAC test

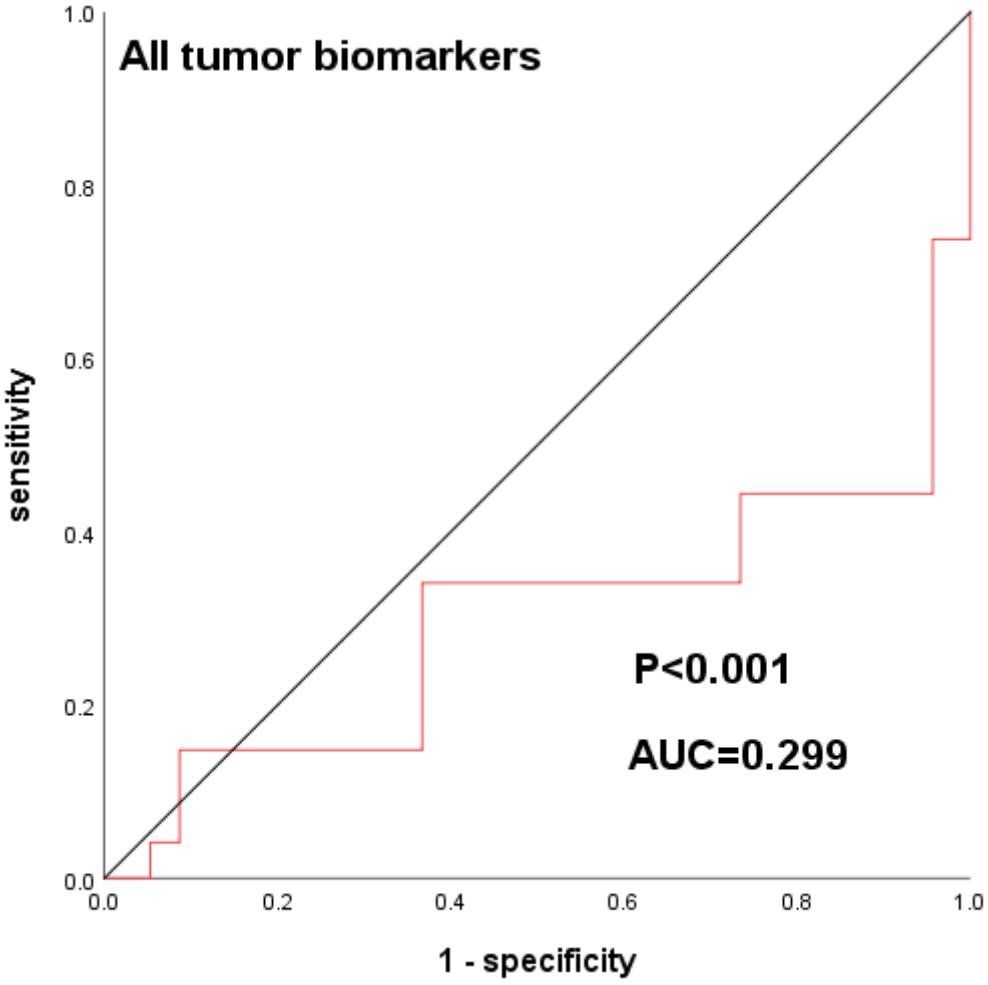

Figure 2

All Tumor biomarkers

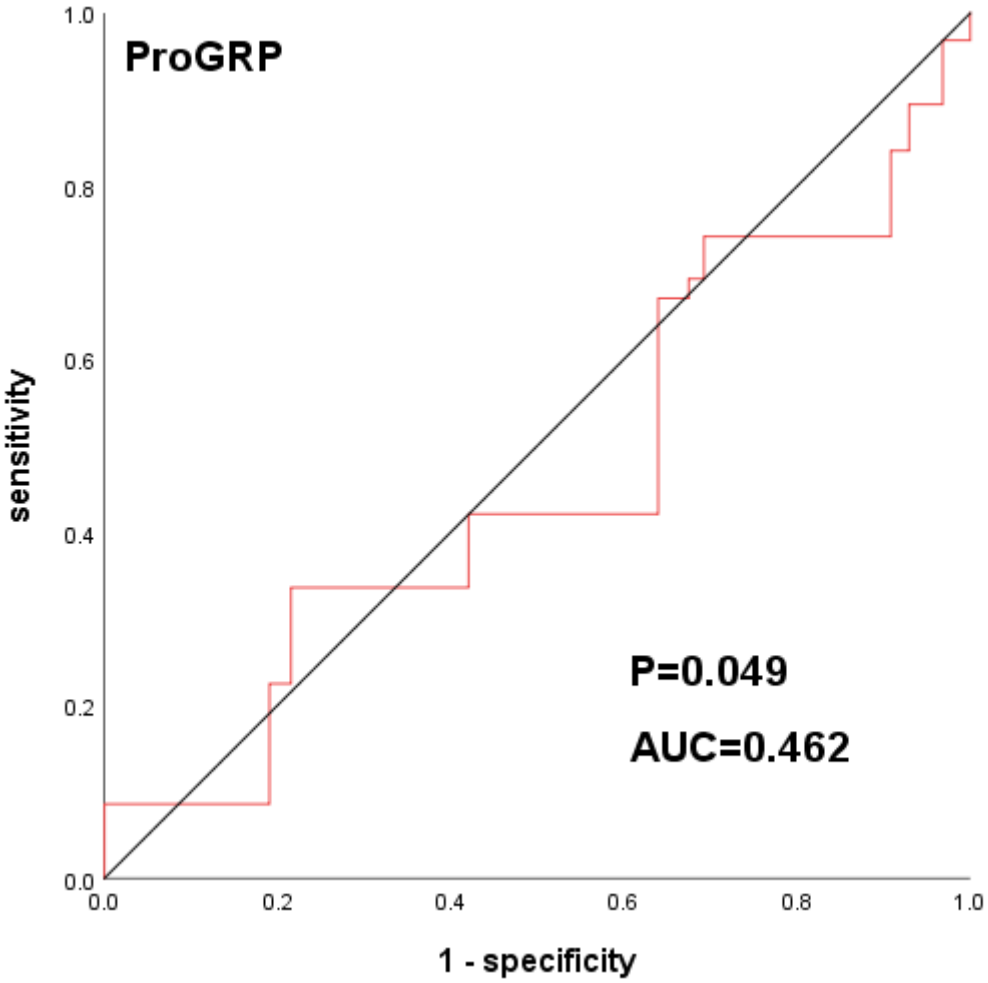

Figure 3

ProGRP

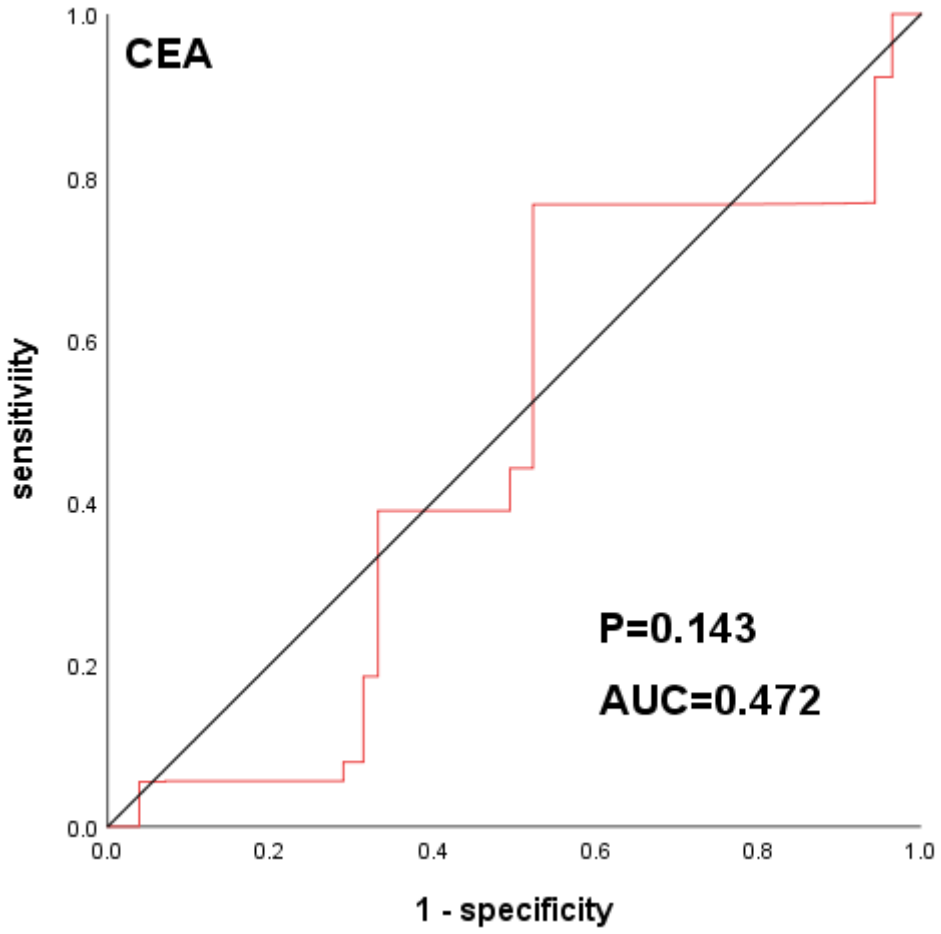
CEA
sensitivity
1 - specificity
P=0.143
AUC=0.472
0.0
0.2
0.4
0.6
0.8
1.0

Figure 4

CEA

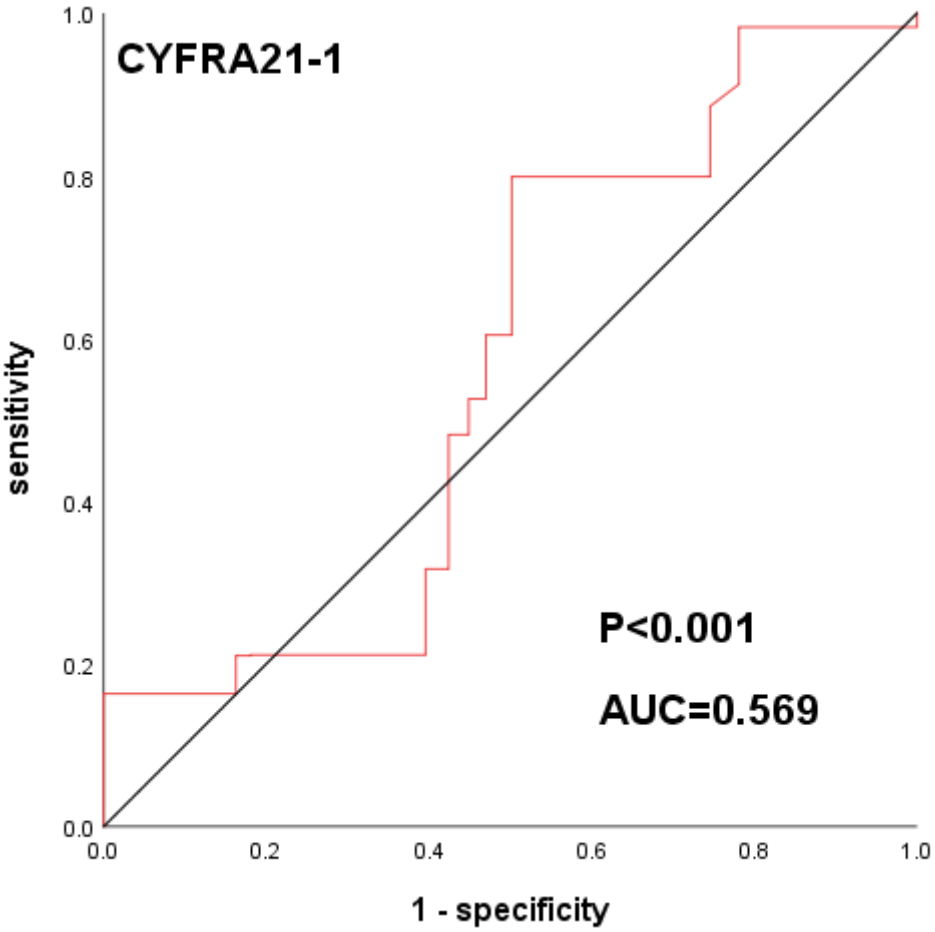

Figure 5

CYFRA21-1

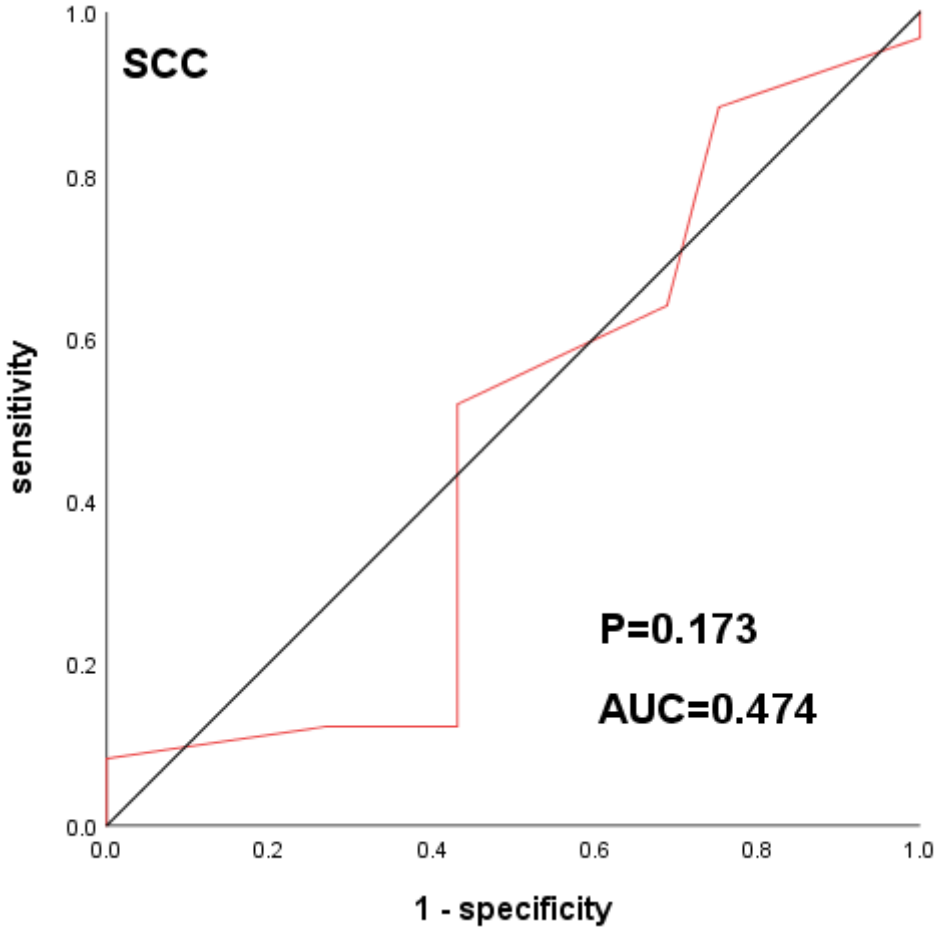
SCC
sensitivity
1 - specificity
P=0.173
AUC=0.474
0.0
0.2
0.4
0.6
0.8
1.0

Figure 6

SCC

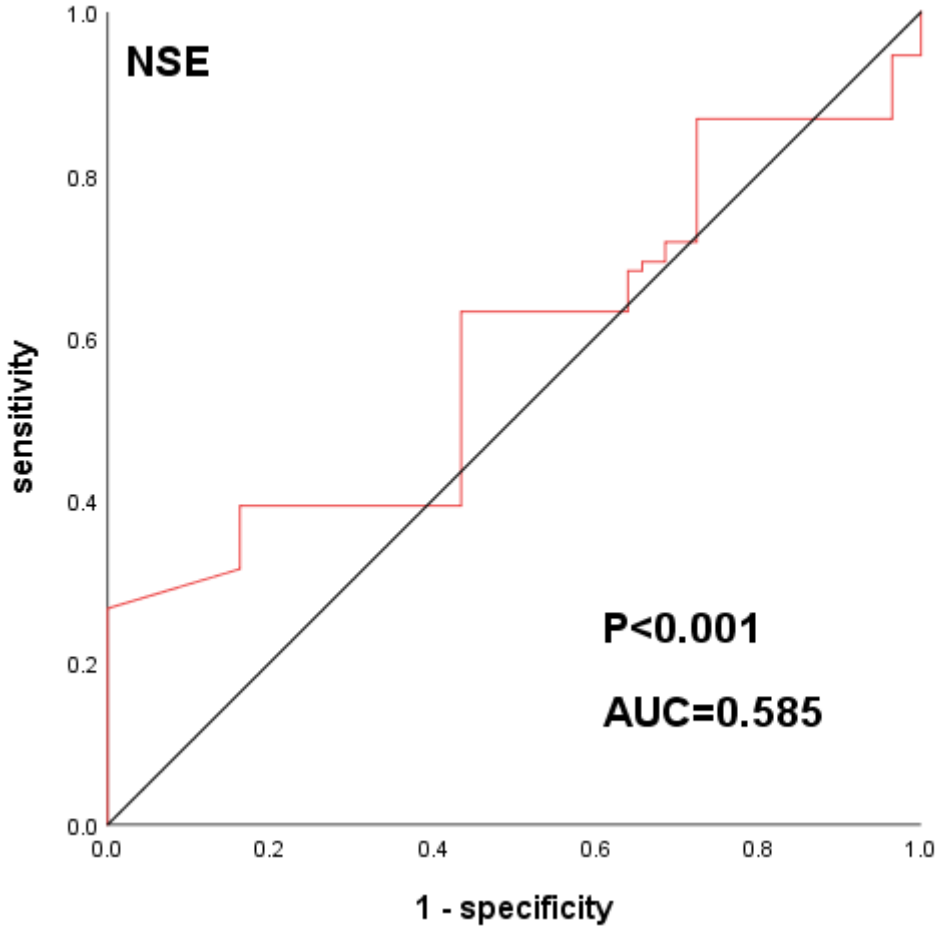
NSE
sensitivity
1 - specificity
P<0.001
AUC=0.585
0.0
0.2
0.4
0.6
0.8
1.0

Figure 7

NSE

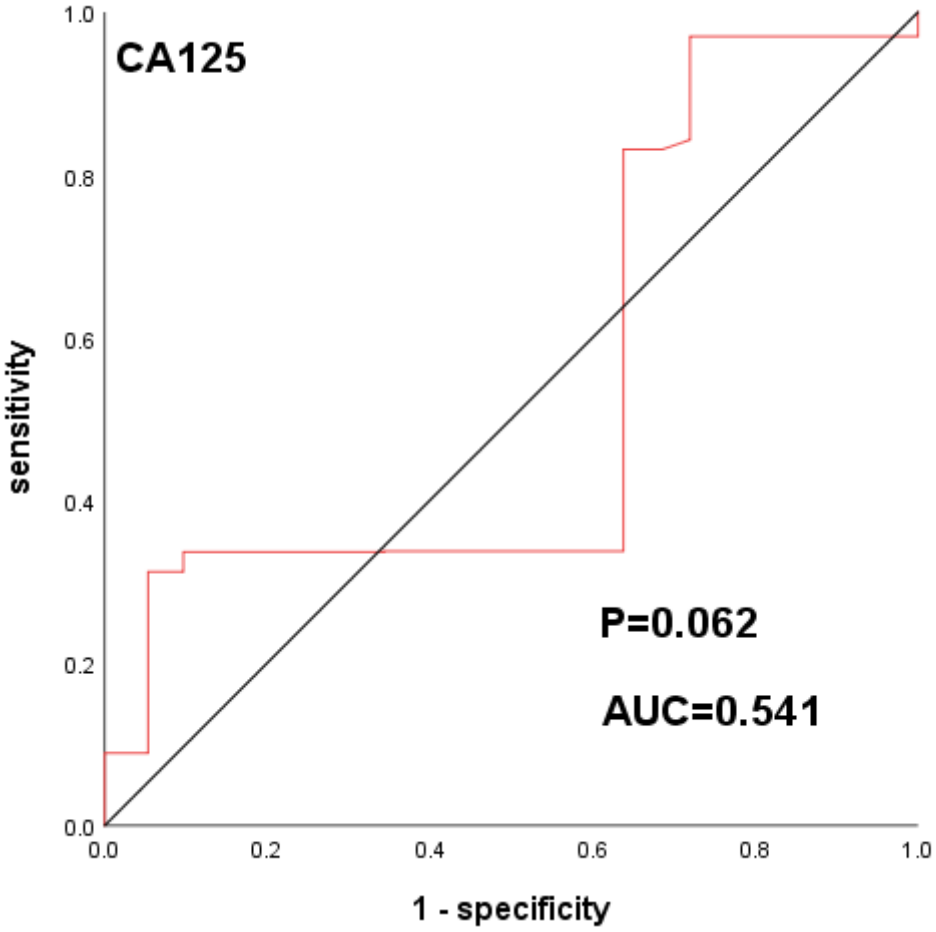

Figure 8

CA125