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BRIEF REPORT

Apo10 and TKTL1 in blood macrophages as biomarkers for differentiating lung cancer from benign lung lesions: a comparative study with conventional biomarkers

Chuanbo Xie[1] · Shuqing Wang[1] · Chi Guo[2] · Yuying Liu[1] · Musheng Zeng[3,4]

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Abstract

The detection of biomarkers in blood macrophages is a new non-invasive cancer screening method, but its performance in early stage lung cancer screening remains undetermined. We evaluated the Apo10 and TKTL1 levels in blood macrophages of 156 early-stage lung cancer patients and 153 controls. APT (combination of Apo10 and TKTL1) level was significantly higher in the lung cancer group than that in the control group ($P < 0.001$). AUROC analysis showed that APT has high diagnostic value in differentiating early-stage lung cancer ($AUC = 0.9132$) and can be considered a biomarker for screening lung cancer patients from individuals with lung nodules.

Keywords Lung cancer · Screening · Apo10 · TKTL1

1 Introduction

The use of flow cytometry to detect biomarkers in blood macrophages (Epitop Detect in Macrophages, EDIM) has opened a new era for early and non-invasive cancer diagnosis. It can detect biomarkers in macrophages carrying tumor substances in the blood. EDIM technology's high sensitivity in cancer screening stems from two key factors [1]. First, tumor substances within macrophages are not diluted as the macrophages return from the tumor through the bloodstream, and the second is the active detection and phagocytosis of tumor cells by macrophages.

Apo10 and TKTL1 are two biomarkers in the EDIM detection system. Apo10 is an antigenic epitope of DNaseX (deoxyribonuclease) that plays a key role in cell apoptosis. The accumulation of Apo10 can be used as a marker for detecting the formation of tumor/proliferative disease [2]. Comparatively, TKTL1 mainly regulates glycolysis metabolism, and its high expression in malignant tumor cells is closely related to tumor invasiveness, therapeutic resistance and prognosis [3]. Thus, analysis of these two markers can give a good illustration of a patient malignancy.

Previous research [4, 5] showed that the accuracy of combined detection of serum Apo10 and TKTL1 (APT) in distinguishing oral squamous cell carcinoma from healthy controls were over 95%. However, the effects of Apo10 and TKTL1 in differentiating early stage lung cancer remain to be further studied. This study aimed to evaluate the performance of Apo10 and TKTL1 in differentiating lung cancer patients from patients with other lung lesions or healthy controls.

✉ Chuanbo Xie
xiechb@sysucc.org.cn

✉ Musheng Zeng
zengmsh@sysucc.org.cn

1 Cancer Prevention Center, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, 651 Dongfengdong Road, Yuexiu District, Guangzhou 510060, China

2 IMB (China) Medical Technologies CO., Ltd, Beijing, China

3 Department of Experimental Research, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, 651 Dongfengdong Road, Yuexiu District, Guangzhou 510060, China

4 Guangdong-Hong Kong Joint Laboratory for RNA Medicine, Guangzhou 510060, China

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2 Methods

From November 2020 to February 2022, 156 lung cancer patients at the Department of Thoracic Cancer Prevention Center of Sun Yat-sen University Cancer Center (SYSUCC) and 153 controls (pulmonary nodules, n = 28; healthy, n = 124) from our Physical Examination Center (SYSUCC) were included in this study. The research protocol was approved by the institutional review board of SYSUCC (ID: G2022-005-01), and all participants provided written consent for the anonymous use of their data for the research purposes. All the controls underwent chest CT examinations within 3 months before Apo10 and TKTL1 testing to distinguish whether they were true healthy controls or controls with pulmonary nodules. We collected 2.7 mL EDTA-anticoagulation venous blood for all the participants 60 min after their last meal. The blood samples were stored at room temperature (15–25 °C) before testing. The detailed methodology of testing Apo10 and TKTL1 can be found elsewhere [6]. Other serum tumor markers (carcinoembryonic antigen [CEA], neuron-specific enolase [NSE], cytokeratin 19 fragment antigen 21 − 1 [CyFra21-1], and squamous cell carcinoma [SCC]) were prospectively measured just before the start of therapy for all the participants. All analyses were performed using the Statistical Package for Social Sciences (SPSS version 25.0; IBM, Chicago, IL) software. We compared the Apo10, TKTL1, APT, and the other lung cancer-related biomarker levels between the lung cancer patients and patients with pulmonary nodules, the lung cancer patients and the true healthy controls using independent t-tests or Spearman's rank tests. Receiver operating characteristic (ROC) curves were generated, and the area under the curve (AUROC) was calculated to compare the diagnostic values of Apo10, TKTL1, APT and traditional cancer biomarkers in distinguishing lung cancer patients from controls. Differences were considered statistically significant at $P < 0.05$.

3 Results

Table 1 shows the comparisons of characteristics between lung cancer patients and controls. The lung cancer group comprised 84 males (53.8%) and 72 females (46.2%), with an average age of 56.42 (± 10.96) years. Of them, 102 (65.4%) did not smoke, 39 (20%) were past smokers, 15 (9.6%) were active smokers (data not shown), and 127 (81%) had stage I or a carcinoma in situ (Tis) lesion. The lung cancer cohort contained 142 patients with adenocarcinoma (91%) and 14 (9%) with squamous cell carcinoma. Comparatively, the control group comprised 81 males (52.9%) and 72 females (47.1%), at an average age of 56.05 (± 10.97) years. No significant difference in the baseline characteristics of sex and age was observed between the lung cancer patients and the controls. From Fig. 1, we also observed that Apo10, TKTL1, and APT levels in the lung cancer group were significantly higher than that in the benign pulmonary nodules group and healthy control group (Fig. 1A), while there were no significant differences in SCC, NSE, CyFra21-1, CEA across the lung cancer, pulmonary nodules, and healthy control groups (Fig. 1B). The diagnostic values of Apo10, TKTL1, and APT were high, with AUCs of 0.8606 (95%CI = 0.7837–0.9375), 0.8196 (95%CI = 0.7377–0.9015) and 0.9132 (95%CI = 0.8560–0.9704), respectively (Fig. 2). The AUROC of the other tumor markers (SCC, NSE, CyFra21-1 and CEA) for screening early stage lung cancer were 0.6249 (95%CI = 0.5196–0.7302), 0.577 (95%CI = 0.4671–0.6869), 0.5363 (95%CI = 0.4333–0.6393), and 0.6472 (95%CI = 0.5486–0.7458), respectively (Fig. 2).

Table 1 Comparisons of the baseline characteristics between lung cancer patients and controls

Variables	Lung cancer patients	Controls			P-value 1	P-value 2	P-value 3
		Total	Lung nodules	Healthy			
Sex, n (%)							
Male	84 (53.8)	81 (52.9)	15 (51.7)	66 (53.2)	0.909	0.833	0.525
Female	72 (46.2)	72 (47.1)	14 (48.3)	58 (46.8)			
Family history of cancer, n (%)							
No	116 (74.4)	129 (84.3)	23 (79.3)	106 (85.5)	< 0.001	< 0.001	< 0.001
Yes	40 (25.6)	20 (13.1)	6 (20.7)	14 (11.3)			
Missing	-	4 (2.6)	0 (0.0)	4 (3.2)			
Age (years), mean (± SD)	56.42 (10.96)	56.05 (10.97)	60.69 (10.02)	54.97 (10.93)	0.7704	0.052	0.272

Abbreviations: SD, standard deviation. P-value1, lung cancer patients versus total controls; P-value2, lung cancer patients versus lung nodule controls; P-value3, lung cancer patients versus healthy controls (For sex and family history of cancer were compared using chi-square tests and for age were compared using t-test)

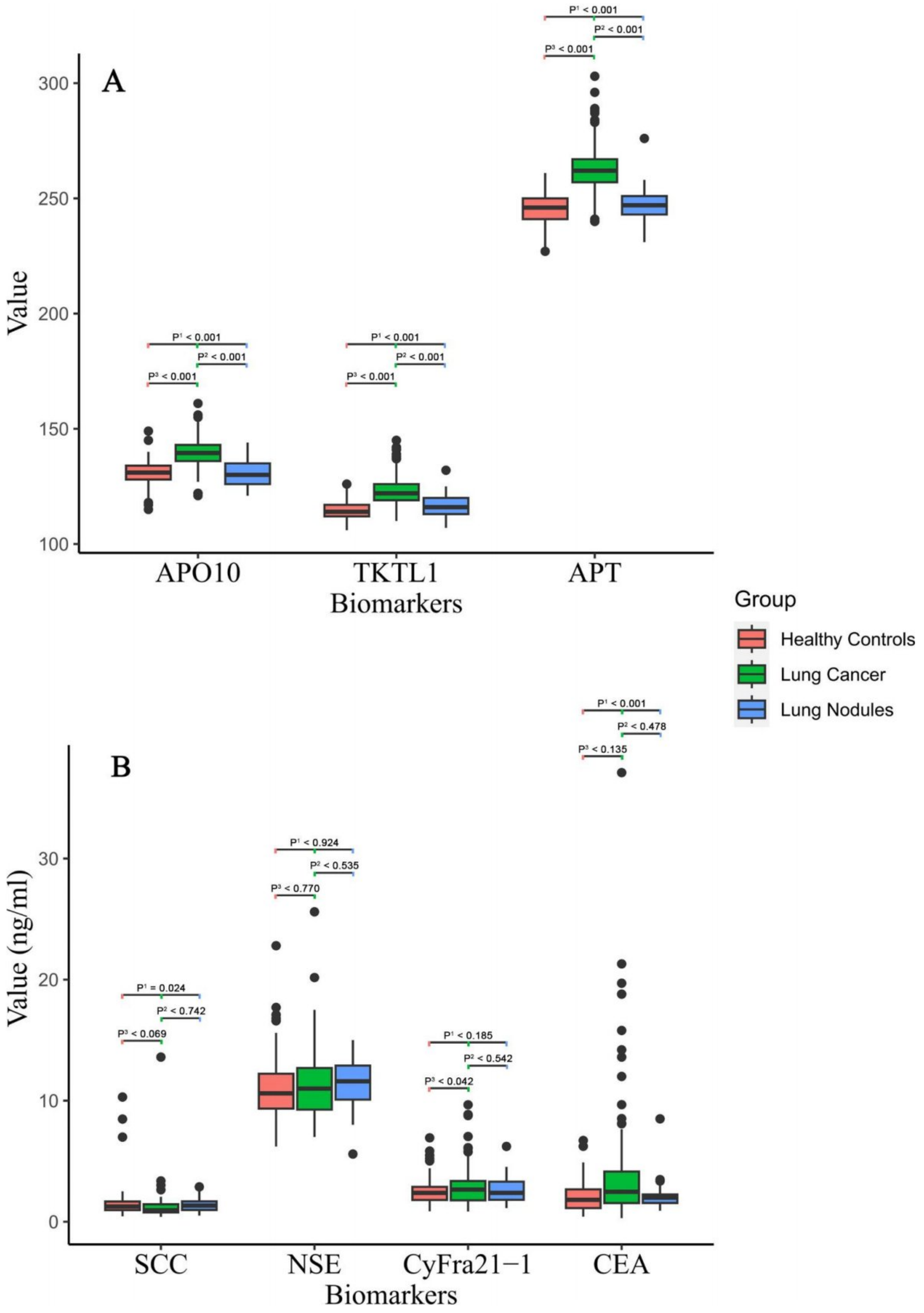

Fig. 1 The distributions of Apo10, TKTL1, APT, and four traditional cancer biomarkers in the lung cancer group, lung nodules group, and healthy control group. Abbreviations: TKTL1, transketolase-like 1; APT, the addition of the two scores of Apo10 and TKTL1; SCC, squamous cell carcinoma; NSE, neuron-specific enolase; CyFRA21-1, cytokeratin 19 fragment antigen 21 – 1; CEA, carcinoembryonic antigen

4 Discussion

Our results showed that APT had greater diagnostic reliability in differentiating lung cancer cases from those with pulmonary nodules and healthy persons compared to existing makers such as SCC, NSE, CyFra21-1 and CEA.

Annual CT examination has become the standard screening method in the world since the results of the National Lung Screening Trial (NLST) were published [7]. However, one of the major clinical concerns of LDCT-based screening is the appropriate management of pulmonary nodules detected on a scan. Previous studies have shown that the risk of major complications was 4.5 per 10,000 persons screened, and 25% of the surgical procedures in the NLST were performed on nodules determined to be initially benign [8]. Hence, biomarkers that can accurately triage malignant from benign pulmonary nodules are important for guiding

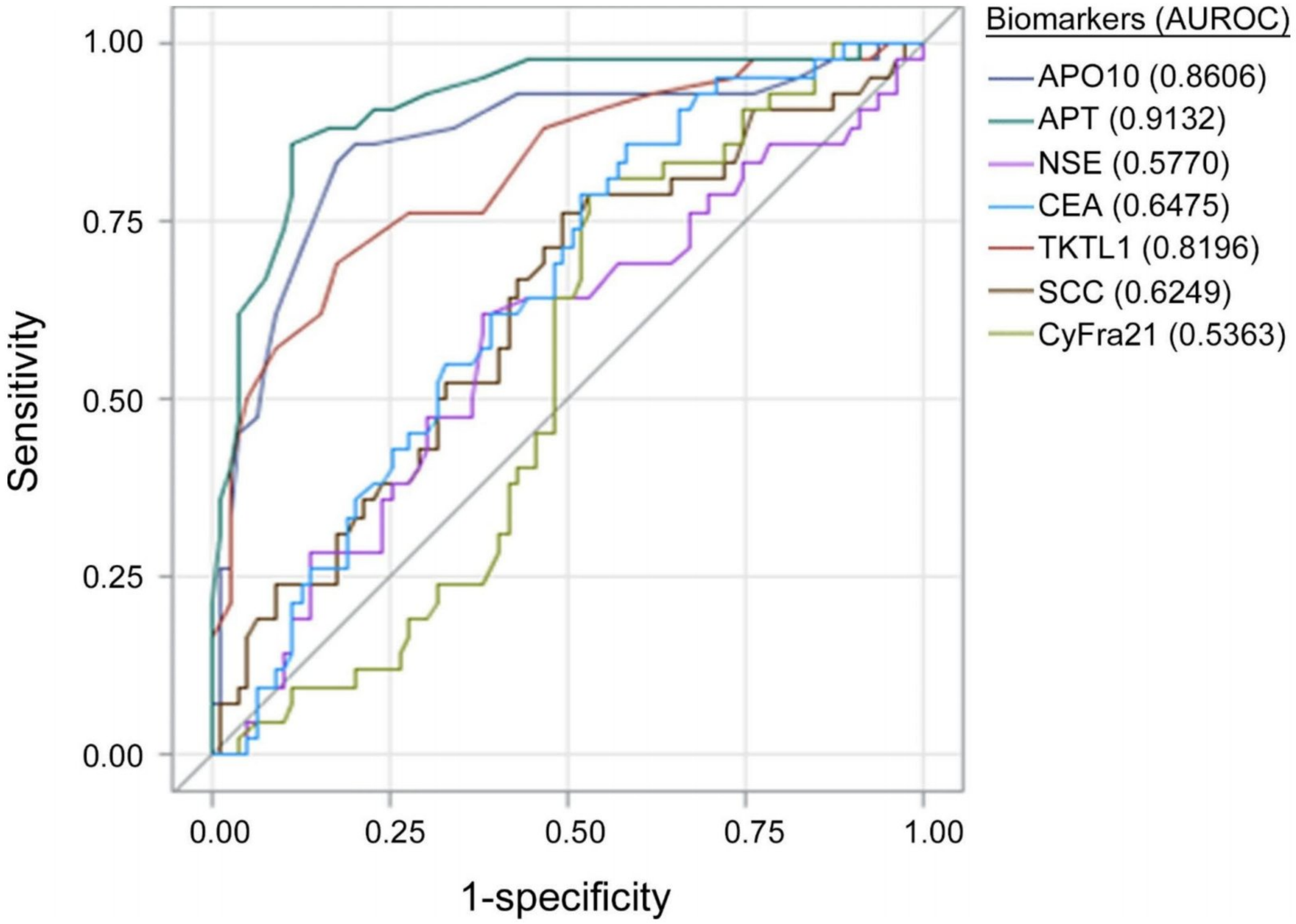

Fig. 2 The performance of Apo10, TKTL1, APT, and other tumor biomarkers in differentiating lung cancer patients from controls. Abbreviations: AUROC, area under the receiver operating characteristic; CEA, carcinoembryonic antigen; SCC, squamous cell carcinoma; NSE, neuron-specific enolase; CyFRA21-1, cytokeratin 19 fragment antigen 21 – 1; TKTL1, transketolase-like 1; APT, the addition of the two scores of Apo10 and TKTL1

clinical decision-making and of potential to reduce costs and the risk of morbidity and mortality in screening programs.

Given this, previous studies have developed models to predict the probability of malignancy for nodules detected by CT and without information on nodule growth [9]. These models are mainly established on radiologic features and patient-related attributes. Although some of these models (e.g., PanCan models) have high accuracy, variables such as the type of nodules used in these models are difficult to standardize across populations which might hinder model generalizability. In this study, we found that APT had higher accuracy than the traditional biomarkers and models in distinguishing early-stage lung cancer patients from patients with benign pulmonary nodules or true healthy controls and was not affected by population variation. Therefore, it could be used as an adjunct to LDCT for pulmonary nodule surveillance.

However, APT as a diagnostic tool also has one potential limitation. Although previous research demonstrated that APT had a extremely high detection rate of cancers of different origin [10], APT may not be sensitive enough to detect all cancers particularly for those that do not produce a strong immune response.

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Authors' contributions Chuanbo Xie, Shuqing Wang, Yuying Liu and Chi Guo were responsible for data acquisition and analysis. Chuanbo Xie was responsible for preparing the first draft of the manuscript. Chuanbo Xie and Yuying Liu were responsible for recruiting study participants. Chi Guo was responsible for Apo10 and TKTL1 testing. Musheng Zeng supported the study design development and interpretation of the data. All authors had an opportunity to review and revise the manuscript and approved its final submitted version.

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Data Availability Chuanbo Xie, Yuying Liu, and Musheng Zeng had

full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. The data used for the analyses are available by request to the corresponding author (Chuanbo Xie).

Declarations

Competing interests The authors declare no competing interests.

Ethics approval and consent to participate This study was approved by the institutional review board of Sun Yat-sen University Cancer Center (SYSUCC, ID: G2022-005-01), and all procedures were conducted according to the principles expressed in the Declaration of Helsinki. The authors enrolled the participants using a chart review blinded to their personal identifying information (name, address, etc.). Therefore, this study did not violate the right and welfare of the subjects. All participants provided written consent for the anonymous use of their data for their research purposes.

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